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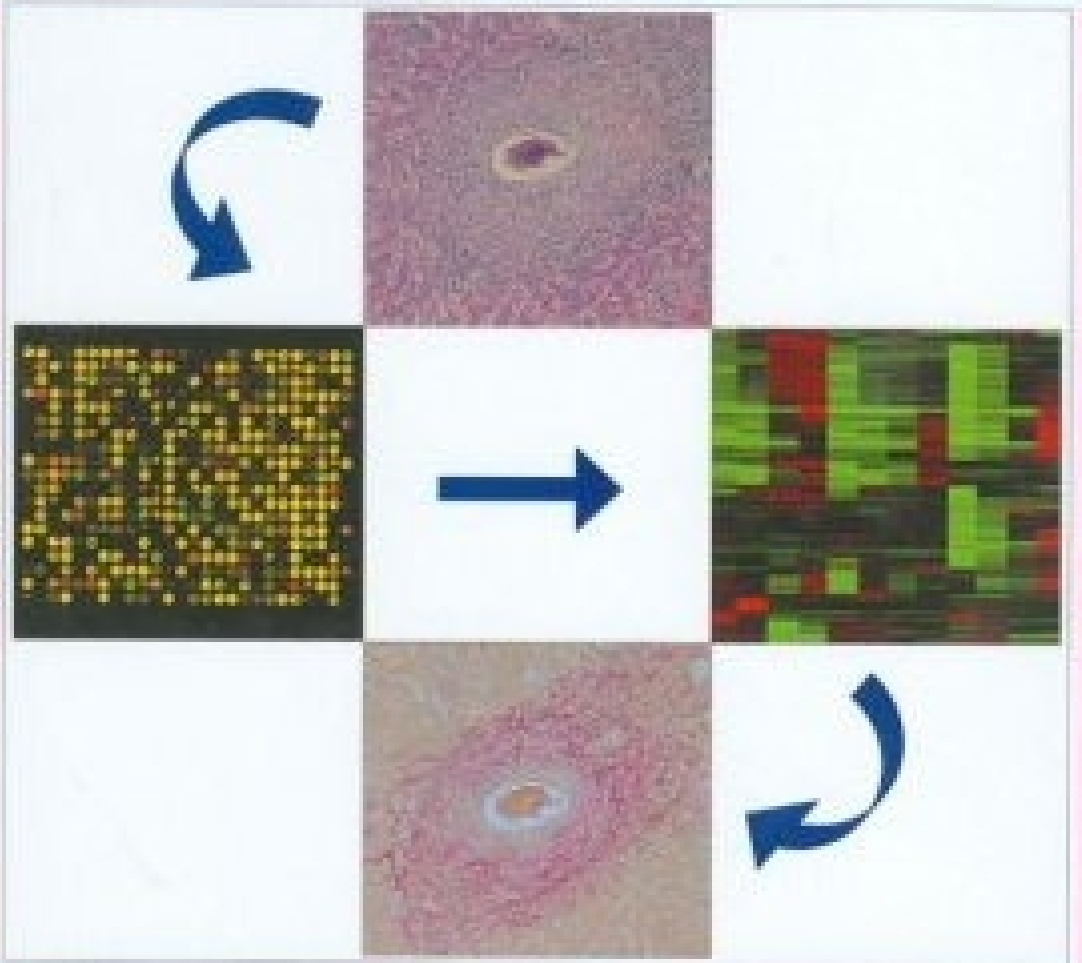
# ADVANCES IN PARASITOLOGY



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# PREFACE

It is rare for one parasitologist to have the opportunity to continue investigations over half a century. Don Thomas (University of Sussex, UK) has had the insight to realize how important this kind of observation can be and has contributed a fascinating chapter concerning the ecology of helminth parasites and their fish hosts. He first carried out ecological studies involving salmonid fish in Welsh rivers in the early nineteen fifties, his interest being their feeding behaviour, helminth parasites and intermediate hosts. An opportunity to follow up these studies in 1998 made it possible to ascertain how changing environmental conditions might have impacted on the salmonid hosts and their parasites. Don uses this study as a backdrop to delve into many important questions concerning interactions between populations of helminth parasites, their hosts and their environment. There is much to stimulate the reader here together with ideas for future research.

Almost twenty years have passed since the last detailed review on avian schistosomes within the genus *Trichobilharzia* by Blair and Islam (1983). So what has been happening in the interim? In a comprehensive chapter Petr Horák, Libuše Kolářová (Charles University, Czech Republic) and Coen Adema (University of New Mexico, USA) bring together a wealth of new information concerning recognized *Trichobilharzia* species, their biology, interactions with their hosts and the pathology associated with infection. Particular attention is given to problems concerning the systematics of the genus and to morphological and molecular methods available for species characterization. Due to the non-specific penetration of the cercariae through the skin, contact with water bodies containing infected snails and cercariae can lead to cercarial dermatitis in man. Cercarial dermatitis is a common condition, which with few exceptions occurs globally. Of perhaps greater concern is that developing schistosomula have now been reported from various organs and nervous tissue of mammals, which may lead to further complications. The chapter ends by considering the epidemiology of cercarial dermatitis including the identification of potential transmission sites and possible control measures.

In the third chapter, Bob Snow and Kevin Marsh, from the Kenya Medical Research Institute, discuss the complicated relationships between intensity of malarial transmission, which can range from fewer than one infective mosquito bite per year to several thousand such bites, and the burden of mortality and morbidity. At the lower end of the range, all age groups are at risk of

developing severe malaria with, mainly, cerebral involvement, whereas at the higher end it is predominantly infants who are at risk of severe disease, and malarial anaemia rather than cerebral malaria dominates the clinical picture. This of course reflects the increasingly rapid development of immunity as transmission intensity increases. The authors conclude that it is in areas of moderate to high transmission that interventions such as the use of insecticide-treated bed nets (ITNs) will have the greatest effect in reducing disease and mortality. In areas of high transmission intensity, the data suggest that malarial and all-cause mortality appear to saturate, although the authors agree that this is contentious. If this is so, initial reductions in mortality may be difficult to sustain as even the reduced mortality rate may lie within the saturated region of the curve. However, the overall benefits of using ITNs in reducing the disease burden regardless of transmission intensity and in producing even limited reduction of infant mortality rates emphasize that their use should be encouraged in all areas of Africa where malaria is endemic.

Most helminth infections induce immune responses characterized by production of Th-associated cytokines and antibodies. These have usually taken to be host-protective, with a highly regulated and controlled host inflammatory response directed against parasite antigens and leading to a chronic infection with mild symptoms. The final review by Karl Hoffmann and David Dunne of the Department of Pathology of the University of Cambridge UK, and Thomas Wynne of the Immunology Section, The National Institutes of Health USA, using schistosomiasis as a model demonstrates that, while most chronic induced type-2 associated mediated responses are held in check by regulated control mechanisms, prolonged cytokine biases can be not only undesirable but, in fact, lethal. Uncontrolled polarized type-1 immune responses can also lead to a serious increase in host immuno-pathology.

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# The Ecology of Fish Parasites with Particular Reference to Helminth Parasites and their Salmonid Fish Hosts in Welsh Rivers: A Review of Some of the Central Questions

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## ABSTRACT

Ecological studies carried out in Welsh rivers on the feeding behaviour of salmonid fish, their helminth parasites and intermediate hosts in the early 1950s and in 1998 have been used as a basis to review the literature dealing with the following questions. First, how are the helminth populations dispersed in space–time? Second, to what extent are the distributional patterns and the life history strategies of the parasites influenced by physicochemical factors? Third, to what extent are populations of helmith parasites in salmonid fish influenced by host characteristics including the genome, sex, age, size, social position and feeding behaviour? Fourth, are the populations of parasites regulated in a density-dependent manner? Fifth, do the parasites influence the survival and wellbeing of their salmonid hosts and the evolution of sex? Sixth, to what extent is the parasite community influenced by environmental changes including those of an anthropogenic nature and can the parasites be used as bioindicators of pollution?

As with most parasites the helminth species found were highly overdispersed thus making it necessary to undertake a $\log_{10}(1 + x)$ conversion for statistical analyses. Statistical analyses confirm that the genome, age and sex of salmonid fish hosts, the station and seasonal change in radiation levels were significant factors in predicting the number of parasites. The evidence given supports the hypothesis that the feeding behaviour and habitat selection by the host fish, their position in the social hierarchy and the overdispersed nature of the transmission sites are the key factors in causing differences in the parasitic fauna related to host species, age, size and sex. Differences in the helminth parasite community related to station can be explained on the basis of differences in water types, sediments and chemistry. Although the evidence presented is in accord with the consensus view that temperature is correlated with seasonal changes in the abundance of many species of helminth parasites, it is argued that it may not be the direct causative mechanism. It is postulated that the life history strategy that results in a decline in abundance of the more vulnerable adult parasites in the gut of the salmonid hosts during the summer has arisen as a result of evolutionary pressures. At this time, the gut environment is particularly inhospitable because of the temperature-related enhancement of the host's immune mechanism and the increased gut turnover rate. In contrast, the larval stages in the immunologically and metabolically more benign intermediate host would be under less intensive selective pressures. It is postulated therefore that evolutionary pressures have caused the parasites to leave the definitive host and concentrate their reproductive efforts in the intermediate hosts during the warmer months.

Evidence is given in support of the hypothesis that the parasite populations are regulated in a density-dependent manner and that the regulatory mechanisms may involve the host's immune mechanisms and intraspecies competition and interspecies competition of an exploitative or interference nature. Quantitative studies using 'K' factor analysis and biochemical research to elucidate the nature of the interference mechanisms are required to test this hypothesis. The absence of age-related resistance indicates an old and stable relationship in which the immunosuppressive and immunoavoidance mechanisms of the parasites and hosts, respectively, are in balance. This indicates that the introduction of novel parasites or new genetic strains of host fish could result in harmful epidemics.

Despite causing tissue damage, there was no evidence of parasite-induced mortality among the salmonids in the Teifi.This finding is in accord with the generally accepted view that most freshwaters are not troubled by parasite problems, although parasites are present in abundance. In fact, parasite abundance in the salmonid fish in theTeifi was positively correlated with the condition factor and the adipose index. Two testable hypotheses were advanced to explain these observations. First, the more dominant well-conditioned fish in the hierarchy are more likely to acquire parasites because they ingest more

food items and spend more time in sheltered habitats with depositing sediments where transmission mainly occurs. Second, the parasites may release factors that stimulate the host's immune and endocrinological systems to produce factors that enhance somatic growth and inhibit reproduction of the host. This benign relationship is considered to be indicative of long-term coevolution.

The sex of the fish had a sigificant influence on the abundance of the parasites in total and also on particular species with the bias in all cases being in favour of the female fish. This review shows that sex bias in parasitism is generally not strong and that male bias in parasitism is not a general rule. Taken as a whole, the results fail to support most of the predictions based on the Hamilton–Zuk and the immunocompetence hypotheses. Possible hypotheses to explain why parasitism tends to be higher in female than in male trout include testosterone immunosuppression, corticosteroid-based immune suppression and differences between the size and behaviour of the sexes. However, the latter two hypotheses have more credence, although testosterone levels are higher in female than male trout.

Between the early 1950s and 1998 there has been a marked decline in the prevalence, abundance and diversity of the helminth parasite communities in salmonid fish as well as their intermediate hosts. Possible reasons for these declines include heavy metal pollution, increased acidity and habitat degradation linked to changes in land use. It is concluded that although helminth parasites can provide supplementary information on pollution, the use of biotic indices based on the Biological monitoring working party (BMWP) or River invertebrate prediction and classification system (RIVPACS) methods are preferable. However, as these methods were designed to measure the impact of organic pollution they lack the sensitivity for measuring metal pollution. It is advocated therefore that new biomonitoring methods should be developed to measure the impact of heavy metal pollution using biotic indices based on the sampling of the susceptible invertebrate communities inhabiting depositing sediments in the transmission sites of helminth parasites.

## 1. INTRODUCTION

Earlier studies of fish parasites were of necessity mainly concerned with taxonomy, geographical distribution, host specificity and parasites' life cycles. These pioneering studies raised many questions regarding the nature of the interactions between the parasites, the host and their environment and the mechanisms responsible for causing the observed patterns. Some of the most important of these, which are summarised in Figure 1, are as follows. First, how are the parasite species distributed spatiotemporally. Second, to what

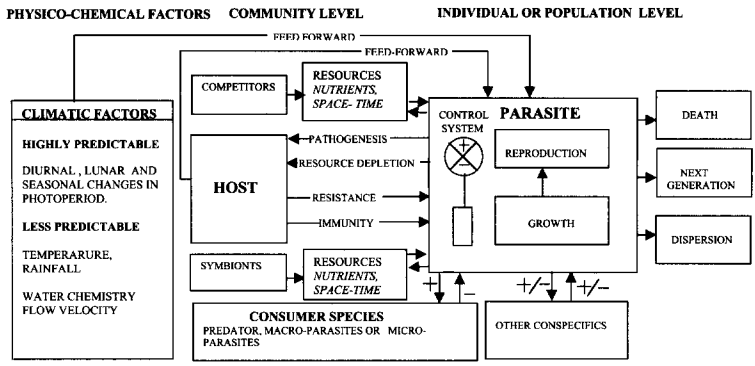


*Figure 1* Ecological factors that may influence the distribution and abundance of helminth parasites and their hosts.

extent are parasite populations influenced by physicochemical factors including those that vary seasonally in a predictable way? Third, is there any evidence that the life history strategies of the parasites have evolved to respond to predictive or feedforward signals? Fourth, to what extent are parasite populations influenced by the genome, sex, age, length, weight, social position and feeding behaviour of the host? Fifth, is there any evidence that the parasite populations are regulated in a density-dependent manner by competition for resources, predation, parasitism and the host's defensive mechanisms? Sixth, is there any evidence that the parasites influence the health and survival of the hosts, drive their population cycles (Freeland, 1976; Anderson and May, 1979; Dobson and Hudson, 1992), determine host genetic structure (Shykof and Schmidt-Hempel, 1991) and favour the evolution of sex (Hamilton, 1980; Hamilton and Zuk, 1982; Folstad and Karter, 1992)? Seventh, to what extent may the helminth community be influenced by environmental changes, including those of an anthropogenic nature, and are they suitable bioindicators of pollution?

Some of these questions were addressed during an earlier investigation carried out on the trout, (*Salmo trutta* L.) and the salmon parr (*Salmo salar* L.) at three stations on two river systems in West Wales in the early 1950s (Thomas, 1956, 1957, 1958a,b, 1962, 1964a,b,c). The following developments have made it possible to re-examine these questions. Firstly, new, more powerful statistical software has made it possible to reanalyse quantitative data more rigorously. Secondly, research by fish parasitologists carried out subsequently has made further valuable contributions to our knowledge and understanding thus making it possible to reinterpret the data. Thirdly, two of the rivers studied, namely the Teifi and the Pysgotwr, have undergone many detrimental changes as a result of increased levels of abstraction and acid deposition, canalisation and changes in land use since the 1950s. An opportunity to revisit

these sites in 1998 made it possible to ascertain how changing environmental conditions might have impacted on the salmonid hosts and their parasites. In this review, the necessary background will be included to make it possible to address fully the questions outlined above.

## 2. ECOLOGICAL BACKGROUND

### 2.1. *Topography*

The area studied (Figure 2) is geologically very uniform, consisting mainly of sedimentary Silurian grits and shales, except where anticlinal folding and faulting brings out Ordovician strata in the eastern region. The region as a whole is traversed by the Teifi and Towy anticlines. Weathering has resulted in an area that is predominantly bleak, high moorland with a capping of poor, calcium-deficient soil. As a result, except in the valleys, land is poorly developed, supporting only a sparse population. Both rivers under consideration have their sources at high altitudes in peat bogs or lakes in upland moors characterised by rough, yellow mountain pasture in dry places and *Sphagnum* in the wetter regions.

The Teifi has its source in Llyn Tefi, one of six upland lakes at an altitude of 455 m OD on the southern edge of the Plynlimmon plateau in the western region of the Cambrian Mountains. It is approximately 117.5 km long with a catchment of approximately 1007 km$^2$. After being joined by over 70 tributaries it flows into Cardigan Bay. In its first 7 km the channel falls 215 m but for the next 15 km, as it passes through the Tregaron Bog (Cors Goch Glan Teifi), the channel gradient is negligible. In this raised bog the river wanders sluggishly through a vast quagmire with a substratum consisting mainly of fine gravel and silt. When not in flood the water flow is barely detectable and it consists of a long series of deep stagnant pools connected by short stretches of shallow water. At the southwest end the river swells into broad and muddy shallows. Along the river banks the vegetation forms a well-defined river terrace, the most abundant plants being *Juncus effusus*, *Phalaris arundacea*, *Deschampia caespitosa*, *Carex acuta*, *Galium palustre*, *Ranunculus acris* and *Equisitum* species (Godwin and Conway, 1939). Submersed vegetation is also abundant. *Nuphar lutea* grows by the edges of deep pools while *Potomogeton natans*, *Myriophyllum*, *Ranunculus penicillatus* (Dumart) var. *penicillatus* and *Callitriche obtusangula* thrive in the shallows. *Glyceria fluitans* grows patchily on the margins. According to Brooker (1984) *Apium inundatum*, *Luronium natans* and *Nuphar lutea* are restricted to the Tregaron Bog. Stations 1 and 2 for the faunistic studies and Station A for the fish collection are located on the southern end of the bog (Figure 2).

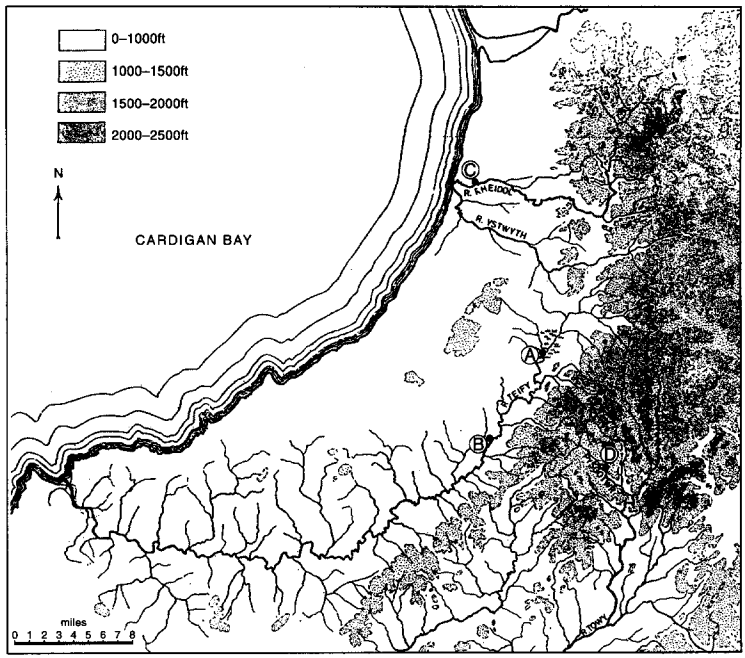


*Figure 2* Map showing the river systems investigated. The grid references and site landmarks for Stations 1–8 on the Teifi where the invertebrate samples, including intermediate hosts, were collected are as follows: (1) SN 6750 6186; Tregaron Bog; shallows 1.5 km N of Pont Eynon. (2) SN 6727 6148; 200 m north of Pont Eynon. (3) SN 6638 5775; below Tyndomen. (4) SN 6645 5717; near mouth of River Carfan. (5) SN 6528 5691; near Pontllanio Bridge. (6) SN 6489 5685; at bend below Pontllanio Bridge. (7) SN 6468 5636; near Old Mill, Llanio Isaf. (8) SN 6423 5440; at bend below Pontgoyan Bridge. Fish samples used to collect helminth parasites were obtained from station A in the Tregaron Bog area which encompasses Stations 1–2 above and also from Station B located below the bog area which encompasses Stations 3–7 above. Station D is located on the Pysgotwr, a tributary of the River Towy.

Downstream of the bog the gradient is relatively constant falling at a rate of about 150 m in 100 km. The river, now swollen to a width of 20–30 m by numerous tributaries, meanders through farmland dominated by sheep and dairy farming, forming a succession of pools, riffles, fast reaches and backwaters. The substratum consists of cobbles, pebbles, coarse gravel and silt depending on the water type. Submersed macrophytes consist mainly of ubiquitous *Ranunculus penicillatus*, *Potomogeton natans*, *Myriophyllum* sp., *Callitriche hamulata* and *C. stagnalis* on gravel beds and *Fontinalis antipyretica* on stony substrate. Over the last 50 years both the submersed macrophytes and riparian trees, such as the alder and willows, have become

less abundant. As a result the main river and particularly some of the tributaries, which have been subjected to canalisation, have become more erosive in character. Stations 3–8 for the faunistic studies are located in the upper region of the Teifi between Tyndomen and Pont Goyan. Salmonid fish were collected at Station B (Stations 3–7 in faunistic study).

The Pysgotwr, one of the small headwaters of the River Towy (Figure 2), is situated at an altitude of 430 m on impermeable mudstones and shales of the Silurian series (Station D). During the 1950s its catchment, which consisted of peat bogs and rough, virtually treeless mountain pasture, was used for sheep farming. At that time the characteristic bankside species were *Drosera rotundifolia*, *Molinia caerula* and *Juncus squarrosus*. Often the smaller feeder streams were almost completely shaded by the latter species. As a result of the steep gradient the small 1–4.5 m wide stream had many small waterfalls with scour pools followed by fast reaches and riffles. The substrate was strongly erosive in character, consisting mainly of small boulders, cobbles and coarse gravel usually colonised by epilithic algae and *Fontinalis antipyretica*.

Since then, however, virtually the entire catchment has been planted with coniferous woodland.

## 2.2. *Physicochemical Characteristics of Study Sites*

As the Teifi and Towy catchments are located in one of the wet areas of the British Isles, with a mean annual rainfall of 1430 mm, the rivers exhibit spate characteristics. Thus, the average flow, the maximum instantaneous flow and the minimum daily mean flows for the Teifi are 28.38, 448.80 and 0.73 cumecs, respectively. Following changes in land use including land drainage, increased erosion due to a doubling of the sheep population since 1950, and canalisation of parts of the main river and some of the feeder streams, the river has become more susceptible to spates and consequently more erosive in character.

As might be expected from the geology the waters from the upper reaches of the Teifi and the Tywi have low conductivities, low base contents (with $Na^+ > Ca^{2+}$), low alkalinities and low micronutrient content (Table 1). It is of interest that the Teifi retains its dystrophic, mountain stream characteristics throughout its length. In the 1980 National Water Quality Survey most of the river was classified as 1A except for some sections of the tributaries and the main river upstream of Tregaron. These were placed in Grade 2 because of elevated concentrations of zinc, originating in disused mine-workings in the upper catchment (Brooker, 1984). It is of interest that measurable concentrations of zinc and lead were found in the water columns below Tregaron during the present investigation (Table 1).

*Table 1* Chemistry of water columns in River Teifi and Pysgotwr

| | Teifi (2) | Teifi (2) | Teifi (4) | Teifi (5) | Teifi (6) | Teifi (7) | Teifi (8) | Pysgotwr |
|---|---|---|---|---|---|---|---|---|
| Date | 1.4.77–1.7.98 | 23.5.98 | 23.5.98 | 22.6.98 | 22.6.98 | 22.6.98 | 22.6.98 | 1.4.97–1.6.2000 |
| pH | 5.5–7.7 | 6.85 | 6.94 | 6.67 | 6.67 | 6.73 | 6.78 | 4.8–6.9 |
| $Na^+$ | 0.9–18.7 | 11.5 | 10.1 | 7.5 | 7.0 | 7.1 | 5.9 | 3.37–6.9 |
| $K^+$ | 0.10–1.84 | 0.91 | 1.08 | 0.59 | 0.61 | 0.624 | 0.746 | 0.10–0.27 |
| $Ca^{2+}$ | 2.61–10.21 | 8.24 | 8.24 | 5.70 | 6.00 | 6.3 | 6.1 | 1.0–2.04 |
| $Mg^{2+}$ | 0.98–3.5 | 2.6 | 2.64 | 1.90 | 1.95 | 2.02 | 1.93 | 0.62–1.25 |
| $Cu^{2+}$ | 0.0017–0.005 | – | – | 0.001 | 0.001 | 0.002 | 0.002 | 0.001–0.002 |
| $Zn^{2+}$ | 0.011–0.109 | 0.019 | 0.0125 | 0.032 | 0.024 | 0.025 | 0.017 | 0.0018–0.0178 |
| Pb (D) | 0.002–0.005 | 0.0029 | – | 0.004 | 0.004 | 0.003 | 0.003 | 0.002 |
| Al (D) | 0.012–0.151 | – | – | 0.083 | 0.025 | 0.054 | 0.097 | 0.035–0.123 |
| $Cl^-$ | 6.0–32.0 | 19.6 | 18.0 | 12.9 | 11.8 | 11.9 | 12.5 | – |
| $SO_4^{2-}$ | 4.9–28.5 | 7.95 | 8.24 | 8.0 | 7.7 | 8.1 | 6.6 | – |
| $HCO_3^-$ | 7.9–15.9 | 23.3 | 22.0 | 15.9 | 15.1 | 15.7 | 16.6 | 1.28–9.68 |
| Ammonia | 0.01–0.27 | 0.01 | 0.01 | 0.06 | < 0.01 | < 0.01 | < 0.01 | 0.01–0.07 |
| $NO_3^-$ (as N) | 0.078–2.41 | 1.17 | 1.54 | 0.803 | 0.97 | 1.04 | 1.05 | 0.034–0.516 |
| $NO_2^-$ (as N) | 0.002–0.074 | 0.006 | 0.008 | 0.007 | 0.008 | 0.006 | 0.005 | 0.002–0.016 |
| $PO_4^{3-}$ | 0.002–5.79 | 0.002 | 0.004 | 0.007 | 0.014 | 0.013 | 0.008 | 0.002–0.232 |
| Silica (Si) | – | 0.4 | 0.68 | 1.13 | 1.25 | 1.34 | 158 | – |
| Conductivity (μS) | 48–118 | 92 | – | – | 90 | 90 | 92 | 31.1–43 |
| $O_2$ (% sat.) | 68.2–128.0 | 94.0 | 98.0 | 97.8 | 95.8 | 100.1 | – | 76.5–109.0 |
| BOD* | 0.50–2.10 | – | – | – | – | – | – | 0.6–2.10 |

*Biochemical oxygen demand (BOD)

Concentrations are given in mg $l^{-1}$ unless otherwise stated; D = dissolved.

### 2.3. *Methods Used for Investigating the Helminth Parasites and their Fish Hosts*

Table 2 shows the number of salmonid fish and eels collected at the three stations for the investigation of helminth parasites and feeding behaviour as well as the dates of collection. These fish were captured by angling during the earlier investigation and by electrofishing in 1998. After giving each fish a reference number the following information was recorded on cards: the date and location of capture, species of fish, weight in grams, the absolute length in centimetres, sex and age of the fish (following scale examination at a later date). The numbers of each helminth parasite species found on the skin, gills, cardiac region of stomach, pyloric region of stomach, pyloric caecae, pyloric caecae region of intestine, post-pyloric region of intestine, rectum and cloacal region of each fish were counted. The adipose index, which ranged from 0 to 3, gives a measure of the amount of fat deposited in the region of the pyloric caecae and the stomach region and was assessed visually. Identification of parasites was carried out as far as possible using living material but all the individual parasites were also fixed prior to future examination. In the case of the trematodes this involved fixing a large representative sample in Gilson's fluid under slight pressure from a cover glass and then staining *in toto* preparations in Coelestine Blue B and Mayer's paracarmine. Transverse and sagittal serial sections were stained in Ehrlich's haematoxylin and eosin. These procedures made it possible to demonstrate the coexistence of two sympatric species, *Crepidostomum metoecus* and *C. farionis* in the alimentary canals of the same fish species (Thomas, 1957; 1958b). When observing living *Crepidostomum* it was noted that some individuals, which turned out to be *C. metoecus*, were more elongated and mobile than *C. farionis*. This observation and the tendency of the two species to be segregated in the alimentary canal facilitated identification (Thomas, 1958b). Similar methods were used for identifying the other Platyhelminthes species (Thomas, 1956; 1958a; 1964a, b, c).

*Table 2* The number of fish examined and periods of investigation

| Habitat | No. fish examined | | | Period of investigation |
|---|---|---|---|---|
| | Trout | Salmon parr | Eels | |
| Teifi Station A | 215 | 15 | 88 | Mar–Jun 1951 |
| Teifi Station A | | | | Sep–Nov 1951 |
| Teifi Station B | 685 | 274 | 155 | Jan–Dec 1951 |
| Teifi Station B | 16 | 2 | | Jun 1998 |
| Pysgotwr Station D | 273 | | | Jun–Aug 1951 |
| | | | | Jan–Feb 1952 |

Nematodes and Acanthocephala were examined as described in Mahoney (1966). All the helminth parasites were fixed in Gilson's fluid and stored in formol-alcohol (Mahoney, 1966). The results are expressed in terms of percentage prevalence (% fish infected), mean abundance or mean intensities (Bush *et al.*, 1997). The community structure of the helminth parasites was quantified at the component and infra-population levels using the Berger–Parker index, the Simpson diversity index, the Shannon–Wiener index and Brillouin's index (Magurran, 1988; Bush *et al.*, 1997).

The weight and length was recorded for each fish, thus making it possible to calculate the condition factor (Thomas, 1964c). The stomachs of all the host fish were also examined for food items. These were identified at least to genera and quantified as described by Thomas (1962, 1964c). Unfortunately, it was not possible to obtain fish from the Pysgotwr in 1998 due to their demise as a result of acid deposition that was exacerbated by afforestation with coniferous trees.

## 2.4. *Methods Used for Investigating the Invertebrate Fauna Including Intermediate Hosts*

The methods used in the earlier investigation for collecting the invertebrate fauna, using a fine-mesh net with a semicircular opening, over as wide a range of habitats as possible have been described (Thomas, 1962). These studies made it possible to identify sites where *Sphaerium corneum* occurred in abundance in backwaters and under banks. Laboratory experiments revealed that this was the molluscan host of *Phyllodistomum simile* (Thomas, 1956, 1958a). *Sphaerium corneum* and other sphaeriid species have also been implicated as the molluscan hosts for *Crepidostomum* species (Brown, 1927; Moravec 1982). However, when the River Teifi was revisited in 1997 *S. corneum* could not be found in study sites where it had formerly been abundant. It was determined therefore to undertake further work to elucidate the possible causes of its decline. This work, which was made possible by assistance from the Environment Agency, involved sampling at Stations 1–8 (Figure 2) using nets, as described above, and also a cylindrical, plastic sampler to obtain quantitative samples. The sampler measured 65 cm in length and had a diameter of 10 cm. Penetration of depositing sediments was facilitated by having wooden handles, held in position by a steel sleeve, 15 cm from the top of the cylinder. The provision of flexible ducting, secured to the top end of the cylinder and held above the water surface, made it possible to extract the water column above the core by means of a pump. This procedure greatly facilitated the extraction of the core from the sediment. Following extraction the cores were divided into three sections, each up to 20 cm in depth. These were then washed and sieved (125 μm) to facilitate the extraction of bivalve molluscs and other invertebrates.

The sediments were then placed in plastic bags and taken to the

Environment Agency (EA) laboratory for the analysis of heavy metals including copper, zinc, lead and chromium using Automated Concentration Analysis System (ACAS) accredited methods.

### 2.5. Species of Helminth Parasites

The brown trout at Stations A and B on the Teifi had a much richer fauna of nine and eight species, respectively, compared with only four species ofbrown trout at Station D (Table 3). The only difference between the specific composition of the parasitic communities of trout from Stations A and B was that the plerocercoid larvae of the allogenic cestode, *Diphyllobothrium ditremum*, only occurred in the former station. In contrast to the trout, the salmon parr harboured only four and six species from Stations A and B, respectively. Of the six species of parasites found in eels, two, namely *Bothriocephalus claviceps* and *Paraquimperia tenerrima*, were unique to this fish species. As *Cucullanus (T.) truttae* constituted more than 90% of the total number of individual nematodes found in the trout the statistical data presented subsequently refer mainly to this species. In contrast, *C. ephemeriderum* occurred more commonly than *C. truttae* in the salmon parr.

### 2.6. The Life History Strategies of the Parasites

The salmonids are an ancient group of teleost fish and have probably coevolved with their helminth parasites and their intermediate hosts since before the Cretaceous period, 70–140 million years ago (Norman, 1963). Their geographic distribution today is circumpolar and it is likely that they colonised freshwater ecosystems in Britain following the retreat of the glaciers after the end of the last ice age about 13 000 years ago (Ferguson, 1989). Of the factors that limit or influence the distribution of the parasites (Table 3) the host species (Table 4) are clearly obligatory. Tables 3 and 5 show that *D. sagittata* is restricted to salmonid species (Hoffman, 1998) whereas the other species of fish parasites found are less specific with the exception of *Paraquimperia tenerrima* in eels. According to Moravec (1994) the latter is an example of a monoxenous species occurring in isolated, endemic or relict species. The low levels of specificity exhibited by the other helminths in the definitive hosts may be partly due to the fact that some of the fish harbouring the parasite species are incidental (or postcyclic, paradefinitive, euparatenic or metaparatenic hosts), rather than true definitive hosts according to Moravec (1994). This would not be surprising in view of the predatory, piscivorous and cannibalistic behaviour of many species of freshwater fish. There is some circumstantial evidence that the eel may have been acting as a postcyclic host rather than the

*Table 3* A list of the helminth parasites encountered, their hosts, microhabitats in the hosts and stations where found

| Taxon | Parasite species | Hosts | Location | Station |
|---|---|---|---|---|
| Monogenea | *Discocotyle sagittata* (Leuckart, 1840) | *Salmo trutta* L.<br>*Salmo salar* L. | Gills<br>Gills | A, B, D<br>A, B |
| Digenea | *Crepidostomum farionis* (Mueller, 1785) | *Salmo trutta* L.<br>*Salmo salar* L.<br>*Anguilla anguilla* L. | Intestine<br>Intestine<br>Intestine | A, B, D<br>A, B<br>A, B |
| Digenea | *Crepidostomum metoecus* (Braun, 1900) | *Salmo trutta* L.<br>*Salmo salar* L.<br>*Anguilla anguilla* L. | Int.,pyl.caecae<br>Int.,pyl.caecae<br>Intestine | |
| Digenea | *Phyllodistomum simile* (Nybelin, 1926) | *Salmo trutta* L. | Urinary bladder | A,B |
| Cestoda | *Bothriocephalus claviceps* (Goeze, 1782) | *Anguilla anguilla* L. | Duodenum and intestine | A,B |
| Cestoda | *Diphyllobothrium ditremum* (Creplin, 1825) | *Salmo trutta* L. | Mesentery, coelum | A |
| Nematoda | *Raphidascaris acus* (Bloch, 1779) | *Salmo trutta* L.<br>*Anguilla anguilla* L. | Intestine<br>Intestine | A, B<br>A, B |
| Nematoda | *Paraquimperia tenerrima* (Linstow, 1878) | *Anguilla anguilla* L. | Intestine | A, B |
| Nematoda | *Cystidicoloides ephemeriderum* (Linstow, 1872) | *Salmo trutta* L.<br>*Salmo salar* L. | Stomach, int. and pyloric caecae<br>Int. and pyl. caecae | A, B<br>B<br>A, B |
| Nematoda | *Cucullanus (Truttaedacnitis) truttae* (Fabricius, 1794) | *Salmo trutta* L. | Int. and pyl.caecae | A, B |
| Nematoda | *Capillaria* sp. | *Salmo trutta* L. | Int. and pyl.caecae | A, B, C |
| Acanthocephala | *Neoechinorhynchus rutili* (Mueller,1790) | *Salmo trutta* L.<br>*Salmo salar* L.<br>*Anguilla anguilla* L. | Int. and pyl.caecae<br>Duodenum, intestine | A, B |

true definitive host for the two *Crepidostomum* species in the Teifi as it is known to prey on both salmonid species. Thus, the values for prevalence and abundance of these parasites in the eels were found to be much lower than in salmonid species (Thomas, 1964c), despite the fact that the dietary niches of all three species overlap. However, it is possible that these differences may have been partly due to the fact that the eel is inactive and ceases to feed in the winter months when the temperature is below 10 °C (Thomas, 1962).

*Table 4* A summary of the ecological conditions which are obligatory for the survival of helminth parasites and also those which are favourable to them

1. Hosts
   - 1.1. Parasites not requiring intermediate hosts
   - 1.2. Parasites requiring one or more intermediate hosts as well the definitive host

2. Environmental conditions which are favourable to parasites and hosts
   - 2.1. Low or zero current velocities for transmission. These are typically found in lentic ecosystems and in lotic ecosystems in dead spaces in backwaters, undercut banks, pools, the interstitial pore water and very close to the sediment surface
   - 2.2. Optimal temperature range; this is 10–16°C for the growth of trout. Parasites have a wide range of tolerance but a temperature of > 10°C is generally necessary to stimulate reproductive activity
   - 2.3. pH > 5.5 for both hosts and parasites. Trout and intermediate hosts, such as *Pisidium*, are tolerant to pH conditions of 5.5–6.5 but as the optimal pH range for molluscan and arthropod hosts is generally 6.5–8.3 the species richness of parasites tends to be higher in this range
   - 2.4. Low levels of organic loading and high oxygen concentrations

It is not unlikely that the record of *Phyllodistomum* in the eel by Kennedy (1974) may also have been due to postcyclic infection as it was absent from the eels examined from the Teifi (Thomas, 1964c). The record of *Phyllodistomum* in the salmon parr by the same author is also unexpected in view of its absence from the salmon parr in the River Teifi where it was commonly found in the trout.

The information, which is currently available regarding the parasites and their intermediate hosts of the parasites listed in Table 5 is an essential prerequisite towards an understanding of their ecology. Correct identification comes first. In this connection it should be noted that Kakaji (1969) considered *P. simile* to be a synonym of *Phyllodistomum folium*. However, this author failed to take into account diagnostic features, such as the distribution of the sensory papillae in the adults, and the differences between the life cycles and larval anatomy of *P. folium* (Ginetsinskaya, 1961) and *P. simile* (Thomas, 1958a). It is therefore safer at this stage in our knowledge to consider these two species to be distinct. Without due care sibling species can easily be confused as were *C. farionis and C. metoecus* until they were distinguished by Thomas (1957, 1958b).

Digenetic trematodes generally show a fairly high level of specificity towards their molluscan hosts. There is a general consensus that freshwater bivalves of the family Sphaeriidae serve as hosts for *Crepidostomum* species including *C. cornutum*, *C. farionis*, *C. isostonum* and *C. metoecus* (Nöller, 1928; Brown, 1927; Baylis, 1931; Hopkins, 1934; Moravec, 1982; Hoffman,

*Table 5* Information on the life cycles of salmonid and eel parasites

| Parasite species | Definitive hosts | Intermediate hosts |
|---|---|---|
| *Discocotyle sagittata* | *Salmo* spp., *Onchorhynchus, Coregonus, Salvelinus, Prosopium* (Kennedy, 1978; Hoffman, 1998) | None |
| *Crepidostomum farionis* | Species of *Coregonus, Salmo, Salvelinus, Thamallus, Perca, Etheostoma, Lepomis, Lota, Leucichthys, Notropis, Oncorhynchus Cristivomer, Prosopium, Gasterosteus* (Hoffman, 1998). | First intermediate host: *Pisidium sp., Sphaerium corneum* (Brown, 1927, Hoffman, 1998), *Pisidium casertanum*, Poli (Awachie, 1968). Hopkins (1934) suggested that due to widespread distribution of this species, it may adapt to other invertebrate hosts, although it will probably remain dependent on Sphaeriidae as molluscan host<br>Second intermediate hosts: *Sialis lutaria, Ephemera danica* (Brown, 1927; Crawford, 1943; Hopkins, 1934; Robertson, 1953), *Gammarus pulex* (Baylis, 1931; Awachie, 1968), mayfly nymph (Hoffman, 1998) |
| *Crepidostomum metoecus* | Species of *Coregonus, Cottus, Esox, Lota, Salvelinus, Thymallus* and *Salmo* | First intermediate hosts: *Pisidium* sp. (Nöller, 1928; Moravec, 1982), *Lymnaea peregra* (Awachie, 1968)<br>Second intermediate hosts: *Gammarus pulex* (Awachie, 1968), *Ramellogammarus vancouverensis* (Margolis and Moravec, 1982), *Ephemera danica* (Moravec, 1982), dead and dying metacercariae in *Ecdyonurus torrentis, Baetis rhodani, Paraleptophlebia submarginata, Leuctra* spp. and *Sialis lutaria* (Awachie, 1968) |
| *Phyllodistomum simile* | *Salmo trutta, Salmo salar* and *Anguilla anguilla* in UK (Kennedy, 1974), *Thymallus thymallus* (Bykhorskaya-Pavlovskaya, 1962) | First intermediate host: *Sphaerium corneum* (Thomas, 1956, 1958a,b, 1964b,c) metacercariae also encyst in sporocysts |

*Table 5 continued*

| Parasite species | Definitive hosts | Intermediate hosts |
|---|---|---|
| *Neoechinorhynchus rutili* | Circumpolar distribution, in species of *Ambloplites, Carrasius, Catastomas, Cottus, Couesius, Culaea, Cyprinus, Dallia, Esox, Fundulus, Gasterosteus, Gila, Hyperhychus, Ictalurus, Lota, Micropterus, Morone, Mylocheilus, Notemigonus, Notropis, Onchorhynchus, Perca, Pimephales, Prosopium, Ptychocheilus, Pungitius, Richardsonius, Salmo, Salvelinus, Semotilus, Stizostedion, Thymallus* and *Umbra* (Hoffman, 1998) | Intermediate hosts include ostracods such as *Cypria reptans* (Dezfuli, 1996) and *Sialis lutaria* (Lassiere, 1988) |
| *Diphyllobothrium ditremum* | Fish-eating birds including cormorants | Procercoids in copepods (Hoffman, 1998; Heckmann and Ching, 1987) plerocercoids in species of *Salmo, Salvelinus, Onchorhynchus, Gasterosteus* |
| *Bothriocephalus claviceps* | Adults in *Anguilla anguilla, Anguilla rostrata,* species of *Ambloplites, Chaenobryttus, Lepomis, Micropterus Percopis, Stizostedion and Gasterosteus* | Procercoids in copepods; small fish as carriers or paratenic hosts |
| *Raphidascaris acus* | Species of *Ptychocheilus, Abramis Onchorhyncthus, Esox, Salmo, Lota, Salvelinus, Stizostedion, Ictalurus* (Hoffman, 1998); *Perca, Hucho, Salmothymus,* (Moravec, 1994) | Only fish or amphibia act as obligate intermediate hosts. Larvae encyst in liver. Fish hosts include 70 species, mainly cyprinids, bullheads, loaches, chub, minnows, pike (Moravec, 1994), and young trout (Alvarez Pellitero, 1979d). Invertebrates including oligochaetes, *Asellus, Macrocyclops,* snails, chironomidae, Trichoptera and Ceratopogonidae act as paratenic hosts |

*Table 5 continued*

| Parasite species | Definitive hosts | Intermediate hosts |
|---|---|---|
| *Cycullanus (T.) truttae* | Species of *Onchorhynchus, Prosopium, Salmo, Salvelinus, Hucho, Coregonus, Thymallus, Brachymystax, Salvelinus, Stenodus, Onchorhynchus, Coregonus,* adult *Lampetra* (gut or adominal cavity) In other fish such as *Anguilla anguilla, Perca fluviatilis* and *Lota lota* do not develop to maturity (Moravec, 1994) | Larval *Lampetra planeri, Lampetra fluviatilis* and *Petromyzon* sp. (Moravec, 1994) act as obligatory intermediate host. Attempts to infect various invertebrate and fish species unsuccessful. Other salmonids act as paratenic hosts or as postcyclic hosts<br>Postcyclic hosts include predatory fish, mainly salmonids (homopost-cyclic hosts), also pike, perch, catfish and burbot (heteropost-cyclic hosts) feeding on adult lampreys or salmonids (the definitive hosts) |
| *Cystidicoloides ephemeridarum* | Species of *Salmo (S .tratta, S. salar), Salmothymus, Onchorhynchus, Salvelinus, Hucho, Thymallus, Brachymystax, Coregonus, Prosopium.* Postcyclic hosts include *Esox, Lota, Anguilla, Leuciscus, Barbus* and *Acipenser* | Ephemeropterans such as *Ephemera* spp., *Leptophlebia, Habrophlebia, Hexagenia* and *Polymitarcys.* Forage fish such as species of *Cottus, Noemacheilus, Phoxinus* as paratenic or paradefinitive hosts |
| *Capillaria* sp. | Circumpolar distribution. Parasites of freshwater fish including *C. salvelini* in salmonid fish. | Paratenic in oligochaetes (Hoffman, 1998). Oligochaete worms act as obligate intermediate hosts or homoxenous (or direct development) in fish without intermediate host may occur |
| *Paraquimperia tenerrima* | *Anguilla anguilla* (monoxenous species; Moravec, 1994) | Life cycle not known (Moravec, 1994) |

1998). The claim made by Awachie (1968) that *Lymnaea peregra* is also a host of *C. metoecus* therefore requires verification. However, as there is evidence that several species of *Pisidium* and *Sphaerium* may be implicated as alternative intermediate hosts of another allocreadiid trematode, *Bunodera luciopercae* (Andrews and Chubb, 1980), this low level of specificity may also apply in the case of *Crepidostomum* species.

The level of specificity showed by digenetic parasites to the second intermediate hosts is generally lower than that to the molluscan host. In the case of *Crepidostomum* species they include *Gammarus pulex* and *Ramellogammarus vancouverensis* as well as insects belonging to the orders Plecoptera, Ephemeroptera and Megaloptera (Table 5). The presence of *Crepidostomum farionis* in Arctic Charr from Greenland and Iceland (Due and Curtis, 1995), where amphipods and Ephemeroptera are absent, indicates that this parasite has some flexibility in its choice of second intermediate host.

During 1951–1952 it was shown that the freshwater bivalve *Sphaerium corneum* acted as the molluscan host of *Phyllodistomum simile* (Thomas, 1956, 1958a, 1964c). Although it was shown that the metacercariae in the sporocysts infected the trout it is possible that insect larvae, including anisopteran dragonfly larvae, may also have been implicated as an optional secondary intermediate host as is the case with other *Phyllodistomum* species (Thomas, 1958a; Ginetsinskaya, 1961). In contrast, the sporocysts of *Phyllodistomum dogieli*, which develop in *Dreissena polymorpha*, leave the host after the metacercariae develop and are presumably swallowed directly by the definitive hosts, *Rutilus rutilus* or *Abramis brama* (Ginetsinskaya, 1961). As *Phyllodistomum simile* was also found in the trout in the Teifi in 1998, in the apparent absence of *Sphaerium corneum*, it would appear that this trematode might also use *Pisidium* species as alternative molluscan hosts.

Small crustacea, namely ostracods, serve as the intermediate hosts of *Neoechinorhynchus* species, including *N. cristatus*, *N. cylindricus*, *N. saginatus* and *N. rutili* (Merritt and Pratt, 1964; Walkey, 1967; Valtonen, 1979; Dezfuli, 1996; Hoffman, 1998). Larvae of *N. rutili* in the alder-fly *Sialis lutaria* have also been shown to be infective to rainbow trout (Lassiere, 1988; Hoffman, 1998) but it is possible that these may be acting only as paratenic hosts (Walkey, 1967). Lassiere and Crompton (1988) also demonstrated the involvement of the stickleback *Gasterosteus aculeatus* in the postcyclic transmission of *N. rutili* into rainbow trout. Other small crustacea, the copepods, also serve as hosts for the procercoids of the two cestode species, *D. ditremum* and *B. claviceps*. Small fish act as carrier or paratenic hosts for *B. claviceps* while the plerocercoids of *D. ditremum*, the only allogenic parasite in the community, develop in species of salmonidae (Table 7).

Larger benthic invertebrates are implicated as hosts of the nematode species *Capillaria* and *Cystidicoloides ephemeridarum*. According to Moravec (1994) oligochaete worms may be obligate intermediate hosts for *Capillaria* sp. or

alternatively development may be homoxenous or direct in the fish host. Several species of mayflies may act as intermediate hosts of C. *ephemeridarum* (Table 5) while small forage fish may act as paratenic hosts. Moravec (1994) showed that ammocoete larvae of lampreys are the obligatory intermediate hosts of *Cucullanus T. truttae* the dominant nematode parasite of the trout in the Teifi, but salmonid fish may act as paratenic and as postcyclic hosts. During 1950 the ammocoete larvae were found to coexist in depositing sediments with *Sphaerium corneum* in the River Teifi. Fish species, including *Salmo trutta*, also serve as intermediate hosts for *R. acus* although a number of invertebrate species (Table 5) may act as paratenic hosts (Alvarez Pellitero, 1978; Moravec, 1994).

According to Moravec (1994) the life cycle of *P. tenerrima* remains to be elucidated. Much work remains to be done to achieve a complete identification of the intermediate hosts that are involved in the transmission of the helminth parasites in the Teifi, Pysgotwr and other river systems. This problem is greatly exacerbated by the low level of specificity. Presumably this has arisen as a result of the stochastic and unpredictable nature of existence in freshwater ecosystems. However, such information is essential for a full understanding of the mechanisms that are involved in regulating their population dynamics.

## 3. DISPERSION PATTERNS OF THE HELMINTH PARASITES

The frequency patterns for the parasites of the two salmonid species and the variance/mean ratios and the dispersion indices (Table 6) indicate that all the parasite populations have strongly clumped, aggregated, overdispersed or contagious distributions. However, it is noteworthy that the values for the

*Table 6* The variance/mean ratios and the dispersion indices ($I_D = S^2\ (n-1)/\bar{x}$) for the parasites of the brown trout and salmon parr

| Parasite | Brown trout | | Salmon parr | |
|---|---|---|---|---|
| | $S^2/x$ | $I_D$ | $S^2/x$ | $I_D$ |
| *Discocotyle sagittata* | 3.68 | 4 338.3 | 1.19 | 326.2 |
| *Crepidostomum farionis* | 6.58 | 7 756.7 | 2.24 | 611.0 |
| *Crepidostomum metoecus* | 35.23 | 41 533.8 | 11.87 | 3 241.5 |
| *Phyllodistomum simile* | 5.72 | 6 747.7 | – | – |
| *Neoechinorhynchus rutili* | 17.62 | 20 737.7 | 11.16 | 3 045.4 |
| Nematodes | 13.42 | 15 825.7 | 9.47 | 2 586.0 |
| Total parasites | 30.44 | 35 885.7 | 11.83 | 3 229.3 |

*Table 7* The percentage prevalence of the dominant brown trout parasites at the three stations during 1951–1952 and the $\chi^2$ values

| | *D. sagittata* | *C. farionis* | *C. metoecus* | *P. simile* | *N. rutili* | Nematodes | Cestodes | Total parasites |
|---|---|---|---|---|---|---|---|---|
| Teifi Station B | 24.82 | 22.66 | 75.62 | 25.44 | 90.36 | 68.47 | 0.0 | 98.68 |
| Teifi Station A | 23.44 | 4.31 | 63.64 | 17.70 | 92.34 | 70.81 | 1.60 | 99.04 |
| Pysgotwr Station D | 9.67 | 20.44 | 82.16 | 0.00 | 0.00 | 1.86 | 0.0 | 84.76 |
| $\chi^2$ value | 27.53 | 35.85 | 21.87 | 84.11 | 809.85 | 375.21 | 91.21 | 94.85 |
| *P* value | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

variance/mean ratios and dispersion indices are consistently higher for the parasites of the brown trout than those of the salmon parr. In view of these dispersionary patterns it was necessary to normalise the data as far as possible before undertaking parametric statistical analyses. To overcome difficulties with zero counts the $\log_{10}(1 + x)$ transformation, where $x$ is the number of parasites, was used.

In order to explain these distribution patterns it is necessary to consider the behaviour and physiology of the host and the distribution patterns of the infective stages. If every potential host were equally exposed to a chance of being infected by the same numbers of infective stages at a time and if the microenvironments inside all the hosts were identical, both physiologically and immunologically, the frequency distribution should follow the Poisson series. Each host will therefore have the population mean as its expected frequency. Such random distributions have been described for some parasites, such as *Ascaridia galli* in chickens, provided they were genetically identical, fed with the same number of infective eggs and kept under identical conditions (Northam and Rocha, 1958). Hopkins (1959) also found this to be the case with populations of *Proteocephalus filicollis* in *Gasterosteus aculeatus* under natural conditions. He postulated that this distribution was due to the random distribution of copepods, infected with only one procercoid, and to random feeding by the sticklebacks.

As in the present case, however, most parasite distributions exhibit overdispersion and conform to the negative binomial model (Thomas, 1965). In the case of bird parasites variance/mean ratios are much higher than those for trout with most of the values in the 10–100 and 100–1000 categories while some are higher than 100 000 (Goater and Holmes, 1997). The distributional pattern of the trout and salmon parr parasites may be due to inequalities in the microhabitats within the host or in the chance of the host being infected. On first inspection the first possibility seems improbable, as populations of both hosts are likely to be genetically fairly uniform. However, the possibilty that the fish hosts may have different levels of immunity or resistance as a result of intervention by the parasites cannot be excluded. It seems more probable, however, that the overdispersion is mainly due to the two fish species being unequally exposed to infestation as a result of variation in their dietaries. The availability of infected food items to the fish will be influenced by a number of factors (Thomas, 1962). Of primary importance is the spatiotemporal distribution of the intermediate hosts. As populations of these are patchily distributed and unequally infested (Thomas, 1958 a, b; 1964 a, b, c) the parasites must therefore be regarded as having foci of infestation. This fact, coupled with the occurrence of multiple infections in individual intermediate hosts, must contribute to the overdispersion. The other major contributory factors are differences in the dietaries of individual fish due to differences in age, sex, season and feeding territories due to their position in the social hierarchy. It

may be suggested that the variance/mean ratios are lower in the case of salmon parr than the trout because their feeding territories overlap to a much greater extent than those of trout (Armstrong *et al.*, 1999). Anderson (1974) also attributed overdispersion of *Caryophyllaeus laticeps* in the bream, *Abramis brama*, to contagious distribution of the tubificid intermediate host in the sediment and to differences in the feeding behaviour of individual fish. This author also points out that the total fish population could have an overdispersed parasite population by compounding a series of essentially random processes.

In the case of parasites, such as monogenetic trematodes, that are transmitted directly by host aggregation, favourable temperatures and overdispersion in the distribution of the infective larvae can result in highly overdispersed distributions. These factors may result in epizootics and host death (Dogiel, 1961; Kennedy, 1975).

## 4. SPATIOTEMPORAL ASPECTS OF THE PARASITIC FAUNA

### 4.1. Quantitative Data

The stations are a major factor in determining the percentage prevalence of the dominant trout parasites (Table 7). Thus the parasitic fauna of the trout from the Pysgotwr (Station D) is characterised by the absence of *P. simile*, *N. rutili*, nematodes other than *Capillaria* sp., and also by having a significantly lower prevalence of *D. sagittata* than the trout from the other two stations. The prevalence rates for the three trematode species are also significantly lower at Station A on the Teifi than at Station B on the Teifi. Comparisons of the parasite abundance (Table 8, Figure 3) show similar trends. When transformed the abundance values for *C. farionis*, *C. metoecus* and *P. simile* were all significantly lower at Station A on the Teifi than at Station B on theTeifi while the converse is the case with *N. rutili* and the nematodes (mainly *C. truttae*). Plerocercoids of *D. ditremum* were only found in trout at Station A on the Teifi. However, the values for both prevalence (1.5%) and mean abundance (0.3) for this parasite were very low (Tables 7 and 8). The maximum number of plerocercoids in any fish was only four. *D. sagittata* was significantly ($P < 0.001$) less abundant and *C. metoecus* significantly ($P < 0.001$) more abundant in the brown trout in the Pysgotwr (Station D) than in the brown trout from the two stations on the Teifi (Table 8).

*Phyllodistomum simile* did not occur in the urinary bladders of salmon parr. The prevalence values for *D. sagittata* are higher for salmon parr at Station A on the Teifi A than for those at Station B whereas the converse is the case with *C. farionis* and *C. metoecus* (Figure 4.). In these respects the salmon parr resemble the trout. Although the abundance values of the dominant parasites in salmon parr at Stations A and B are much lower than corresponding values for

*Table 8* The means of transformed $\log_{10}(1 + x)$ numbers of trout parasites and the results of statistical analyses

| | *D. sagittata* | *C. farionis* | *C. metoecus* | *P. simile* | *N. rutili* | Nematodes | Cestodes | Total parasites |
|---|---|---|---|---|---|---|---|---|
| Teifi Station B (1) | 0.248 | 0.238 | 2.101 | 0.321 | 1.902 | 1.126 | 0.000 | 5.927 |
| Teifi Station A (2) | 0.269 | 0.041 | 1.690 | 0.220 | 2.096 | 1.272 | 0.0166 | 5.604 |
| Pysgotwr Station D (3) | 0.098 | 0.203 | 2.376 | 0.0 | 0.0 | 0.014 | 0.000 | 2.692 |
| *F* value (1,2,3) | 12.04 | 16.05 | 12.42 | 35.77 | 506.16 | 171.28 | 8.27 | 157.23 |
| *P* value (1,2,3) | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| *F* value (1/2) | 0.300 | 31.94 | 12.02 | 4.52 | 5.94 | 3.28 | 11.88 | 2.15 |
| *P* value (1/2) | 0.585 | < 0.001 | < 0.001 | < 0.03 | 0.014 | 0.071 | < 0.001 | 0.143 |
| *F* value (1/3) | 22.81 | 1.03 | 6.63 | 11.51 | 967.28 | 322.43 | 4.66 | 302.51 |
| *P* value (1/3) | < 0.001 | 0.301 | 0.01 | < 0.001 | < 0.001 | < 0.001 | < 0.031 | < 0.001 |
| *F* value (2/3) | 17.93 | 24.45 | 24.43 | 46.61 | 1129.61 | 386.46 | 0 | 229.23 |
| *P* value (2/3) | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0 | < 0.001 |

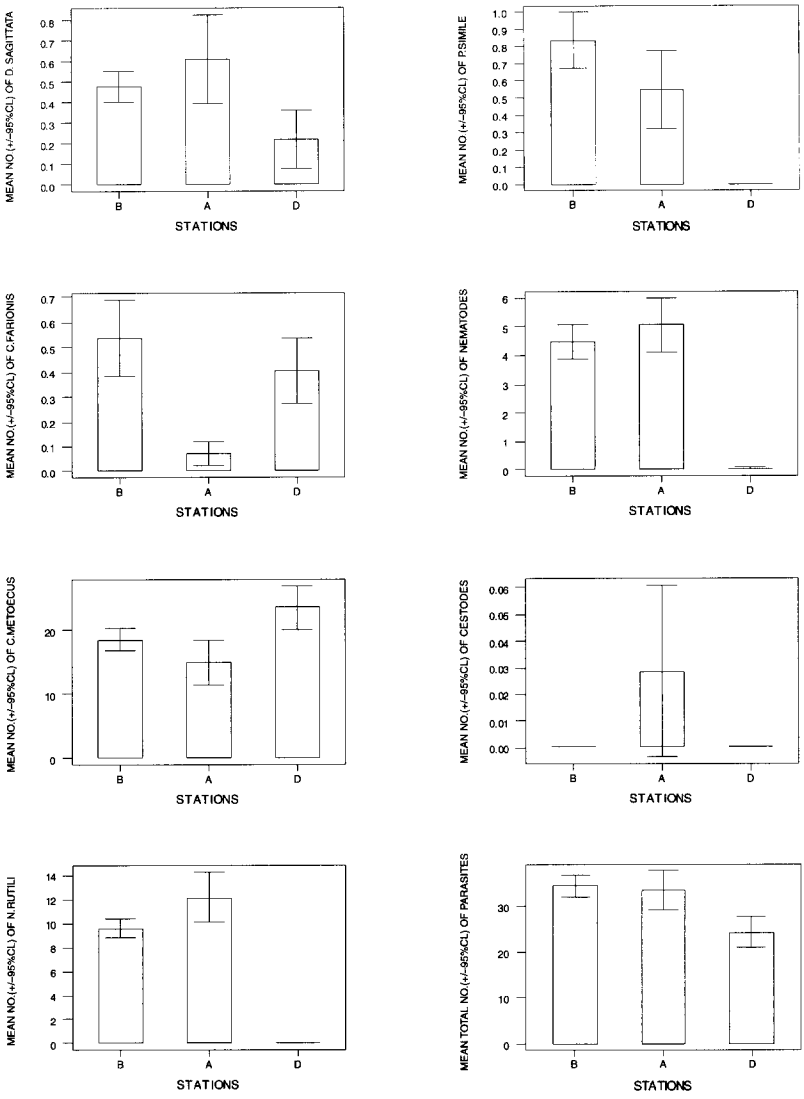


*Figure 3* The mean abundance (± 95% C.L.) of parasites found in brown trout at the three stations. (Station B on the Teifi; Station A on the Teifi; Station D on the Pysgotwr).

trout they show similar spatial trends (Table 9; Figure 5). Thus *D. sagittata*, *N. rutili* and nematodes are more abundant in salmon parr at Station A on the Teifi A than at Station B whereas the converse is the case with *C. metoecus* and *C. farionis* (Table 9; Figure 5). However, statistical analyses (Table 9) revealed that the differences were only significant in the case of *D. sagittata*.

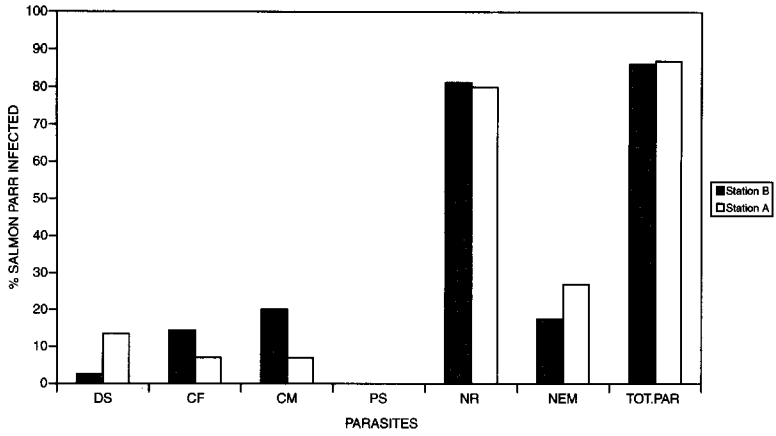


*Figure 4* The percentage prevalence of the dominant parasites of the salmon parr at Stations A and B on the Teifi. DS, *D. sagittata*; CF, *C. farionis*; CM, *C. metoecus*; PS, *P.simile*; NR, *N.rutili*; NEM, nemanodes; TOT. PAR, total parasites.

*Table 9* The means of transformed $\log_{10}(1 + x)$ numbers of salmon parr parasites and the results of statistical analyses

| | *D. sagittata* | *C. farionis* | *C. metoecus* | *N. rutili* | Nematodes | Total parasites |
|---|---|---|---|---|---|---|
| Teifi Station B | 0.0167 | 0.130 | 0.303 | 1.729 | 0.186 | 0.835 |
| Teifi Station A | 0.092 | 0.073 | 0.092 | 1.922 | 0.258 | 0.885 |
| *F* values | 5.34 | 0.39 | 1.36 | 0.41 | 0.31 | 0.15 |
| *P* values | 0.022 | 0.53 | 0.24 | 0.52 | 0.58 | 0.70 |

The abundance of the helminth parasites may be determined by a number of interactive factors such as stations, seasons, the age, size, sex and wellbeing (as measured by the adipose index or condition factor) of the host fish (Figure 1). In view of the complexity of these interactions multiple regression analysis was used to evaluate the extent to which these could predict parasite abundance. The results of these analyses show that the main predictors of the transformed total number of parasites in trout, as the response, are stations, seasons and age of the trout as these contribute 38.0%, 40.8% and 20.0%, respectively, to the total variance (Table 10). The sex of the trout and the adipose index are also significant predictors ($P = 0.001$ and 0.011, respectively) although their contribution to the total variance is minimal (0.72% and 0.44%, respectively). In contrast the condition factor was not a significant predictor ($P = 0.867$). When individual parasite taxa were included as the response in the equation, significant relationships

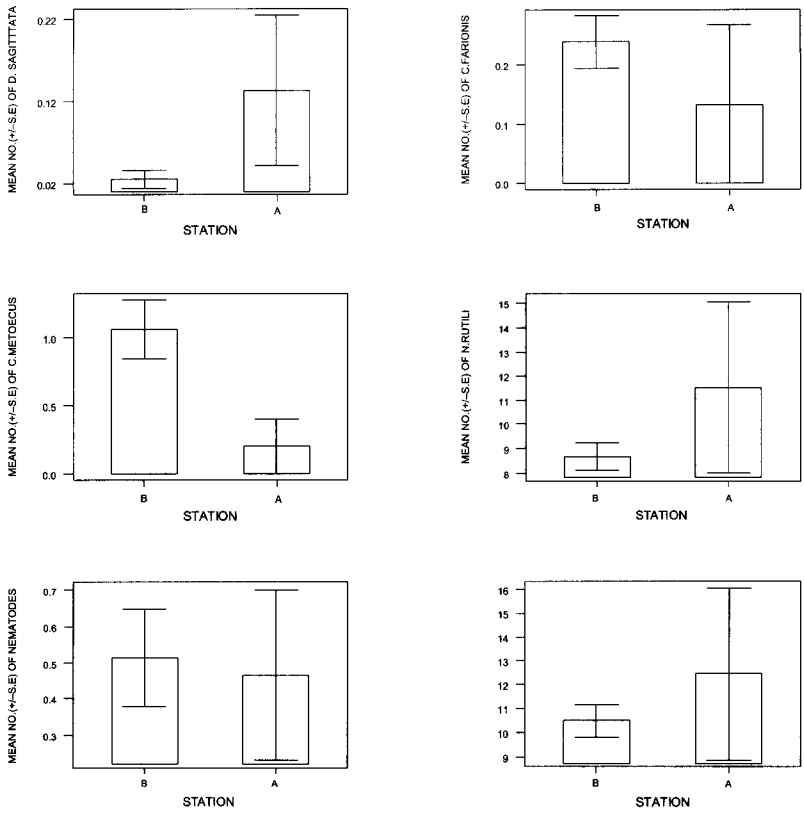


*Figure 5* The mean abundance (± 95% C.L.) of parasites found in salmon parr at the two stations on the Teifi (= Teifi B) and Station 2 (= Teifi A).

between the transformed parasite numbers and the condition factor or adipose index ($P = 0.044$ and $P = 0.018$, respectively) were only found in the case of the most abundant parasite, *C. metoecus* (Table 10). Both of these relationships were positive but they contribute little to the total variance (0.48% and 0.46%, respectively). In contrast, stations, seasons and age were also highly significant predictors of numbers of *C. metoecus*, contributing 4.0%, 88.2% and 6.3%, respectively, to the total variance. The sex of the trout was also a significant predictor of the numbers of *C. metoecus* ($P = 0.005$) although its contribution to the total variance was minimal (0.54%). The seasons also contributed 48.78%, 52.5%, 33.7%, 5.4% and 3.2% of the total variance, respectively, when *D. sagittata*, *C. farionis*, *P. simile*, *N. rutili* and nematodes were the main responses in the multiple regression equation. The relatively low variance values in the last three taxa can be attributed to their absence or dearth at Station D. As a result the

*Table 10* The results of multiple regression analyses of transformed numbers of parasites (total numbers of parasites and *C. metoecus*) of brown trout as the response, and stations, seasons, age, sex, condition factor and adipose index as predictors (note that the % of variance accounted for by each factor is conditional on the factors entered earlier in the table)

| Predictors | Coefficients | S.D. | *P* | % variance |
|---|---|---|---|---|
| **Total parasites** | | | | |
| Constant | 4.013 | 0.976 | 0.000 | |
| Stations (3) | –3.125–0.190 | 0.182–0.187 | 0.296–0.000 | 38.00 |
| Months (12) | –3.079–0.798 | 0.355–0.300 | 0.000–0.008 | 40.83 |
| Age | 1.077 | 0.071 | 0.000 | 19.98 |
| Sex | –0.379 | 0.116 | 0.001 | 0.72 |
| Condition factor | 0.014 | 0.086 | 0.867 | 0.02 |
| Adipose index | 0.239 | 0.094 | 0.011 | 0.44 |
| R.Sq.= 56.1% ; $F$ = 85.96; $P$ = < 0.000 | | | | |
| ***C. metoecus*** | | | | |
| Constant | 0.867 | 0.532 | 0.103 | |
| Stations (3) | –0.041–0.598 | 0.099–0.102 | 0.678–0.000 | 4.04 |
| Months (12) | –0.041–0.550 | 0.181–0.194 | 0.002–0.000 | 88.21 |
| Age | 0.316 | 0.039 | 0.000 | 6.26 |
| Sex | –0.178 | 0.063 | 0.005 | 0.54 |
| Condition factor | 0.095 | 0.047 | 0.044 | 0.48 |
| Adipose index | 0.122 | 0.051 | 0.018 | 0.46 |
| R.Sq.=51.9%; $F$ = 72.71; $P$ =< 0.000 | | | | |

R.Sq. = Sum of squares.

stations contributed 46.2%, 94.2% and 50.1% of the total variance in the cases of *P. simile*, *N. rutili* and nematodes, respectively.

The results of multiple regression analyses carried out with the salmon parr data show that the only significant predictors of the transformed number of total parasites were seasons, condition factor and the adipose index. These contributed 79.0%, 8.2% and 6.6% of the total variance respectively (Table 11). In contrast stations, age, sex and weight were not significant predictors of parasite abundance. When individual parasites were investigated as the response in the multiple regression equation significant relationships between the transformed parasite numbers and the condition factor or adipose index ($P$ = 0.000 and $P$ = 0.023, respectively) were only found in the case of the most abundant parasite, *N. rutili* (Table 11). As was the case with total parasites both of these relationships were positive. Multiple regression analyses showed, however, that seasonal change was a major factor in predicting the numbers of all the individual parasites in the case of the salmon parr. Thus it contributed

*Table 11* The results of multiple regression analyses of transformed numbers of total parasites and *N. rutili* in salmon parr, as the response, and stations, seasons, age, sex, weight, condition factor and adipose index as predictors (note that the % variance accounted for by each factor is conditional on the factors entered earlier in the table)

| Predictors | Coefficients | S.D. | *P* | % variance |
|---|---|---|---|---|
| **Total parasites** | | | | |
| Constant | 0.016 | 0.433 | 0.971 | |
| Stations (2) | –0.016 | 0.018 | 0.356 | 2.00 |
| Seasons (12) | 0.884–0.109 | 0.170–0.285 | 0.521–0.000 | 78.96 |
| Age | 0.192 | 0.110 | 0.083 | 1.30 |
| Sex | –0.043 | 0.057 | 0.444 | 2.05 |
| Weight | –0.006 | 0.004 | 0.140 | 0.88 |
| Condition factor | 0.080 | 0.033 | 0.016 | 8.17 |
| Adipose index | 0.100 | 0.047 | 0.033 | 6.60 |
| R.Sq.= 20.4%; $F = 4.33$; $P = < 0.000$ | | | | |
| ***N. rutili*** | | | | |
| Constant | –1.226 | 1.026 | 0.233 | |
| Stations (2) | –0.032 | 0.042 | 0.439 | 0.27 |
| Months (12) | –1.585–0.167 | 0.65–0.402 | 0.860–0.000 | 63.34 |
| Age | 0.321 | 0.261 | 0.221 | 0.05 |
| Sex | –0.113 | 0.135 | 0.400 | 3.04 |
| Weight | –0.018 | 0.010 | 0.075 | 1.30 |
| Condition factor | 0.277 | 0.078 | 0.000 | 22.40 |
| Adipose index | 0.254 | 0.111 | 0.023 | 9.62 |
| R.Sq.= 16.8 %; $F = 3.42$, $P = < 0.000$ | | | | |

R.Sq. = Sum of squares.

48.3%, 71.2%, 69.7%, 63.34% and 61.3% to the total variance in the cases of *D. sagittata*, *C. farionis*, *C. metoecus*, *N. rutili* and nematodes, respectively.

Multiple regression analyses shows that the stations are clearly major factors in determining the abundance of individual parasites as well as total parasites in trout (Table 10). Thus, the stations contribute 17.5%, 29.3%, 4.0%, 46.2%, 50.1%, 94.2% and 38.0% of the total variances in the cases of *D. sagittata*, *C. farionis*, *C. metoecus*, *P. simile*, nematodes, *N. rutili* and total parasites, respectively. As salmon parr were restricted to the two Teifi stations, stations are much less important predictors as they contribute only 0.3%, 6.7%, 25.4%, 0.3%, 27.9 and 2.0% of the total variances in the cases of *D. sagittata*, *C. farionis*, *C. metoecus*, *N. rutili*, nematodes and total parasites, respectively (Table 11).

The mean abundance of each of the dominant trout parasites varies significantly on a seasonal basis (Figure 6 and Table 12). In the cases of *D. sagittata*, *C. farionis*, *C. metoecus*, *P. simile*, nematodes (mainly *C. truttae*) and total

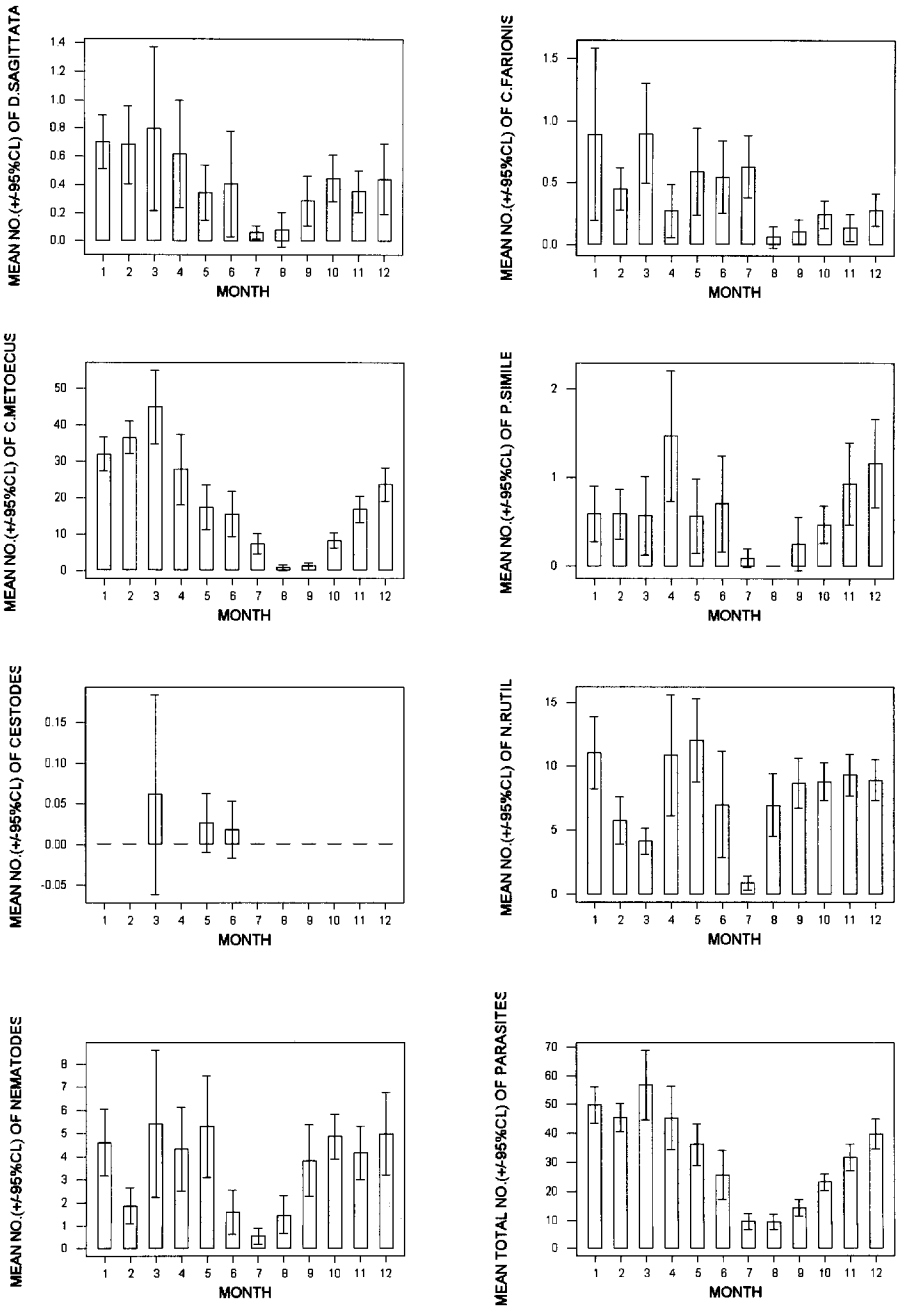


*Figure 6* The mean monthly changes (± 95% C.L.) in the abundance of the dominant parasites of the brown trout in the Teifi.

*Table 12* The monthly means of transformed numbers $\log_{10}(1 + x)$ of brown trout parasites and the results of statistical analyses

| | Months | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | *F* value | *P* value |
| Parasites | | | | | | | | | | | | | | |
| *D. sagittata* | 0.377 | 0.309 | 0.346 | 0.288 | 0.193 | 0.178 | 0.038 | 0.041 | 0.144 | 0.214 | 0.182 | 0.215 | 5.36 | < 0.001 |
| *C. farionis* | 0.286 | 0.225 | 0.432 | 0.140 | 0.260 | 0.276 | 0.303 | 0.085 | 0.056 | 0.131 | 0.074 | 0.211 | 5.61 | < 0.001 |
| *C. metoecus* | 3.140 | 3.267 | 3.469 | 2.717 | 1.977 | 2.024 | 1.163 | 0.196 | 0.220 | 1.298 | 2.341 | 2.938 | 92.62 | < 0.001 |
| *P. simile* | 0.223 | 0.221 | 0.236 | 0.523 | 0.229 | 0.240 | 0.040 | 0.000 | 0.091 | 0.207 | 0.355 | 0.468 | 6.42 | < 0.001 |
| *N. rutili* | 1.584 | 0.981 | 1.355 | 1.919 | 1.938 | 1.117 | 0.236 | 1.421 | 1.824 | 1.890 | 1.932 | 2.037 | 27.06 | < 0.001 |
| Nematodes | 0.966 | 0.558 | 1.240 | 1.102 | 1.167 | 0.525 | 0.184 | 0.528 | 0.939 | 1.256 | 1.097 | 1.232 | 14.15 | < 0.001 |
| Cestodes | 0 | 0 | 0.283 | 0 | 0.182 | 0.121 | 0 | 0 | 0 | 0 | 0 | 0 | 1.98 | < 0.026 |
| Total parasites | 6.576 | 5.520 | 7.115 | 6.689 | 5.783 | 4.372 | 1.964 | 2.219 | 3.275 | 4.986 | 5.981 | 7.086 | 46.61 | < 0.001 |

*Table 13* The monthly means of transformed number $\log_{10}(1 + x)$ of salmon parr parasites and the results of statistical analyses

| | Months | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | *F* value | *P* value |
| Parasites | | | | | | | | | | | | | | |
| *D. sagittata* | 0.087 | 0.000 | 0.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.012 | 0.046 | 0.039 | 0.00 | 0.87 | 0.573 |
| *C. farionis* | 0.347 | 0.173 | 0.480 | 0.053 | 0.000 | 0.693 | 0.078 | 0.055 | 0.133 | 0.094 | 0.039 | 0.244 | 2.35 | < 0.01 |
| *C. metoecus* | 0.825 | 0.949 | 1.404 | 1.330 | 0.000 | 0.693 | 0.162 | 0.055 | 0.030 | 0.151 | 0.344 | 0.497 | 9.59 | < 0.000 |
| *N. rutili* | 2.311 | 2.250 | 1.824 | 2.161 | 0.597 | 2.890 | 0.927 | 1.380 | 1.849 | 1.843 | 1.852 | 1.964 | 3.00 | < 0.000 |
| Nematodes | 0.677 | 0.173 | 0.321 | 0.818 | 0.000 | 0.693 | 0.866 | 0.108 | 0.113 | 0.138 | 0.252 | 0.156 | 3.69 | < 0.000 |
| Total parasites | 1.265 | 1.106 | 1.028 | 1.236 | 0.259 | 1.322 | 0.470 | 0.655 | 0.833 | 0.850 | 0.886 | 0.976 | 4.96 | < 0.000 |

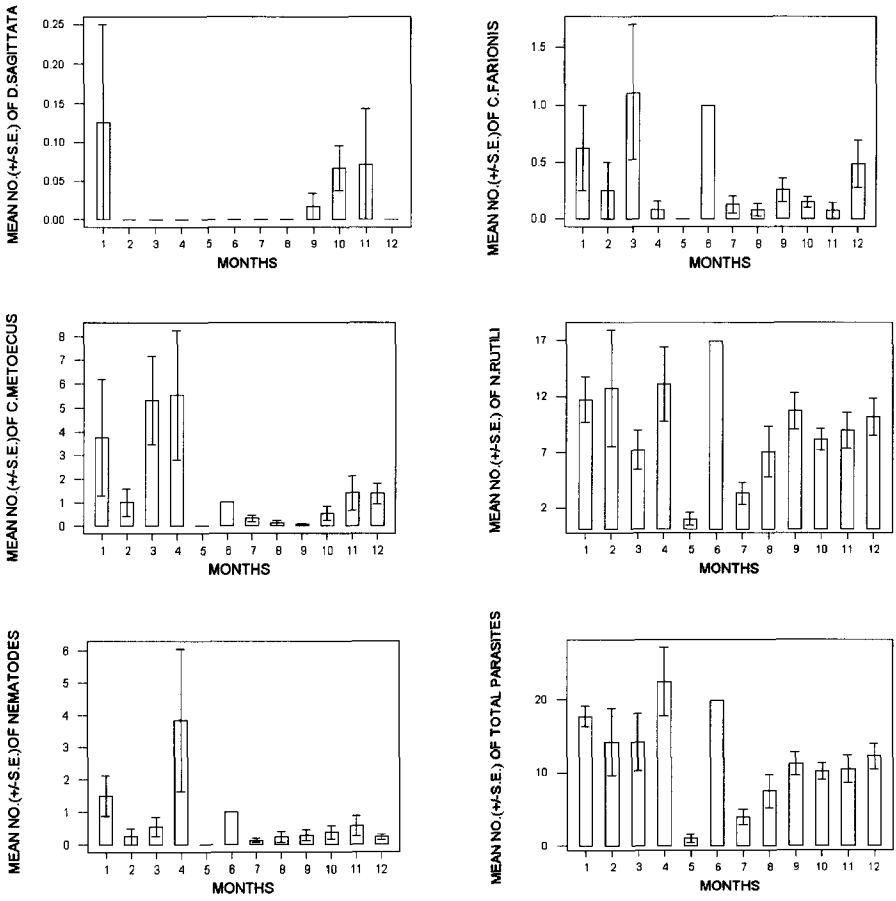


*Figure 7* The mean monthly changes (± 95% C.L.) in the abundance of the dominant parasites of the salmon parr in the Teifi.

parasites the abundance values decline from winter highs in the late spring and summer months but begin to increase again in the autumn. However, these trends are not apparent in the case of *N. rutili.*

With the exception of *D. sagittata* the mean abundances of individual parasites in the salmon parr, although much lower than those of trout, also show significant seasonal fluctuations (Table 13, Figure 7). The prevalence of *D. sagittata* was much less in salmon parr than the trout and it was only encountered during the months of January, September, October and November.

However, with the exception of *C. metoecus*, there were no summer troughs in abundance values of salmon parr parasites.

Multiple regression analyses confirms that seasonality was also a major factor in predicting the abundance of individual parasites such as *D. sagittata*, *C. farionis*, *C. metoecus* and *P. simile* in trout as it contributed 48.78%, 52.46%, 88.21% and 33.74%, respectively, to the total variance in each case (Table 10). In contrast, the abundances of *N. rutili* and nematodes, chiefly *C. truttae*, were much less influenced by seasonality as it contributed only 5.45% and 3.22%, respectively, to the total variance. Multiple regression analyses also showed that seasonality was a major factor in predicting the numbers of *D. sagittata*, *C. farionis*, *C. metoecus*, *N. rutili* and nematodes in salmon parr as it contributed 48.3%, 71.2%, 69.7%, 63.3% and 61.3% to the total variance, respectively (Table 11). However, there were no well-defined seasonal trends, with winter highs and summer lows except in the case of *C. metoecus* (Figure 7).

## 4.2. Physicochemical Factors that may Influence the Distribution and Abundance of the Parasites and their Fish Hosts

The nature of the parasitic community in aquatic ecosystems is determined by interactions involving the definitive host fish, the intermediate hosts and the physicochemical factors (Wisniewski, 1958; Dogiel, 1961; Chubb, 1970; Kennedy, 1978c; Esch *et al.*, 1988; Kennedy and Hartvigsen, 2000). The major physicochemical factors that may influence the distribution and abundance of the parasitic fauna in aquatic ecosystems are summarised in Table 4 and Figure 1 and their possible involvement is discussed below.

### *4.2.1. Water flows and water types*

As a result of evolutionary pressure, trout and salmon have become adapted to live in the sediment-producing erosion zone, which is the first of three major zones in river classification systems (Dobson and Frid, 1998). In this zone, which is the major source of both water and sediment, the slope is typically steep and deposition of sediment is therefore localised or ephemeral because of the high shear velocity. The water types in this zone are diverse and include successions of riffles, fast reaches, small pools and backwaters with depositing sediments. In the older classification systems, based on biological parameters, which were developed for temperate-zone rivers, this was described as the trout zone (Carpenter, 1928). The second major zone, known as the sediment transfer zone, is characterised by a reduction in gradient. As a result the sediments, consisting of sand and gravel, are transported with little net loss or gain. Here

the pools and backwaters are larger, the flow more uniform and riffles and fast reaches are absent. This zone is inhabited by minnows, grayling and some trout and is succeeded by the sediment deposition zone, where roach, bream and barbel predominate.

The presence of localised, often ephemeral, depositing zones in reaches occupied by trout in rivers is of paramount importance because it determines the distribution of both the free-living stages of the parasites and their hosts. The parasitic stages, which emerge from the eggs, are planktonic in nature and are therefore particularly vulnerable to currents. This consideration may explain the following gradation in the prevalence and intensities of infection of *D. sagittata* in brown trout at the three stations: Station A > Station B > Station D. Thus, the water flow in Station A in the Tregaron Bog area is hardly detectable much of the time because of the very gentle gradient. The Teifi at Station B is a much larger river than the Pysgotwr at Station D and because it has a gentler gradient and a reduced shear force it has a much greater surface area of sheltered microhabitats consisting of backwaters, pools and undercut banks with depositing sediments. It is likely that the low flow at station A is also responsible for the fact that it is the only station where *D. ditremum* was found. The almost lentic conditions prevailing at this station are favourable to both the planktonic coracidium larvae of this species and its copepod intermediate host. The completion of the life cycle of *B. claviceps* may also be facilitated by the fact that the eel has a preference for sheltered habitats, with depositing sediments, frequented by copepod intermediate hosts.

The intermediate hosts of other parasites including *C. farionis*, *C. metoecus*, *P. simile*, *N. rutili*, *C. truttae* and *Capillaria* species (Table 5) are also generally found in association with depositing sediments. The highly erosive nature of the substrate may, therefore, be partly responsible for the absence of *P. simile*, *N. rutili* and *C. truttae* from Station D. In contrast to the other parasites the trout at Station D are more heavily infected with *C. metoecus* than the trout at the two stations on the Teifi. This suggests that the second intermediate hosts may be mayflies or stoneflies that are well adapted to live in more turbulent waters with eroding sediments. The species involved might include *Ecdyonurus torrentis*, *Baetis rhodani*, *Paraleptophlebia submarginata* and *Leuctra* spp. as Awachie (1968) found these to be infected with dead or dying metacercariae of *C. metoecus*. However, *Gammarus pulex*, which Awachie (1968) considered to be the major second intermediate host of *C. metoecus*, was absent from the Pysgotwr.

It would appear, therefore, that some trematode species, such as *C. metoecus* are better adapted for life in fast-flowing waters than others. This is also the case with some of the nematode parasites of freshwater fish. Thus, Moravec (1994) lists *Cucullanus truttae*, *Paraquimpera tenerrima* and *Cysdicoloides ephemeridarum* as being adapted to live in regions of rivers where the water is fast-flowing, while *Raphidascaris acus* is better adapted to still or fast-flowing

waters. However, these parasites may be locally distributed because the intermediate hosts have preferences for particular water types. Thus, *C. ephemeridarum* is abundant in the more erosive habitats that support mayflies but tends not to coexist with *Cystidicola farionis*, which uses gammarids as intermediate hosts. Likewise, *C. truttae* is restricted to stretches of river where depositing conditions provide favourable habitats for its intermediate host, which are larval lampreys. It is not surprising that none of the nematode species described by Moravec (1994) as being planktophilous because they use copepods or brachiurids as intermediate hosts and therefore are characteristic of still or very slow-flowing waters were found in the Teifi or Pysgotwr.

The increase in availability of depositing sediments in the second and third major river zones favours the distribution and abundance of macrophytes, the molluscan and crustacean intermediate hosts of helminth parasites and cyprinid and other non-salmonid fish. It can therefore be hypothesised that species richness and diversity of the helminth parasites would increase at the supra-community level but that the converse would be the case with the helminth parasites of trout because of the reduction in the densities of both definitive and suitable intermediate hosts.

Lentic water bodies may be more favourable for the transmission of the helminth parasites in some fish. Thus, Landry and Kelso (1999) found that median parasite abundances in the largemouth bass, *Micropterus salmoides*, were higher in lakes than in rivers or swamps although the abundance of *Proteocephalus amploplitis* was higher in riverine sites. As species diversity is higher in the littoral zone of lentic water bodies than in the profundal zone it can be postulated that the helminth parasites of fish occupying these zones would show a similar trend. There is evidence in support of this hypothesis as Knudsen *et al.* (1997) found that the normal morph of the Arctic charr, *Salvelinus alpinus*, which feeds in the littoral zone, has a much richer helminth fauna than the dwarf morph that inhabits the profundal zone.

Fish inhabiting lentic water bodies also tend to have a higher prevalence of allogenic helminth species than fish in lotic water bodies. There are two main reasons for this. Firstly, the larvae of allogenic species are more planktonic in nature than those of autogenic species and birds, which are generally the definitive hosts. Secondly, birds are more abundant in lentic than in lotic habitats. As trout have evolved to exploit lotic habitats their helminth communities are characterised by a dominance of autogenic species. In contrast, allogenic species are more prevalent than autogenic species in cyprinid fish as they have evolved to exploit lentic habitats and the depositing conditions encountered in lowland reaches of lotic ecosystems.

Kennedy (1978c) attempted to relate the parasitic fauna of brown trout in nine British lakes to various physicochemical parameters. However, the only significant correlations were those between the size of the lake and the number of parasite species harboured by trout and between the altitude of the lake and

the number of species present. The relationship between the parasitic fauna of the fish and the size of the water body is in accord with the findings of Dogiel (1961) and is probably attributable to greater habitat and biological diversity. The negative relationship between lake altitude and parasitic fauna was attributed to the higher altitude lakes being relatively smaller than those at lower altitude. However, there were no significant relationships between the number of parasitic species and geographical position, age, degree of isolation or $CaCO_3$ levels of the lakes. Kennedy (1978c) and Esch *et al.* (1988) therefore concluded that helminth communities of freshwater fish were stochastic assemblages whose compositions are largely determined by high rates of dispersion of the parasites and host fish by birds and human activity. Although these conclusions were based on relatively small samples it is evident that research aimed at relating the parasitic fauna to limnological conditions should be carried out in relatively undisturbed habitats.

### *4.2.2. Water temperature and photoperiod*

As temperature limits the growth and development of poikilothermic organisms including helminth parasites it would seem logical to hypothesise that the helminth communities should be richer in tropical freshwater fish than in temperate zone fish because the average temperatures will tend to be higher in the former case. However, Choudury (2000) and Poulin (2001b) found the opposite trend in contrast to the majority of animal and plant assemblages. The reasons for this apparent anomaly are not known and require investigation. Observations made by Thomas (1966) in a tropical lake in Ghana may provide some clues. These show that species richness is much higher among taxa with high dispersive potential such as the Odonata, Hemiptera, Coleoptera and Diptera than among taxa with poorer dispersive powers such as the Ephemeroptera, Trichoptera, Annelida, Crustacea and Mollusca. The most plausible explanation for this phenomenon is that water bodies in savanna areas of Africa are often ephemeral due to severe seasonal drought. The selective mechanisms would therefore favour taxa with high dispersive powers. In contrast Platyhelminthes and the organisms involved as intermediate hosts of helminth parasites, such as the Mollusca and Crustacea, would be disadvantaged.

As both the fish hosts and parasites are poikilothermic it is to be expected that their reproduction and population dynamics would also be affected by seasonal changes in temperature in the temperate zones. In the case of the salmonid hosts there is ample evidence in support of this hypothesis. Thus, experimental work on the brown trout has shown that although growth occurs in the temperature range of 4–19 °C the optimum growth occurs between 13 and 14 °C (Elliot, 1989a). A growth model for the brown trout (Elliot, 1989b) which assumed that the initial size of the fry and the water temperature are the

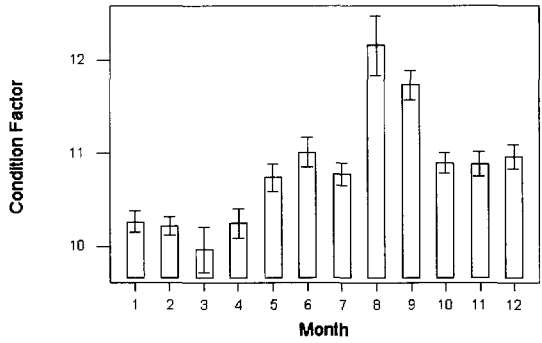


*Figure 8* Seasonal changes in the condition factor (± 95% C.L.) of brown trout over a 12-month period.

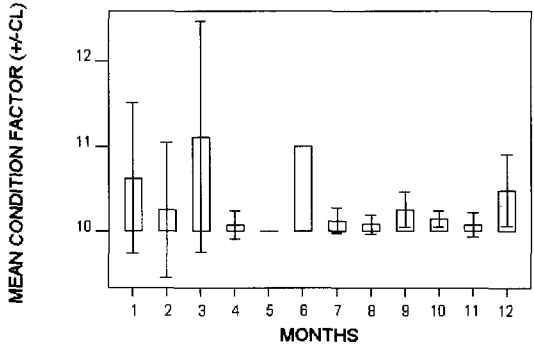


*Figure 9* Seasonal changes in the condition factor (± 95% C.L.) of the salmon parr over a 12-month period.

chief factors affecting growth when food is not limiting gave an excellent fit to the data. During the course of the present investigation on the Teifi it was found that the water temperatures were favourable for growth from mid-April until September (Thomas, 1962). This period coincided with the time when the condition factors of the trout reached maximum values (Figure 8) and when their immune response is most effective (Secombes and Chappell, 1996). However, temperature is not the only climatic factor that may influence growth and the timing of reproduction in salmonid fish as there is also evidence that photoperiod is also implicated (Porter *et al.*, 1998). These authors consider that although there is evidence for an endogenous, circannual clock this mechanism

is synchronised or entrained by photoperiod changes. Changes in photoperiod provide a more reliable feed-forward or predictive signal than temperature for organisms that live in the temperate areas of the world. In the case of salmonids it ensures that the fry emerge at a time when local enviromental factors such as food, water flow and temperature are optimal for their survival.

The present results show that the seasonal cycle in radiation levels, manifested by changes in temperature and photoperiod, is the major factor in predicting the total number of parasites of both trout and the salmon parr as it contributed 40.8% and 79.0% to the total variance, respectively. Seasonality was also a major factor in predicting the abundance of individual parasite species such as *D. sagittata*, *C. farionis*, *C. metoecus* and *P. simile* in trout as it contributed 48.78%, 52.46%, 88.21% and 33.74%, respectively, to the total variance in each case. In contrast, seasonality was less influential in determining the abundance of *N. rutili* and nematodes, chiefly *C. truttae*, in trout as it contributed only 5.45% and 3.22%, respectively, to the total variance in each case. Although, with the exception of *C. metoecus*, there were no well-defined cyclical seasonal trends in the abundance of salmon parr parasites, seasonality was nevertheless an important factor in predicting their numbers.

In view of the fact that parasite abundance values in the definitive host can be influenced by a number of factors, including stations as well as the age and sex of the host care must be taken in evaluating claims that they show statistically significant seasonal trends. However, there have been a number of attempts at classifying species of helminth parasites into those that may exhibit irregular fluctuations, without marked seasonal changes, and those that show marked seasonal cycles in prevalence or abundance (Kennedy, 1975; Chubb, 1977, 1979, 1980,1982; Forbes *et al.*, 1989). Seasonal cycles are more likely to occur in the case of species that are univoltine. This is the case with populations of smaller monogeneans such as *Dactylogurus vastator* in carp which build up in spring and summer and decline in winter when they may overwinter as eggs (Dogiel, 1961; Chubb, 1977). In contrast, *D. sagittata* may have different life history strategies with life spans of 1, 2 to 3 years depending on geographical location (Dogiel, 1961; Paling, 1965). Egg production and development take place at all times although they are optimal when the temperatures are higher in the summer (Gannicott and Tinsley, 1998b). However, mortality of both the larvae and adults is also higher at this time (Paling, 1965; Gannicott and Tinsley, 1998a). If mortality exceeds recruitment in the summer then a summer trough in abundance may occur, as was the case with the *D. sagittata* populations in trout in the present investigation. However, tendencies for seasonal fluctuations to occur will be buffered in those populations with longer life spans. As a result marked seasonal cycles in abundance or prevalence of *D. sagittata* populations are not generally observed (Paling, 1965; Campbell, 1974). As a result

of their relatively long-term survival rates, extending over many years, this is also often the case with larval parasites such as species of *Diplostomum* and *Diphyllobothium* (Chubb, 1980).

Populations of *N. rutili* are characterised by having gravid individuals present throughout the year in the temperate zone although peak egg or larval production occurs during the warmer season (Chubb, 1982). Populations of another acanthocephalan, *Pomphorhynchus laevis* in dace behave in a similar manner (Kennedy, 1975). Despite the reduction in abundance of *N. rutili* in the trout during July it must be concluded that there was no evidence of a marked seasonal cycle in abundance and prevalence of this parasite in salmonid populations in the Teifi. This suggests that mortality and recruitment are in a state of equilibrium most of the time. This process is aided by the presence of adults throughout the year. In contrast, the prevalence and abundance of *N. rutili* in barbel, *Barbus barbus*, in the Danube basin showed marked seasonal change with peaks from February to April and troughs from July to August (Moravec and Scholz, 1994). As recruitment peaks from May to July it would appear that the troughs from July to August were caused by high mortality.

*Phyllodistomum* species resemble *N. rutili* in not having a distinct annual cycle of reproductive activity. Thus, Chappell (1969) found that although the rate of egg production of *P. folium* was higher in the summer than in the winter the stickleback, *Gasterosteus aculeatus* became infected throughout the year. These findings are in accord with those of Pietrock *et al.* (1999) as they found juveniles and gravid *Phyllodstomum folium* in blue bream, *Abramis ballerus* and ruffe, *Gymnocephalus cernuus* in all seasons. They therefore concluded that infection might occur at all times. However, these authors found that *P. folium* in the ruffe exhibited a well-defined seasonal cycle with the prevalence and abundance values being significantly higher in the winter than in the summer months as was the case with *P. simile* in the trout in the Teifi in the present investigation. In contrast, the prevalence and abundance values for this parasite in the blue bream varied irregularly throughout the year without exhibiting an annual cycle.

According to Chubb (1982) nematodes such as *Capillaria* sp.and *Raphiascaris acus* have two generations a year whereas *Cucullanus truttae* and the two *Crepidostomum* species appear to be univoltine with well-defined periods for maturation, oviposition, senescence and death. It is not surprising therefore that *C. metoecus*, *C. farionis* and *C. truttae* exhibit well-defined annual cycles characterised by high prevalence and abundance values in the definitive fish host during the colder months of the year and low values during the warmer months of the year. As pointed out by Dogiel (1961) and Chubb (1977,1979, 1980, 1982) such annual cycles are typical and widespread in parasites of freshwater fish and other examples are provided by *Bunodera sacculata*, *Bunodera lucioperca*, *Asymphylodora tincae*,

*Allocraedium isoporum*, *Sphaerostoma bramae*, *Camallanus lacustris* and *Echinorhynchus gadi*.

The similarities in the life histories of digenetic trematodes of temperate zone fish such as *C. metoecus*, *C. farionis* and *Bunodera lucioperca* have been discussed by a number of authors (Awachie, 1968; Awachie and Chubb, 1964; Andrews and Chubb, 1980; Forbes *et al.*, 1989). Typically, juvenile worms invade their fish hosts from late summer to autumn and mature during the winter and early spring. During the summer months the gravid worms suffer heavy mortality and disappear from their definitive hosts by August. There has been much discussion regarding the mechanisms that may cause the seasonal cycles in their prevalence and abundance. As these cycles are negatively correlated with temperature, some authors (Awachie and Chubb, 1964; Awachie, 1968; Chubb, 1977, 1979, 1980, 1982) suggested that it is the causative factor. They postulated that the disappearance of *C. metoecus* and *C. farionis* from their trout host in the late summer was caused by an increase in water temperature above 10 °C, which was considered to be the critical upper level for the survival of adult parasites. They therefore proposed that these parasites were of northern origin, stenothermal and probably relicts of the ice age.

Despite these correlations several arguments can be advanced in support of the view that temperature may not be directly responsible for driving the seasonal cycle by causing mortality of the adult worms. Firstly, if temperature-induced mortality reduces the fitness of the species it is to be expected that the parasites would have become adapted to the higher summer temperatures as a result of natural selection. The fact that parasites such as *C. metoecus* and *C. farionis* can survive high summer temperatures in their intermediate hosts indicates that their genome is amenable to these selective pressures. Secondly, it was found that some adult *C. metoecus* and *C. farionis*, albeit in smaller numbers, did survive in the salmonids in the Teifi, particularly in the salmon parr, during the warm summer months although their abundance was significantly less than in the winter months. Thirdly, some *Crepidostomum* species such as *C. cooperi* living in perch, *Perca flavescens* in Lake Opeongo, Ontario, Canada can survive high summer temperatures as adults. Thus, Cannon (1972) found the maximum prevalence of these parasites in the summer and the lowest in the autumn and early winter. Fourthly, as pointed out by Kennedy (1975), this hypothesis is not supported by experimental evidence.

It seems more likely therefore that the annual cyclical changes in worm abundance of parasites such as *C. metoecus* have resulted from selective pressures that favour the avoidance of the definitive host during the summer because the conditions in the gut become highly unfavourable at this time. Although the high summer temperatures are correlated with the increased worm mortality it does not follow that it is the causative mechanism. One

factor that may make it difficult for the parasites to survive in the gut at this time is the enhancement of the host's immune response as a result of increased temperature. This view is supported by laboratory experiments and field observations which show that the immune response of teleost fish to helminth parasites is enhanced by increase in temperature (Kennedy and Hine, 1969; Kennedy, 1972; Rawson and Rogers, 1972a, b; Avtolion, 1973; Secombes and Chappell, 1996; Bernstein *et al.*, 1998). The fact that teleost fish are highly competent immunologically both in innate immunity and in the expression of the combinatorial immune system may have been underestimated in the past (Bernstein *et al.*, 1998). Thus, Chubb (1980) stated that 'it seems unlikely that the immune reaction on the part of the fish host plays any significant seasonal role in the seasonal dynamics of larval parasites'. However, one weakness in the fish armoury is that the rejection time of parasites is much longer at lower ambient temperature than is the case with mammals.

Seasonal changes in the host metabolism and diet may also be important factors in influencing seasonal changes in parasite abundance. Elliott (1994) has shown that the metabolic rate and the turnover of food in the alimentary canal of brown trout increase with temperature and that 13–14 °C is the optimal temperature range for growth. It is possible therefore that the more mobile intestinal parasites such as *C. metoecus* and *C. farionis* which lack strong adhesive structures will be more readily egested when the temperatures are high in the summer. Circumstantial evidence in support of this hypothesis is provided by the fact that *N. rutili*, which is armed with a proboscis that penetrates into the intestinal wall, does not exhibit a marked summer trough in its abundance unlike the *Crepidostomum* species. The decline in feeding rate during the spawning period may also influence parasite abundance. Moravec (1994) has suggested that this may be responsible for the loss of the autumn generation of *Cystidicoloides tenuissima* and a decline in abundance of *Raphidascaris acus* in trout in the autumn and winter.

Older workers have invoked cyclical changes in dietaries to explain cyclical changes in the prevalence and abundance of parasites including *Echinorhynchus truttae* in trout (Awachie, 1965) and *Podocotyle* sp. in flounders (MacKenzie and Gibson, 1970). More recently, Pietrock *et al.* (1999) also concluded that the cyclical changes in abundance of *P. folium* in the ruffe was caused by diet rather than by the annual temperature cycles although the latter was correlated with it. This conclusion was based on the observation that although the infective stages were available throughout the year the seasonal cycles in abundance of *P. folium* was only observed in the ruffe and not in the other host, the blue bream. The most plausible reason for this difference is that the ruffe ingested more of the intermediate hosts than the blue bream during the spring and summer. Prolonged periods of fasting may also have a major impact on parasite abundance. Thus, according to Dogiel (1961) the

catfish, *Siluris glanis*, loses its intestinal parasites in the winter, except for scoleces of *Proteocephalus*, because it does not feed at this time. Kennedy (1972) also found that the rate of loss of parasites increased under conditions of host starvation. The fact that the European eel *Anguilla anguilla* also ceases to feed in the winter when the temperature falls below 9–10 °C (Thomas, 1962) may be partly responsible for its depauperate fauna (Kennedy and Guégan, 1996).

There are also many other examples, cited by Chubb (1977, 1979, 1980, 1982), in which maturation and reproductive activity of the helminth parasites and their host fish are synchronised. The selective advantage of such synchronous behaviour are apparent in the case of parasites where transmission is direct as the host fish aggregate during spawning thus greatly increasing the probability of successful transmission. Examples are provided by monogenetic trematodes such as *Dactylogyrus vastator* in carp and *Mazocraes alosae* in shad. Where intermediate hosts are involved in transmission the selective advantages of synchrony are less obvious. However, it can be postulated that the release of eggs by the parasite during redd formation by salmonids may increase the probability of sediment-dwelling intermediate hosts being infected. This may be the case with *Echinorhynchus salmonis*, *Philonema agubernaculum* and *Philonema onchorhynchi* when their salmonid hosts *Onchorhynchus tshawytscha*, *Salmo salar* and *Onchorhynchus nerka*, respectively, are spawning. There is also evidence that maturation and egg production of *C. truttae* are synchronised with the onset of spawning activity by the host fish at least in *Lampetra* species (Moravec, 1994). As the adult lampreys spawn in microhabitats already occupied by the previous generations of larval lampreys which are the intermediate hosts this would be selectively advantageous. In this connection it would also be of interest to ascertain whether oviposition by *P. simile* might be synchronised with redd formation and spawning by trout as this would increase the probability of the sediment-dwelling, sphaeriid intermediate hosts becoming infected.

Other cases of synchrony between host maturation on the one hand and parasite maturation (e.g., *Caryophyllaeus laticeps* in *Leuciscus leuciscus*, *Proteocephalus filicollis* in *Gasterosteus aculeatus* and *Triaenophorus crassus* in *Esox lucius*), rapid growth (e.g., *Camallanus oxycephalus* in *Morone chrysops*), increased susceptibility to infection (e.g., *Archigetes iowensis* in *Cyprinus carpio*) on the other may also be linked to a lowered host immunity (Chubb, 1979,1980, 1982). Pickering and Pottinger (1987) provide evidence in support of this hypothesis as they found marked reductions in the number of circulating lymphocytes in the blood of sexually mature male and female trout during the spawning season. This effect was attributed to cortisol suppression of lymphoidal activity. Cortisol appears to influence reproduction by sensitising the gonads to steroids and indirectly by regulating metabolism. Pickering and Pottinger (1987) have suggested that the costs of increased susceptibility

to disease resulting from this mechanism may have contributed to the evolution of semelparity in some salmonid species. Møller (1997) has discussed both cost and benefits of reproductive suppression of immunity, which is a common feature in all vertebrates, and suggests that parental suppression of the immune system is a necessary cost that has to be incurred to allow successful oogenesis and reproduction.

The above discussion makes it possible to formulate a unifying hypothesis, based on evolutionary considerations, to account for the seasonal cycles in the prevalence and abundance of univoltine parasites such as *C. metoecus* and *C. farionis*. During the summer months it can be postulated that it will be the adult stages of helminth parasites, inhabiting the gut of fish, which will be under greatest threat as a result of the enhanced immunity and the increased turnover rate of the gut contents. Parasites with relatively poorly developed adhesive structures will be particularly vulnerable. In contrast, the larval stages in the immunologically and metabolically benign intermediate hosts will be under less intense selective pressure and therefore have a higher survival rate than the adults in the summer months. The summer would therefore be the opportune time for the parasites to leave the definitive host and concentrate their reproductive efforts in their invertebrate intermediate hosts. However, with the onset of autumn the gut environment of salmonids and other autumn spawning fish become more favourable because of a lowering of host immunity and gut turnover rates as a result of the onset of reproductive activity and a fall in metabolic rate, respectively. Late autumn would therefore be the optimal time for the more vulnerable parasites to reinvade the gut environment. As the parasites are poikilotherms the ambient temperature will influence their growth and reproduction. On evolutionary grounds it might be expected therefore that somatic growth and reproductive output of both the definitive host and parasites would increase in parallel with the rise in temperature in spring. The predictions based on this model are in accord with the observed cyclical patterns.

A corollary to this hypothesis is that the parasites will use predictive signals to activate the appropriate genes. One possibility is that the parasites may use a rise in temperature to trigger enhanced reproductive activity prior to death. However, as temperatures fluctuate a great deal it may prove to be an unreliable signal. A more reliable signal is provided by changes in photoperiod, possibly modulated by seasonal changes in temperature, and it is for this reason that the majority of organisms in the temperate zone have evolved mechanisms to use it as the key predictive signal. However, as this appears to be unavailable to gut parasites it seems plausible, as suggested by Forbes *et al.* (1989), that they might use the seasonal changes in the host's metabolism, endocrine or immune mechanisms instead. There is empirical evidence that parasites use seasonal changes in the host's endocrine cycle, which are set by changes in photoperiod and modulated by temperature, as a reliable predictive

signal to set their annual reproductive cycle (Kearn, 1998). One of the best examples of a parasite adapting physiologically to a narrow window of opportunity for transmission is provided by *Polystoma integerrimum*. This parasite uses the endocrine mechanism of its host, the common frog, to trigger egg production thus allowing it to synchronise oviposition with its host when the frog enters the water to oviposit (Kearn, 1998). It must be concluded therefore that the present hypothesis is at variance with the suggestion of Verberng and Vernberng (1974) that the thermal tolerances of trematodes should agree closely with that of their respective hosts.

Further research is needed to test this hypothesis which focuses on parasites that become gravid in spring and early summer. Chubb (1982) included three other categories of life history strategies: (1) gravid in late spring–summer; (2) gravid from spring to autumn during the warmer months of the year; and (3) gravid throughout the year although peak egg or larval production occurs during the warmer season. Models designed to account for these differences will have to take into account seasonal changes in climate, the life history strategies of both the parasites and hosts, and in particular the time of spawning of the definitive fish hosts.

### *4.2.3. Water chemistry*

It can be hypothesised that the species richness and abundance of helminth parasite communities in freshwater fish would be positively correlated with the nutrient status of the water bodies, as measured by pH, conductivity, base cation and micronutrient concentrations, up to critical thresholds (Figure 1). This hypothesis, which was tested by reference to commonly used measures of community structure, is supported by the results. Thus, a comparison of the helminth parasite communities of trout in the River Teifi and Pysgotwr shows that the species richness and the Shannon–Wiener and Brillouin diversity indices are much higher in the former case (Table 14). In contrast, the Berger–Parker index, which provides a measure of the relative abundance of the dominant species, is higher for the helminth community in the Pysgotwr than is the case in the Teifi. Although both rivers are oligotrophic or nutrient poor the pH, conductivity, base cation and micronutrient concentrations are much lower in the Pysgotwr than the Teifi (Table 1).

Other workers have also found that water chemistry can be a major factor in influencing the distribution and abundance of helminth parasites in fish. Thus, Cone *et al.* (1993) found that the parasite communities in the American eel, *Anguilla rostrata*, inhabiting acidic waters (pH 4.5–5.0) were characterised by the absence of digenetic trematodes, lower species richness and fewer multiple infections compared with those in less acidic limed waters. Kennedy *et al.* (1994) and Yeomans *et al.* (1996) also found that increased

*Table 14* Community structure indices for the helminth parasites of brown trout and salmon parr in two river systems in West Wales

| | Station | | | | | |
|---|---|---|---|---|---|---|
| | Stat. A | Stat.B | Stat.B | Stat.D | Stat.A | Stat.B |
| Fish species | Trout | Trout | Trout | Trout | S. parr | S.parr |
| Year | 1950 | 1950 | 1998 | 1950 | 1950 | 1950 |
| No. fish examined | 209 | 685 | 18 | 269 | 15 | 273 |
| No. helminth species | 10 | 9 | 5 | 4 | 5 | 5 |
| No. autogenic species | 8 | 8 | 5 | 4 | 6 | 6 |
| No. allogenic species | 1 | 0 | 0 | 0 | 0 | 0 |
| **Component population** | | | | | | |
| Dominant species | CM | CM | NR | CM | NR | NR |
| Berger–Parker index | 0.449 | 0.542 | 0.889 | 0.973 | 0.925 | 0.825 |
| Slope 'a' of geometric series | –0.485 | –0.356 | –0.678 | –0.934 | –0.442 | –0.571 |
| Simpson diversity index (1/D) | 2.806 | 2.565 | 1.258 | 1.055 | 1.167 | 1.441 |
| Shannon–Wiener index (H) | 1.690 | 1.607 | 0.639 | 0.205 | 0.518 | 0.966 |
| Equitability index ($H/H_{max}$) | 0.602 | 0.622 | 0.319 | 0.102 | 0.223 | 0.416 |
| Brillouin's index (BI) | 1.166 | 1.119 | 0.417 | 0.142 | 0.326 | 0.634 |
| $BI_{max}$ | 1.942 | 1.781 | 1.346 | 1.384 | 1.555 | 1.595 |
| BI equitability index | 0.600 | 0.628 | 0.310 | 0.103 | 0.210 | 0.397 |
| **Infrapopulation** | | | | | | |
| Mean no. parasite spp.per fish (± S.D.) | 2.74 ± 1.03 | 3.07 ± 1.24 | 1.50 ± 0.82 | 1.14 ± 0.04 | 1.33 ± 0.64 | 1.34 ± 0.93 |
| Maximum no. parasite spp. | 5 | 6 | 3 | 4 | 2 | 4 |
| Mean no. individual helminths (± S.D.). | 33.60 ± 31.3 | 34.52 ± 31.7 | 12.37 ± 10.0 | 24.27 ± 11.7 | 12.47 ± 14.0 | 10.50 ± 10.1 |
| % Fish with no parasites | 0.96 | 1.31 | 6.25 | 15.24 | 13.33 | 14.29 |
| % Fish with 1 parasite sp. | 9.57 | 8.91 | 50.0 | 58.36 | 60.0 | 51.28 |
| % Fish with 2 parasite spp. | 29.67 | 22.77 | 31.25 | 23.79 | 13.33 | 23.08 |
| % Fish with 3 parasite spp. | 39.23 | 29.78 | 125 | 2.23 | 13.33 | 8.42 |
| % Fish with 4 parasite spp. | 15.31 | 24.23 | 0 | 0.37 | 0 | 2.93 |
| % Fish with 5 parasite spp. | 5.26 | 11.39 | | | | |
| % Fish with 6 parasite spp. | | 1.61 | | | | |
| Mean B.I. ± S.D. (all fish) | 0.557 ±0.319 | 0.602 ±0.322 | 0.109 ±0.204 | 0.066 ±0.134 | 0.132 ±0.245 | 0.142 ±0.232 |
| Mean B.I. ± S.D. (infected fish) | 0.562 ±0.316 | 0.611 ±0.316 | 0.124 ±0.242 | 0.078 ±0.142 | 0.152 ±0.258 | 0.165 ±0.242 |
| Maximum BI | 1.328 | 1.336 | 0.793 | 0.643 | 0.687 | 0.994 |

CM = *C. metoecus*; NR = *N. rutili*.

levels of eutrophication were accompanied by population increases in fish parasites such as *Ligula* and trichodinids, respectively.

The tendency for the richness and diversity of helminth parasite communities to be positively correlated with the nutrient status of water bodies is attributable to the fact that the latter favours a corresponding enhancement in

benthic invertebrates, zooplankton and fish that may serve as intermediate or definitive hosts (Moss, 1988). There is a great deal of cumulative evidence that the distribution and abundance of molluscs, which are obligatory hosts to digenetic trematodes, and crustacea, which are often hosts to digenetic trematodes, cestodes, acanthocephala and nematodes, are strongly correlated with pH and associated base cation concentration (Økland, 1990). As some sphaeriids such as *Pisidium* species are more tolerant to low pH conditions than other molluscs (Økland and Økland,1986; Økland,1990) this helps to explain the preponderance of *Crepidostomum* species in trout inhabiting acidic waters.

However, due to the complexity of the interactions between the physicochemical interactions it is not surprising that the relationship between trophic state and species richness is not always clear-cut. Thus, Chubb (1964, 1970) found that the species richness of helminth fish parasites was 14 for mesotrophic Llyn Tegid compared with 16 for oligotrophic Llyn Padarn. Wisniewski (1958) and Esch (1971) also found that the species richness of helminth parasites of fish was higher in oligotrophic than in eutrophic waters although the latter had proportionately more allogenic species than the former. As the trophic status of water continues to increase from oligotrophic to hypereutrophic it is to be expected that the species richness of helminth parasite communities in particular fish species would reach an optimum level. As the communities of helminth parasites of salmonid and coregonid fish and their intermediate hosts have evolved in oligotrophic waters they are generally intolerant of eutrophic conditions. It would be predicted therefore that the optimum species richness for these helminth communities would precede those for percid and cyprinid fish on the trophic scale as the latter are more tolerant of eutrophication and better adapted to lentic conditions.

## 5. THE RELATIONSHIPS BETWEEN PARASITE ABUNDANCE AND THE AGE AND SIZE OF THE FISH HOSTS

### 5.1. Quantitative Data

There are strong, statistically significant tendencies for the mean abundance of parasites to increase with the length and weight of the brown trout (Table 15). The $F$ values indicate that these relationships are particularly strong in the case of the nematodes. In contrast to the trout the relationships between the size of the salmon parr and the mean abundance of parasites were less clear (Table 16). Thus, there were statistically significant tendencies for the mean abundance of *D. sagittata*, *C. metoecus* and *C. farionis* to increase with the length of the salmon parr ($P < 0.05$, $< 0.05$ and $< 0.002$, respectively). However, there were no statistically significant relationships between the mean abundance of

*Table 15* The regression equations showing the relationships between the transformed number of parasites $\{\log_{10}(1+x)\}$ and $\log_e$ (ln) of the length (cm) and $\log_e$ (ln) weight of the trout (g)

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = –1.46 + 0.575 ln length | 104.55 | < 0.001 |
| Numbers (tr.) of *C. farionus* = –0.834 + 0.353 ln length | 39.57 | < 0.001 |
| Numbers (tr.) of *C. metoecus* = –2.31 + 1.51 ln length | 65.11 | < 0.001 |
| Numbers (tr.) of *P. simile* = –1.64 + 0.642 ln length | 92.88 | < 0.001 |
| Numbers (tr.) of *N. rutili* = –3.90 + 1.85 ln length | 165.33 | < 0.001 |
| Numbers (tr.) of nematodes = –7.20 + 2.78 ln length | 759.51 | < 0.001 |
| Numbers (tr.) of *D. ditremum* = –0.036 + 0.013 ln length | 3.87 | < 0.049 |
| Numbers (tr) of all parasites = –17.3 + 7.71 ln length | 698.19 | < 0.001 |
| Numbers (tr.) of *D. sagittata* = –0.587 + 0.191 ln weight | 96.95 | < 0.001 |
| Numbers (tr.) of *C. farionus* = –0.291 + 0.116 ln weight | 35.53 | < 0.001 |
| Numbers (tr.) of *C. metoecus* = –0.275 + 0.432 ln weight | 44.05 | < 0.001 |
| Numbers (tr.) of *P. simile* = –0.695 + 0.220 ln weight | 91.47 | < 0.001 |
| Numbers (tr.) of *N. rutili* = –1.360 + 0.680 ln weight | 191.20 | < 0.001 |
| Numbers (tr.) of nematodes = –3.16 + 0.965 ln weight | 776.49 | < 0.001 |
| Numbers (tr.) of *D. ditremum* = –0.013 + 0.004 ln weight | 2.70 | < 0.100 |
| Numbers (tr.) of all parasites = –5.82 + 2.600 ln weight | 652.84 | < 0.000 |

*Table 16* The regression equations showing the relationships between the transformed number of parasites $\{\log_{10}(1+x)\}$ and $\log_e$ (ln) of the length (cm) and $\log_e$ (ln) weight of the salmon parr (g)

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = – 0.352 + 0.147 ln length | 3.99 | 0.047 |
| Numbers (tr.) of *C. farionis* = – 0.942 + 0.423 ln length | 4.35 | 0.038 |
| Numbers (tr.) of *C. metoecus* = –2.91 + 1.26 ln length | 9.98 | 0.002 |
| Numbers (tr.) of *N.rutili* = 0.351 – 0.700 ln length | 1.68 | 0.299 |
| Numbers (tr.) of nematodes = – 0.502 + 0.273 ln length | 0.89 | 0.347 |
| Numbers (tr.) of all parasites = 0.572 + 0.105 ln length | 0.13 | 0.718 |
| Numbers (tr.) of *D. sagittata* = – 0.127 + 0.046 ln weight | 3.52 | 0.061 |
| Numbers (tr.) of *C. farionis* = – 0.128 + 0.080 ln weight | 1.39 | 0.239 |
| Numbers (tr.) of *C. metoecus* = 0.143 + 0.047 ln weight | 0.12 | 0.731 |
| Numbers (tr.) of *N.rutili* = 2.43 – 0.216 ln weight | 0.93 | 0.335 |
| Numbers (tr.) of nematodes = 0.293 – 0.032 ln weight | 0.11 | 0.738 |
| Numbers (tr.) of all parasites = 0.978 – 0.044 ln weight | 0.21 | 0.651 |

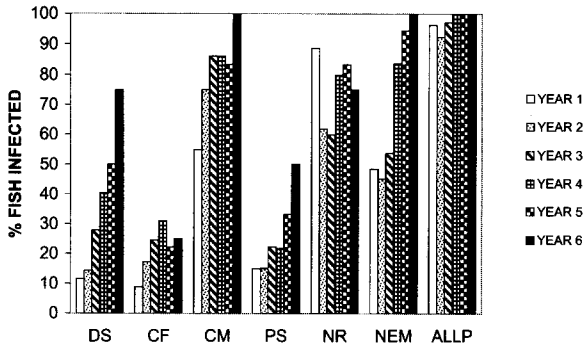


*Figure 10* The relationship between the percentage prevalence of the dominant parasites and the age of the trout. DS, *D.sagittata*; CF, *C. farionis*; CM, *C. metoecus*; PS, *P. simile*; NR, *N. rutili*; NEM, nematodes; ALLP, all parasites.

*N. rutili*, nematodes and the total parasites and the length of the salmon parr (Table 16). Neither were there any statistically significant relationships between the mean abundance of any of the parasite species and the weight of the salmon parr (Table 16). In contrast there were non-significant tendencies for the mean abundance of *N. rutili* and nematodes to decrease with increase in length or weight of the salmon parr (Table 16).

The percentage prevalence values for *D. sagittata*, *C. metoecus* and *C. farionis*, *P. simile* and nematodes in trout tend to increase with the age of the host (Figure 10). However, in the cases of *C. metoecus* and *C. farionis* there is a tendency for the values to reach an asymptote from age 3 years onwards. In contrast, the percentage prevalence values for *N. rutili* tend not to increase with the age of the trout. All the trout of 4 years and over were infected with one or more parasite species and the prevalence values for 1–3-year-old trout were over 90%.

Table 17 shows that, with the exception of *N. rutili* and *D. ditremum*, there were highly significant statistical tendencies for the mean abundance of all the parasite species to increase with the age of the trout ($P < 0.001$ in all cases). These trends (Figure 11) show however that there were tendencies for the mean abundance of the parasite species to reach asymptotic values from the age of 3 years onward. The only exceptions to this rule are *D. ditremum*, probably due to inadequate data, and the nematodes, where the mean abundance continues to increase up to the age of 5 years.

The prevalence data for the parasites of the salmon parr (Figure 12) show similar trends with those observed in the trout. Thus, the prevalence values for all the parasites tend to increase with age. However, *N. rutili* and *C. farionis* provide exceptions to this rule as their mean abundances do not increase significantly ($P = 0.213$ and 0.074 respectively) with the age of the salmon parr (Table 17). The tendencies for the mean abundance to increase with age are

*Table 17* The results of statistical analyses relating the abundance of different parasites (transformed $\log_{10}(1 + x)$) and the age of brown trout and salmon parr

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Brown trout** | | | | | | | | |
| Parasite | *D. sagittata* | *C. farionis* | *C. metoecus* | *P. simile* | *D. ditremum* | *N. rutili* | Nematodes | All parasites |
| *F* value | 13.33 | 5.10 | 52.57 | 5.72 | 0.58 | 3.05 | 57.61 | 73.87 |
| *P* value | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.718 | 0.010 | < 0.001 | < 0.001 |
| **Salmon parr** | | | | | | | | |
| Parasite | *D. sagittata* | *C. farionis* | *C. metoecus* | *N. rutili* | Nematodes | All parasites | | |
| *F* value | 4.87 | 2.34 | 16.44 | 1.51 | 7.47 | 3.60 | | |
| *P* value | 0.003 | 0.074 | < 0.001 | 0.213 | < 0.001 | 0.014 | | |

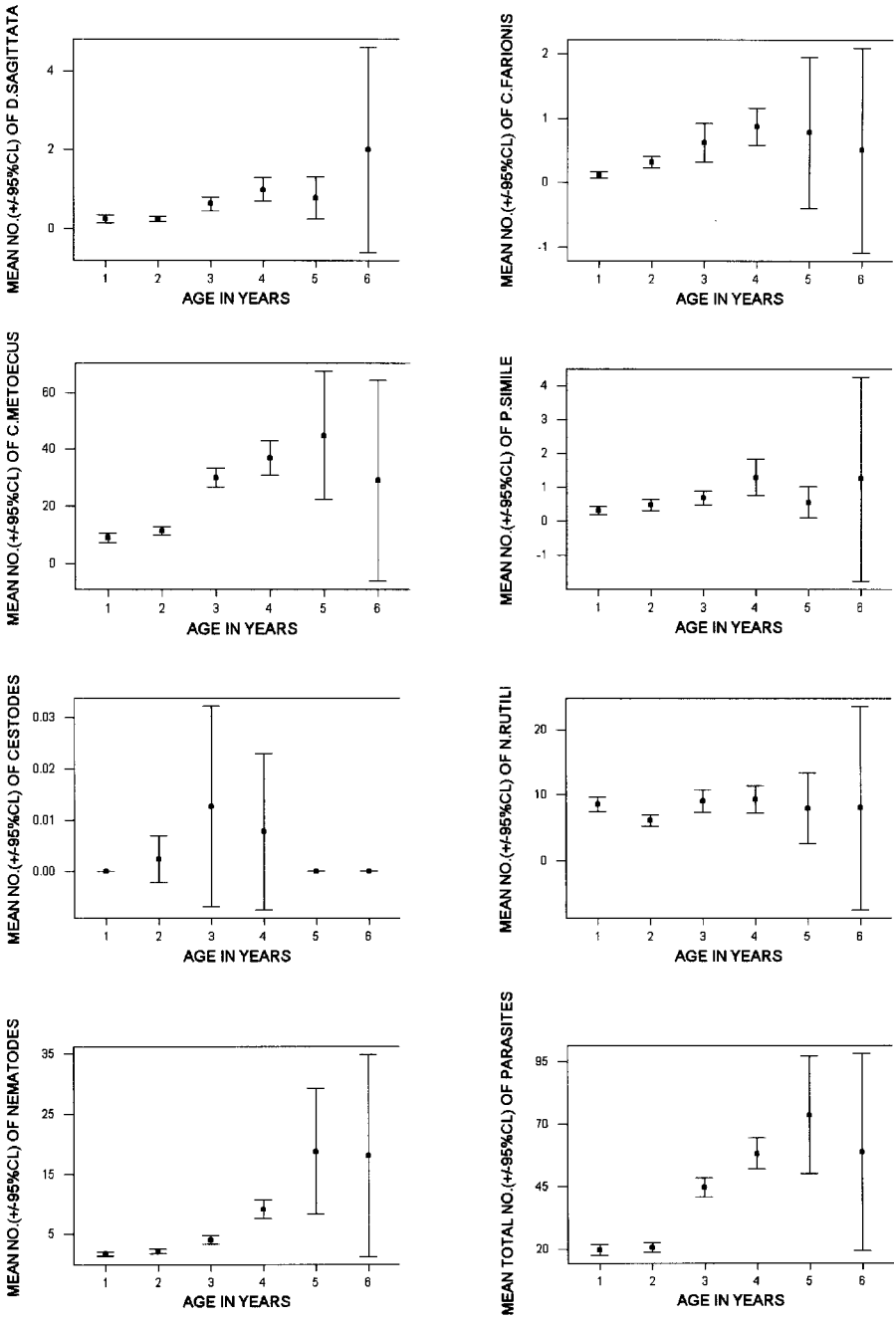


*Figure 11* The relationship between the parasite abundance and the age of the trout.

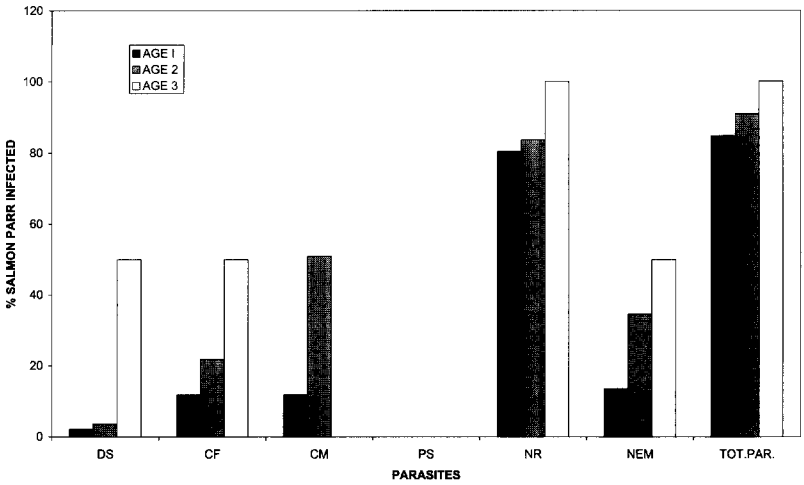


*Figure 12* The relationship between the percentage prevalence of the dominant parasites and the age of the salmon parr. DS, *D. sagittata*; CF, *C. farionis*; CM, *C. metoecus*; PS, *P. simile*; NR, *N. rutili*; NEM, nematodes; TOT.PAR., all parasites.

more marked in the cases of *C. metoecus* and nematodes (Figure 13 and Table 17). However, as *N. rutili* is the most abundant parasite in salmon parr the relationship between the intensity of total parasite infection and host age is only statistically significant at $P < 0.05$.

The present results are in accord with the general rule that the relationship between the age, or size, of fish and the prevalence or abundance of their helminth parasites tend to be positive in most cases (Bangham, 1944; Thomas, 1958b; Dogiel, 1961; Hicks and Threlfall, 1973; Cannon, 1972; Hine and Kennedy, 1974; Zelmer and Arai, 1998; Zhokov, 1998).

## 5.2. Causative Mechanisms

The statistically significant tendencies for the prevalence or abundance of most of the helmith parasites of trout to increase with size or age of the trout may be attributable either to changes in feeding behaviour or to an increase in available space and nutrients in the microhabitat of the parasite. The possibility that the increase in parasite load with age is due to an increased probability of ingesting infected intermediate hosts as a result of increased food intake has been suggested by a number of previous workers (Walkey, 1967; Hine and Kennedy, 1974; Muzzall, 1980; Amin, 1985). However, as the trout age and increase in size their dietary and habitat preferences also change. Thus, they select larger food items, become more piscivorous and tend to

spend more time in transmission sites associated with depositing sediment (Thomas, 1962). These changes further increase the probability of them ingesting infective larvae. The particularly strong trend for nematode abundance to increase with age can be attributed to the increased tendency of the trout to ingest more fish and ammocoete larvae as they get older (Thomas, 1962). Fish, including minnows and salmonids, and larval lampreys are obligate intermediate hosts for *R. acus* and *C. truttae*, respectively. The lack of a significant relationship between the age of trout and the abundance of *N. rutili* suggests that the probability of ingesting infective larvae is approximately the same for trout of all ages. In view of the positive correlation between the size of the prey and the size of the fish (Thomas, 1962) it seems

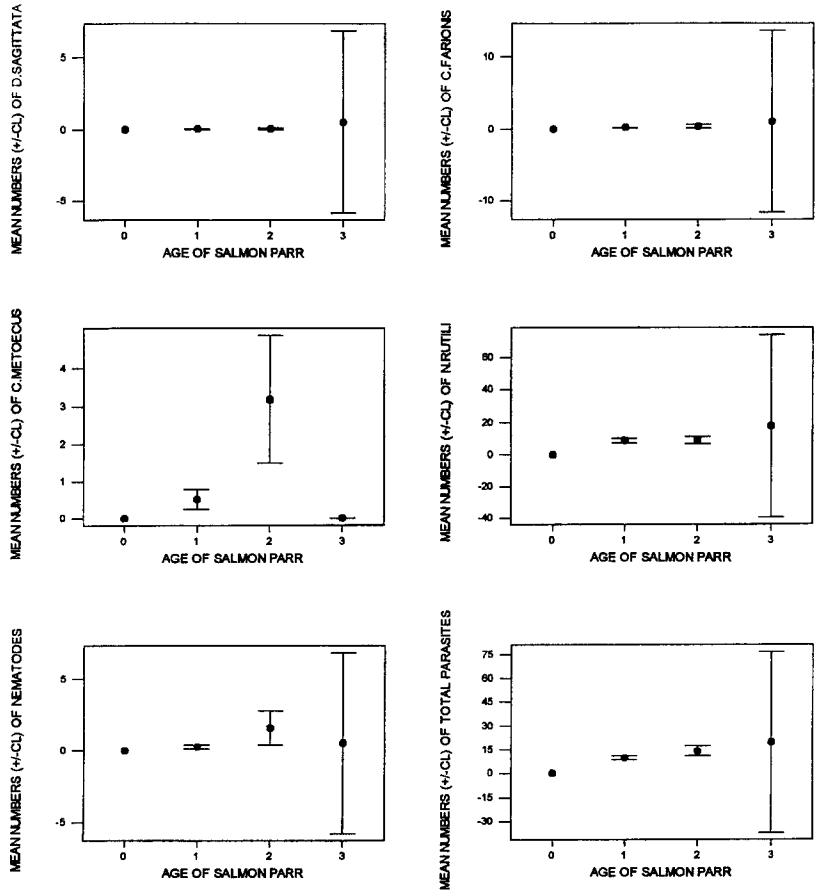


*Figure 13* The relationship between the parasite abundance and the age of the salmon parr.

probable that the younger trout become infected by ingesting smaller ostracod intermediate hosts while the older trout become infected by ingesting larger *Sialis* larvae which inhabit depositing sediment. Divergence in feeding behaviour between different morphs of lake resident Arctic charr (*Salvelinus alpinus*) over an extended period of time has also been used to explain divergence in their parasitic fauna (Curtis *et al.*, 1995). Thus, morphs feeding predominantly in the limnetic zone were infected mainly with cestodes whereas benthic feeders were more heavily infected with *C. farionis* and acanthocephala. Helminthological considerations may make it necessary to question conventional views regarding the feeding behaviour of host fish such as the eel. Thus, 63% of the specialist parasites of the European eel (*Anguilla anguilla* L.) use planktonic intermediate hosts as do several of the commoner generalist species (Kennedy *et al.* 1992). As a result these authors argue that the widely accepted view that eels are exclusively benthic feeders requires reinvestigation. One possible explanation for this apparent anomaly is that 'planktonic' food organisms are much commoner in the interstitial spaces in the sediments of lotic habitats than hitherto believed but that they may have been overlooked during the examination of the stomach contents because they are small and easily digested.

The fact that the relationships between the prevalence and abundance of helminth parasites and the length, weight and age of the salmon parr are less clear-cut than in the trout can be attributed to smaller ranges in the length, weight and age of the latter species. As a result it is to be expected that there would be less variation in the diet of the salmon parr. This hypothesis is supported by empirical evidence (Thomas, 1962). The fact that the feeding territories of the salmon parr overlap to a greater extent than the trout (Armstrong *et al.*, 1999) would also result in the dietaries of the former species being less variable. The results obtained by Hicks and Threlfall (1973) for species of salmonid and coregonid fish resemble those for trout and salmon parr in the present study. Thus, they found that in the case of salmonid species older fish tended to harbour more parasites than younger fish whereas this was not the case with parasites of coregonid fish. They also attributed these differences to the fact that the dietaries of the coregonid fish change very little with age unlike the salmonid species they had studied. However, an increase in dietary input related to size may not be the only factor in determining parasite abundance. There is evidence that the availability of colonising space may be a key factor as well. Thus Poulin *et al.* (1991) found that the size of the brook trout, *Salvelinus fontinalis*, was positively correlated with the number of acquired copepods. In contrast there was no statistically significant relationship between the numbers of copepods acquired and the level of activity of the fish, the time spent near the bottom or the distance from the parasite release site.

Poulin and Valtonen (2001) found that when the fish hosts accumulate

parasites in a predictable way, proportional to their size, the parasite communities conform to a nested pattern. On the other hand, when fish hosts do not accumulate parasites in a predictable way, proportional to their size, possibly as a result of dietary specialisation preventing them from sampling available parasite species, the parasite communities exhibit an anti-nested pattern. Meta-analysis carried out by Poulin (2000) on 76 different host–parasite species revealed that the overall mean correlation between fish length and the intensity of parasite infections was weakly positive but non-significant following corrections for sample size. These results imply approximate equality between the numbers of nested and anti-nested communities. It is not surprising therefore that there are exceptions to the rule that the prevalence or abundance of helminth parasites correlate positively with the age and size of the fish hosts (Dogiel, 1961; Kennedy, 1968; Zhokhov and Pugacheva, 1998; Poulin, 2000). Kennedy (1968) gives a specific example. He found that the abundance of *Caryophyllaeus laticeps* in dace was independent of age, as was the case with *N. rutili* in trout in the present study. These patterns may also be explained on the basis of dietary considerations. When there are profound changes in diet with age as is the case with the pike, *Esox lucius*, there may be conflicting trends. Thus, Dogiel (1961) found that the prevalence or abundance of 11 (55%) of the 18 parasites in this fish increased with age whereas 11% decreased with age and 33% were independent of age.

Changes in helminth fauna of fish can also occur as a result of habitat change resulting from migration. This has been well documented in the case of migratory salmonid fish by Dogiel (1961) and Molloy *et al.* (1993). However, not all the changes in helminth fauna are attributable to change in diet. Thus, according to Paling (1965) trout in Lake Windermere become infected with the monogenetic trematode *D. sagittata* only after migrating into the deeper water of the lake and young trout in nursery streams are uninfected. It is probable that the absence of *D. sagittata* from the trout in the feeder stream is attributable to the vulnerability of the oncomiracidia in turbulent waters. According to Dogiel (1961) *Dactylogyrus vastator* is only found in young carp because they are predominantly surface dwellers unlike the adults. The decrease in prevalence of *Gyrodactylus rarus* in sticklebacks with age (Kennedy, 1970) may be attributable to the short life span of the parasite and to changes in host density, associated with maturation, although the involvement of age resistance cannot be ruled out. In contrast, the larvae of *Diplostomum spathecum*, which also attach to the skin of the fish to gain entry into the body tissues, have a long life span and as a result their mean abundance shows a strong positive relationship with the age of the fish (Kennedy, 1970). This suggests that this parasite does little harm to their fish hosts although Secombes and Chappell (1996) provide evidence to the contrary.

The alternative explanation for the positive relationships between the abundance of parasites and the size, or age, of fish like the trout is that it is

due to helminth parasites in trout being regulated in a density-dependent manner by spatial or nutrient limitation. The fact that the parasite density per square metre of alimentary canal remains virtually constant in 1-, 2-, 3- and 4-year-old trout (Thomas, 1964a) supports this hypothesis as it suggests that space or nutrient availability may be limiting factors. Brown (1986) also found that the number of parasites harboured with increasing infection dosage reached a plateau, proportional to the length of the fish, in laboratory-maintained rainbow trout. He suggested that this effect was due to an increase in absolute surface area available for attachment with increase in fish size. According to Al-Hussaini (1949) the intestinal length of many fish species increases in direct proportion to body length and the absolute surface area available to the parasites for attachment increases as the square of any increase in intestinal length.

## 5.3. Ecological Relevance and Density Dependence

The important question of whether populations of helminth parasites of fish are regulated in a density-dependent manner and whether they conform to the 'r' or 'K' strategies has been widely discussed (Kennedy, 1974, 1977; Holmes *et al.*, 1977; Esch *et al.*, 1977; Kennedy and Rumpus, 1977; Figure 1). In order to resolve this question entomologists and fishery biologists have resorted to using 'K' factor analyses (Elliot, 1994). Unfortunately, the complexity of the infrapopulation, component population and suprapopulation structures and the problems of acquiring life table data over several years has made it very difficult to use this approach to resolve this question with certainty in the case of helminth fish parasites.

When discussing this question Kennedy (1977), like many other reviewers, used the Bradley (1974) classification as a framework for discussion. According to this classification populations can theoretically be regulated by: (1) transmission; (2) causing death of the host; (3) host immunity or premunition; and (4) intraspecific or interspecies competition mediated by exploitation of resources, interference or by exploitation of the host's immune response. There is a general consensus that although the transmission rate can influence population size it does not act in a regulatory manner. Regulation by parasite-induced host mortality appears only to occur commonly in nature when the fish is the intermediate host of the parasite (Kennedy, 1977). As the prevalence and abundance of *D. ditremum* plerocercoids was extremely low in the Teifi trout and there was no evidence of parasite-induced mortality the second of Bradley's options can also be excluded. Kennedy (1977) also excluded both the immune response and interspecies competition from involvement in the regulation of parasite infrapopulations in fish. If these assumptions are correct this leaves only intraspecies competition as a regulatory mechanism. According to

Kennedy (1977) this mechanism operates by causing a density-dependent reduction of the natality rate but he concluded that as it is uncommon the majority of fish parasite populations are unregulated and hence unstable. Esch and Fernandes (1993) have also argued that the mortality rate of parasites in most fish living under natural conditions is density independent. This opinion is also shared by Kennedy *et al.* (1986) and they consider that interspecies competition is a far greater structuring force in the more diverse, species-rich helminth communities in the alimentary canals of birds and mammals than in the comparatively poor communities of freshwater fish. These views are reminiscent of those expressed by Andrewartha *et al.* as they also postulated that the population size of some free-living organisms such as thrips and grasshoppers are determined by non-stabilising factors (Ricklefs, 1973). As it is to be expected that such populations would dwindle to extinction rather frequently Ehrlich and Birch (1967) suggested they could be re-established by colonisation from surrounding sub-populations. Thus, populations could be maintained below resource limitation through the balance of local extinction and recolonisation. However, as pointed out by Ricklefs (1973) the tendency for the populations to increase through colonisation is density dependent and the mosaic model differs from the conventional density-dependent model in that the spatial components, immigration and emigration, replace birth and death rates, respectively.

Kennedy and Guegan (1996) and Kennedy and Hartvigsen (2000) have continued to hold the view that population sizes of fish parasites are determined by non-stabilising factors on the basis of their studies on the structure of helminth communities in eels and trout. According to these authors helminth communities in both of these species are very similar and they consider them to be unsaturated stochastic assemblages and isolationist in nature. According to Bush *et al.* (1997) the use of the term isolationist community (Holmes and Price, 1986) implies that the communities are not in equilibrium as they are unsaturated due to low transmission rates and that species are individualistically dispersed and insensitive to the presence of other guild members.

However, more recent research findings in the fields of immunology and parasitology make it necessary to reassess the views expressed by Kennedy (1977) and Kennedy and Hartvigsen (2000). Although the existence of an immune response in fish has been known for some time it is possible that its importance as a regulatory mechanism has been underestimated. Kennedy and Walker (1969) showed that *Caryphyllaeus laticeps* in dace were rejected after an initial period of establishment. The fact that the period of establishment decreased and the speed of rejection increased with a rise in temperature is suggestive of an antibody mechanism of resistance, although at the time they could find no evidence of circulating antibodies. However, in these earlier studies the use of precipitation techniques to detect antibodies was suspect as fish only possess IgM (Wilson and Warr, 1992). More recent studies, using

agglutination and ELISA assays, or enumeration of antibody-secreting cells, are without such concerns and have been used to demonstrate antibody production in response to infestation by acanthocephala, nematodes, monogenea, digenea and cestodes in fish (Secombes and Chappell, 1996). However, there was no correlation between worm burden and antibody titre.

Protective immune responses to helminths have been demonstrated in a number of cases of the more invasive helminth parasites including *Gyrodactylus salaris* and *D. spathecum* (Secombes and Chappell, 1996). It has been postulated that as the gut is relatively non-aggressive immunologically a stable relationship would be more likely to develop in the case of parasites inhabiting the gut than in those inhabiting tissue (Secombes and Chappell, 1996). Nevertheless, there is now accumulating evidence for the existence of intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) in the teleost gut (McMillan and Secombes, 1997). These authors suggest that by furthering our understanding of the basic immunology of the teleost gut information will ultimately be made available for optimisation of oral vaccination regimens. This would involve targeting the immune cells in the intestine for stimulation and the establishment of mucosal, antigen-specific antibody responses both in the gut mucosa and systemically. It must be concluded therefore that as fish have evolved sophisticated immune mechanisms (Secombes and Chappell, 1996) this may enable them to regulate the density of helminth parasites.

There is also a great deal of accumulating evidence, summarised below, suggesting that exploitative and interference competitive mechanisms and cross immunity may be more important in regulating helminth parasites of fish than was believed in the past. Thus, Brown (1986) showed that asymptotic levels were reached when rainbow trout were infected with increasing numbers of *Pomphorhynchus laevis*. He therefore suggested that the direct relationship between worm burden and the length of the fish host was wholly consistent with the action of density-dependent processes within each fish size class. This mechanism, together with interspecific competition, may also be responsible for the observed positive relationship between fish size and parasite load and for the almost constant parasite density per square metre observed in the alimentary canal of trout (Thomas, 1964a). Density dependence regulation due to space limitation has also been invoked to explain the decline in numbers of *L. thecatus* in the green sunfish (Uganski and Nickol, 1982; Ewald and Nickol, 1989), *Pomphorhynchus laevis* in dace (Kennedy, 1975) and *E. crassum* in trout (Kennedy, 1996).

Bates and Kennedy (1990) have produced direct experimental evidence of asymmetrical interspecies competition or ammensalism involving *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in rainbow trout in which the latter species was disadvantaged but not the former. Spatial exclusion of helminth species resulting from active site selection by the dominant

competitor has also been reported in natural populations (Chappell, 1969; Williams *et al.*, 1987). There are also many reports (Dogiel, 1961; Thomas, 1964a; Chappel, 1969; Whitfield, 1979; Kennedy, 1985; Vidal-Martinez and Kennedy, 2000; Kennedy and Hartvigsen, 2000) of significant negative associations involving pairs of helminth parasites in fish hosts. As a result, Kennedy (1992) and Vidal-Martinez and Kennedy (2000) appear to have modified their views recently. They now postulate that competitive interactions may be potential determinants of community structure even in the low-diversity helminth communities found in fish from the temperate and tropical zones, though it is not yet clear to what extent this potential is realised in nature. Kennedy and Hartvigsen (2000) have cited evidence, based on community structure, suggesting that even the relatively low-diversity helminth communities in the intestines of eels and trout may be saturated. According to Srivastava (1999) saturation suggests the action of interspecies competition. Little is known about the nature of the mechanisms that might be implicated in causing the effect observed during intraspecific competition. They may include exploitation of resources, interference and using the host's immune response to the parasite's advantage. Bates and Kennedy (1990) suggested that the most tenable explanation for the exclusion of *A. anguillae* from the gut of rainbow trout by *P. laevis* was the development of a host-mediated exclusion zone around individuals of *P. laevis*. A similar example is provided by *Dactylogyrus vastator*. This parasite induces cellular reactions in the gills of the fish host which prevent settlement by other species (Holmes, 1973).

Although there are also many examples of positive associations (Rohde, 1991; Dorocu *et al.*, 1995) involving species of helminth parasites these can be attributed to the overlapping and overdispersed nature of favourable transmission sites. This results in an increased probability of a fish acquiring more than one species of parasite at the same time. It is important to realise however that positive associations do not preclude the possibility of interspecies competition occurring.

The occurrence of character displacement and niche diversification in coexisting species of parasites can be cited as circumstantial evidence for the existence of strong selection pressures driven by interspecies competition (Thomas, 1958a; MacKenzie and Gibson, 1970; Whitfield, 1979). On the other hand, the existence of niche diversification and the restriction of species to preferred sites has also been cited as evidence that interspecies competition seldom occurs in nature (Kennedy, 1974). However, although character displacement and competition niche diversification will clearly reduce the intensity of interspecies competition this does not exclude the possibility that it may occur. Further research is clearly needed to ascertain the extent to which interspecies competition between helminth parasites of fish may act as a density regulatory mechanism and to ascertain the causative mechanisms.

The above evidence suggests that the use of the word isolationist to describe populations of helminth parasites in trout in the Teifi is unjustified. It would appear, to the contrary, that the helminth parasites of the Teifi trout conform to the term interactive community (Holmes and Price, 1986). This term was coined to characterise a community that fits the assumptions of the competition hypothesis at the infracommunity level. These include the assumptions that the parasites have a high transmission rate, that host immunity and interspecies competition may act in a density-dependent manner and that communities are in equilibrium. Bush *et al.* (1997) concluded that care should be taken in the use of the terms isolationist and interactive community and that further empirical work is required to evaluate their usage.

It can be concluded therefore that the above arguments support the views held by Anderson (1982) on population regulation of helminth parasites. Anderson considered the host's immune response causes a proportional reduction in the establishment, survival and reproduction of helminth parasites following infection and thus acts in a density-dependent manner. As the distribution of the parasites becomes more aggregated the regulatory impact of the density-dependent processes will become more pronounced. Anderson (1982) also considered that competition between parasites might be important as a density-dependent mechanism. He therefore concludes that density-dependent processes acting on infrapopulations regulate macroparasite abundance and that the severity of these processes is a major determinant of the observed stability of many helminth infections despite severe perturbations due to climate or human intervention. Field studies involving the gathering of life table data are needed to further validate these conclusions.

It was suggested by Thomas (1958b) that the absence of any clear evidence of age-related resistance to the parasites of trout and salmon parr is indicative of an old and stable relationship. This implies that the fish hosts and the parasites have evolved effective immune and immunosuppression or immunoavoidance mechanisms, respectively, and that these are in balance. The introduction of novel parasites or new genetic strains of the hosts could therefore be potentially harmful. An example is provided by the emergence of *Gyrodactylus salmonis*, as a pathogen of salmonids, especially in Norway. Using carefully controlled experimental infections, Bakke *et al.* (1990) demonstrated innate and acquired resistance in Baltic (Neva) stocks where the parasite was indigenous whereas the Norwegian stocks were highly susceptible. That the resistance has a genetic basis is also demonstrated by the heterogeneity of the responses to *G. salaris* shown by different races of rainbow trout and salmonids other than Norwegian *S. salar* (Secombes and Chappell, 1996). Care should therefore be taken during stocking of water bodies so as not to alter the genetic composition of the native fish stock or to introduce novel parasites.

# 6. RELATIONSHIPS BETWEEN PARASITE ABUNDANCE AND THE WELLBEING OF THE FISH HOSTS

## 6.1. Introduction

The symbols + ↔ – are generally used to depict host–parasite relationships implying that parasites always harm their hosts while the parasites themselves benefit. Damage suffered by the host may include mechanical injury, tissue changes as a result of the immune response, toxicity due to toxic metabolic endproducts, sterilisation and loss of nutrients and other resources due to competition. Parasites may also act as vectors of other pathogens. As a result the metabolism and behaviour of the host may be impaired and death may result.

Despite these potentially harmful effects host–parasite systems have continued to coexist and coevolve in nature. However, this balance is being disturbed in a world that is constantly being subjected to increasingly frequent anthropogenic influences. As a result epidemics caused by parasites have tended to become more commonplace. It is predictable therefore that parasitology, linked to human and veterinary medicine, and with its focus on the more strongly negative aspects of host–parasite relationships should have emerged as an applied science. As a result of the focus on man-made epidemics population biologists may well have overemphasised the role of parasites in influencing population structure and genetics in natural habitats.

In order to get a more balanced view of host–parasite relationships it is necessary to investigate more cases where the environment has not been unduly disturbed by man. One such opportunity arose when it became possible to investigate the salmonid fish and their helminth parasites in the River Teifi more than 50 years ago. At this time the environment was relatively undisturbed and the Teifi was considered to be one of the best brown trout rivers in the country.

## 6.2. Pathology

Of the salmonid parasites encountered in the present investigation only *N. rutili*, *C. truttae* and *Diphyllobothrium ditremum* were observed to be causing pathological damage to the host tissues. In the case of *N. rutili* damage occurred at the point of attachment in the intestine as a result of the proboscis penetrating the tunica propria, the stratum granulosum and even into the circular muscle coat. Outwardly, a circular layer of hypertrophied tissue around the proboscis indicated parasitism by *N. rutili*. The hyperaemia of the surrounding tissue often spreads to the muscular layers and in some cases causes acute inflammation and metaplasia. Some encapsulated juveniles were

observed in the gut wall of some trout (Thomas, 1964a, c). When the green sunfish, *Lepomis cyanellus*, was lightly parasitised by *N. rutili* it was observed that the parasites penetrated deeply into the intestinal wall and connective tissue developed around the proboscis as in trout (Adelmeguid *et al.*, 1995). However, when infections were heavy (> 50 cystacanths) the parasites evoked goblet cell hyperplasia and the release of substantial quantities of mucus. Under these conditions penetration was shallower and parasites appeared to change their sites of attachment frequently. Adelmeguid *et al.* (1995) suggested that the mucus covering and the presumed presence of antibodies in the mucus combined to create a protective barrier that reduced the number of parasites that could become established. The fact that the abundance of *N. rutili* does not increase with age in the trout may be due to acquired immunity or to dietary changes associated with age.

Adults of *C. truttae* were mostly found in the pyloric caecae of the salmonids where they perforate the mucosa with their peribuccal teeth to feed on tissue or host blood. According to Moravec (1994) any pathogenicity caused by this species is due mainly to mechanical damage and possibly inflammation of the intestinal mucosa. However, Russell (1980) concluded that the rate of food ingestion and growth of rainbow trout were not influenced by *C. truttae* infections except possibly when they were heavy. According to Moravec (1994) other nematodes such as *C. ephemeridarum* may also cause mucosal damage. *Diphyllobothrium ditremum* larvae were found to harm trout by causing adhesions of the peritoneum (Thomas, 1964c).

There is evidence from other studies that some of the other parasites encountered in salmonids in the Teifi may harm their hosts. Thus, in the case of the sanguinivorous parasite, *D. sagittata*, haematological parameters such as haematocrit counts, erythrocyte counts, mean corpuscular haemoglobin and mean cell volumes all showed significant negative correlation with the intensity of infestation (Ronga, 1995). Parasite-induced damage to the gills may cause epithelial lesions, necrosis and mucus secretion. As a result the osmoregulatory and respiratory mechanisms may be impaired.

There are also reports of free-living gut trematodes being pathogenic. Thus, Davis (1953) observed that mass infestations of trout with *C. farionis* might cause general inflammation of the alimentary canal. Although there is no evidence that *P. simile* is pathogenic it has been reported that the congeneric species *P. lysteri* may cause nephric duct lesions (Hoffman, 1998). In the case of nematodes Moravec (1994) and Hoffman (1998) have summarised the evidence that *Raphidascaris acus*, *C. ephemeridarum* and *Capillaria* spp. may be pathogenic to fish.

It is significant that it is helminth parasites inhabiting tissues such as the eye (*Diplostomum*), the blood system (*Sanguinicola*) and the perivisceral cavity of fish (*Diphyllobothrium* and *Ligula*) that are classed as being amongst the most pathogenic of fish parasites (Pike and Lewis, 1994).

There are also reports of fish mortalities being possibly caused by some of the helminths encountered in the present study including *D. sagittata* (Ronga, 1995), *C. farionis* (Hoffman, 1998), *D. ditremum* (Hickey and Harris, 1947; Chubb, 1980; Hoole, 1994), *Raphidascaris acus*, *C. truttae* and *Capillaria* spp. (Moravec, 1994; Hammar, 2000). However, these parasites appeared not to cause severe host reaction or mortality among the salmonids in the River Teifi.

These results suggest that the salmonids in the River Teifi have evolved mechanisms to prevent hyperinfection and mortality. One of the most important of these is the immune response. Teleost fish are highly competent immunologically, both in innate immunity and in the expression of the combinatorial immune system. When stimulated by antigens they produce antibodies of high specificity and affinity by a process of collaboration between T cells, B cells and antigen presenting cells (Bernstein *et al.*, 1998). They differ, however, from mammals in two important respects. Firstly, they lack lymph nodes and a second class of immunoglobulins comparable to IgG and the mechanism of mutation correlated with the class shift. As a result they cannot mount a secondary immune response involving a switch to a different class of antibodies showing higher affinity for the antigen. Secondly, lower ambient temperatures can result in a longer rejection time compared with mammals. However, fish have excellent immunological memories as evidenced by the fact that they can be successfully vaccinated against particular parasites (Richards and Chubb 1996; Woo, 1997; Bernstein *et al.*, 1998). The immune response has also been invoked to explain the apparent rejection of parasites and resistance to reinfection in wild fish populations (Kennedy and Walker, 1969).

As the immune response is genetically determined host resistance to parasitism can develop by natural or artificial selection. It follows therefore that as the wild trout, salmon parr and their parasites have coevolved in the River Teifi for many generations it is to be expected that they will have acquired a measure of immunity. This explains the absence of host mortality and parasite epidemics. It follows therefore that there is a danger in stocking rivers with new strains of salmonid fish, as they are likely to have a lower resistance to the endemic parasites than the indigenous race. In addition the stocked fish may also introduce novel parasites to which the resident fish might have a low resistance. Fevolden *et al.* (1993) give experimental evidence showing that strains of salmonid species vary in their responses to stress and disease.

## 6.3. Statistical Analyses of the Relationships between Parasite Abundance and the Condition Factor and Adipose Index

As the regression equation, based on a plot of $\log_e$ weight against $\log_e$ length for trout, was $y = -4.17973 + 2.87876x$ the value of 2.87876 was used to

calculate the condition factor ($W/L^{2.87876}$).100, where $W$ = the weight in grams and $L$ = the length in cm. Figure 8 shows that the condition factor varies significantly ($P < 0.001$) on a seasonal basis with the values rising from April until August or September after winter lows and then declining during the spawning and post-spawning period from October to December. Linear regressions, in which the $\log_{10}(1+x)$ transformed values for numbers of parasites were plotted on the $y$-axis against values for condition factors on the $x$-axis. These reveal that with the exception of *N. rutili* the condition factor tends to decline significantly ($P < 0.001$ in all cases) as the number of parasites increases (Table 18). The overall tendency for condition factor to decline with increase in the total number of parasites is shown in Figure 14a. In contrast, the condition factor increases significantly ($P < 0.002$) as numbers of *N. rutili* increase. However, the adipose index was significantly positively correlated with the transformed values for the numbers of *D. sagittata*, *C. metoecus*, nematodes and total parasites (Table 19). As might be expected the adipose index was significantly positively correlated with the condition factor ($y = 10.5 + 0.135\,x$, $P < 0.001$).

*Table 18* The regression equations relating to the transformed number of parasites ($\log_{10}(1 + x)$) to the condition factor (CF) of the brown trout

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = 1.130 – 0.085 CF | 31.88 | < 0.001 |
| Numbers (tr.) of *C. farionis* = 0.867 – 0.062 CF | 17.94 | < 0.001 |
| Numbers (tr.) of *C. metoecus* = 8.78 – 0.622 CF | 178.06 | < 0.001 |
| Numbers (tr.) of *P. simile* = 0.867 – 0.059 CF | 10.98 | < 0.001 |
| Numbers (tr.) of *N. rutili* = 0.131 + 0.127 CF | 10.12 | < 0.002 |
| Numbers (tr.) nematodes = 2.54 – 0.153 CF | 20.83 | < 0.001 |
| Numbers (tr.) of total parasites = 14.3 – 0.865 CF | 85.31 | < 0.001 |

*Table 19* The regression equations showing the relationships between the transformed number of parasites ($\log_{10}(1 + x)$) and the adipose index (AI) of trout

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = 0.0797 + 0.0713 AI | 11.99 | 0.001 |
| Numbers (tr.) of *C. farionis* = 0.130 + 0.0335 AI | 2.77 | 0.096 |
| Numbers (tr.) of *C. metoecus*= 0.1.76 + 0.170 AI | 6.35 | 0.012 |
| Numbers (tr.) of *P. simile* = 0.188 + 0.0204 AI | 0.70 | 0.402 |
| Numbers (tr.) of *N. rutili* = 1.36 + 0.0682 AI | 1.58 | 0.209 |
| Numbers (tr.) of nematodes = 0.420 + 0.246 AI | 29.53 | 0.000 |
| Numbers (tr.) of all parasites = 3.95 + 0.606 AI | 22.09 | 0.000 |

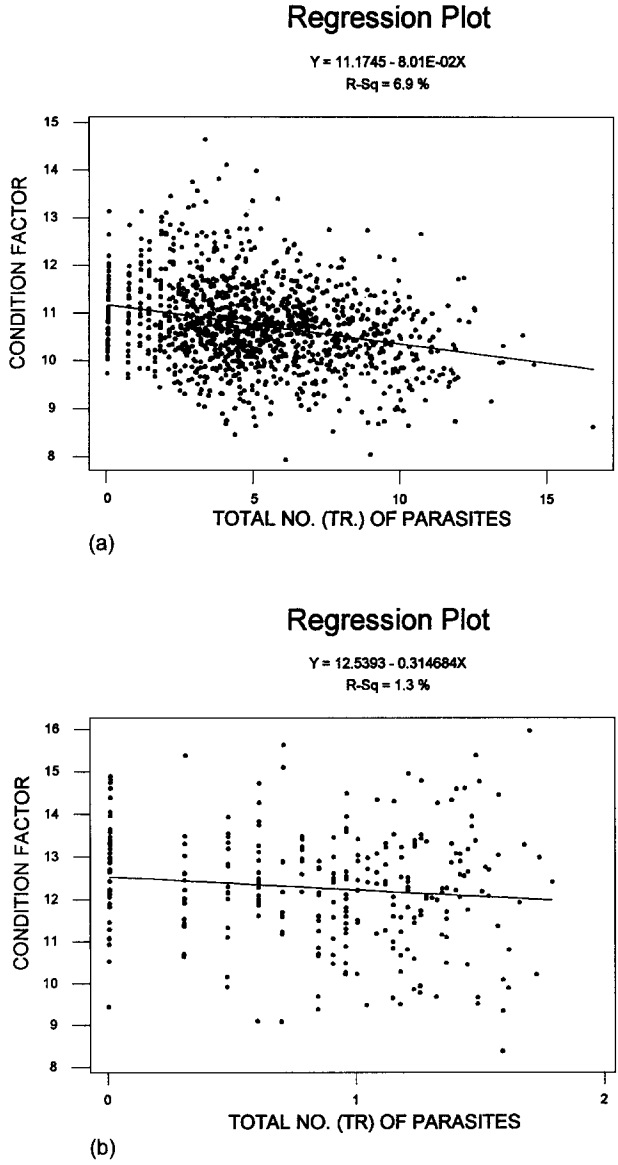


*Figure 14* The relationship between the condition factor of the trout (a) and the salmon parr (b) and the total parasite abundance (transformed $\log_{10} 1+x$).

As the regression equation, based on a plot of $\log_e$ weight against $\log_e$ length for the salmon parr was $y = -3.79918 + 2.76135x$ the value of 2.76135 was used to calculate the condition factor $(W/L^{2.76135}).100$ where $W$ = the weight in grams and $L$ = the length in cm. Unlike the brown trout the condition factor showed no significant seasonal variation although the mean values tended to be slightly higher and more variable in January and March (Figure 9). Regression analyses, where the $\log_{10}(1 + x)$ transformed values for parasite numbers are plotted on the $y$-axis against values for condition factors on the $x$-axis were undertaken. These reveal that with the exception of *N. rutili*, the condition factor tends to decline significantly with increases in numbers of *C. farionis*, *C. metoecus*, nematodes and the total parasites ($P = 0.008$, $< 0.001$, $< 0.001$ and 0.051, respectively) as the number of parasites increase (Table 20). In contrast, the condition factor tends to increase as numbers of *N. rutili* increase although this trend is not statistically significant. The tendency for the condition factor of the salmon parr to decline with increase in the total number of parasites is shown in Figure 14b. In contrast, the regression equations relating the adipose index to the transformed numbers of *N. rutili* and the total numbers of parasites indicate significant positive relationships (Table 21).

*Table 20* The regression equations relating the transformed numbers of parasites ($\log_{10}(1 + x)$) to the condition factor (CF) of the salmon parr

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = 0.0443 – 0.00193 CF | 0.12 | 0.728 |
| Numbers (tr.) of *C. farionis* = 0.619 – 0.0400 CF | 7.12 | 0.008 |
| Numbers (tr.) of *C. metoecus* = 2.85 – 0.208 CF | 56.67 | 0.000 |
| Numbers (tr.) of *N. rutili* = 1.50 + 0.0194 CF | 0.15 | 0.699 |
| Numbers (tr.) of nematodes = 1.20 – 0.0819 CF | 15.07 | 0.000 |
| Numbers (tr.) of total parasites = 1.35 – 0.0420 CF | 3.83 | 0.051 |

*Table 21* The regression equations relating the transformed numbers of parasites ($\log_{10}(1 + x)$) to the adipose index (AI) of the salmon parr

| Regression equations | *F* | *P* |
|---|---|---|
| Numbers (tr.) of *D. sagittata* = –0.0221 + 0.0195 AI | 2.59 | 0.109 |
| Numbers (tr.) of *C. farionis* = 0.134 – 0.0033 AI | 0.01 | 0.921 |
| Numbers (tr.) of *C. metoecus* = 0.547 – 0.116 AI | 3.06 | 0.081 |
| Numbers (tr.) of *N. rutili* = 1.09 + 0.297 AI | 7.37 | 0.007 |
| Numbers (tr.) of nematodes = 0.185 + 0.0021 AI | 0.00 | 0.965 |
| Numbers (tr.) of total parasites = 0.591 + 0.113 AI | 5.70 | 0.018 |

In view of the complexity of the interactions that might influence the condition factor the relationships were also investigated by means of multiple regression analyses. The results for trout (Table 22) show that it is mainly stations, seasons, age and sex of the trout that predict the condition factor values. These account for 5.4%, 79.1%, 14.1% and 1.3%, respectively, of the total variance, making a total of 99.96%, when the transformed total number of parasites is included in the equation. In contrast, the latter fail to make a significant contribution to the total variance ($P$ value = 0.541) and account for only 0.04% of the total. When the individual parasite taxa are included in the equation (Table 22) only one, namely *C. metoecus*, has a significant predictive value ($P = 0.007$). In numerical terms this is by far the most abundant species of parasite. It is noteworthy that the numbers of this species show a significant positive correlation with the values for condition factor. Nevertheless, the contributions to the total variance made by the six parasitic taxa as a whole and by *C. metoecus* in particular are only 1.62% and 0.88%, respectively.

The results for the salmon parr show similar trends to those for the trout (Table 23). Thus, stations, seasons and age are the major predictors of condition factor, as they contribute 18.14%, 78.14% and 2.44%, respectively, to the total variance compared with the non-significant ($P = 0.236$) contribution of only 0.69% made by the total parasites (Table 23). When the individual parasite taxa are included in the equation only the numbers of the most abundant parasitic taxon, *N. rutili*, correlated significantly ($P = 0.013$) with the values for condition factor (Table 23). As was the case with the trout it is of interest that this correlation is also positive. However, the contribution made to the total variance by the five parasitic taxa as a whole and by *N. rutili* in particular are only 2.86% and 1.75%, respectively, compared with 18.14%, 76.9% and 2.44% made by station, season and age, respectively.

When interpreting the possible impact of the parasites on the condition factors and adipose indices of the trout and salmon parr it is necessary to consider the interactions between these and other parameters including station, season, size and age of the fish. The significant negative coefficients obtained for the linear regressions relating condition factor of the trout to the numbers of parasites in the various taxa, except for *N. rutili*, suggest that the increase in numbers of parasites might be the causal factor responsible for the decline in the condition factor. However, this conclusion is invalidated by the fact that the condition factor of the trout and the numbers of all the parasite taxa, except for *N. rutili*, show different seasonal trends. Thus, the condition factor is maximal in August and September (Figure 8) at a time when the abundances of the parasite taxa, except for *N. rutili*, tend to be minimal.

The multiple regression analyses show that the trout's condition factor is predicted mainly by season, age, station and sex, accounting for 79.1%, 14.1%,

*Table 22* The results of multiple regression analyses of the condition factor of the brown trout as the response and stations, seasons, sex, age and parasite as the predictors

| Predictors | Coefficients | S.D. | *P* | % Total variance | |
|---|---|---|---|---|---|
| Constant | 10.834 | 0.114 | 0.000 | | |
| Stations (3) | –0.024 – (–0.197) | 0.062–0.072 | 0.000 | 5.35 | (5.44) |
| Months (12) | –0.304 – 1.566 | 0.119–0.115 | 0.000 | 77.84 | (79.06) |
| Age | –0.234 | 0.027 | 0.000 | 13.8 | (14.12) |
| Sex | 0.142 | 0.040 | 0.001 | 1.30 | (1.35) |
| *D. sagittata* | –0.051 | 0.046 | 0.268 | 0.12 | |
| *C. farionis* | –0.050 | 0.047 | 0.283 | 0.05 | |
| *C. metoecus* | 0.052 | 0.019 | 0.007 | 0.88 | |
| *P. simile* | –0.043 | 0.039 | 0.263 | 0.14 | |
| *N. rutili* | 0.034 | 0.023 | 0.133 | 0.20 | |
| Nematodes | –0.038 | 0.026 | 0.145 | 0.23 | |
| (Total parasites) | (0.006) | (0.010) | (0.541) | | (0.04) |

The values for total parasites are given in parentheses. (Note that the percentage of the variance accounted for by each factor is conditional on the factors entered earlier in the table.)

R.Sq. = 44.1%; D.F. = 21; $F = 42.83$; $P = \leq 0.001$

*Table 23* The results of multiple regression analyses of the condition factor of the salmon parr as the response and stations, seasons, sex, age and parasite as the predictors

| Predictors | Coefficients | S.D. | *P* | % Total variance | |
|---|---|---|---|---|---|
| Constant | 16.617 | 0.955 | 0.000 | | |
| Stations (2) | 0.309 | 0.064 | 0.000 | 18.14 | (18.14) |
| Months (12) | –1.338, – 4.333 | 0.488–0.680 | 0.050–0.000 | 76.90 | (78.63) |
| Age | 1.055 | 0.348 | 0.003 | 2.44 | (2.44) |
| Sex | –0.068 | 0.220 | 0.756 | 0.10 | (0.10) |
| *D. sagittata* | 0.137 | 0.874 | 0.876 | 0.01 | |
| *C. farionis* | –0.407 | 0.322 | 0.207 | 0.50 | |
| *C. metoecus* | –0.244 | 0.181 | 0.178 | 0.49 | |
| *N. rutili* | 0.247 | 0.099 | 0.013 | 1.75 | |
| Nematodes | –0.138 | 0.238 | 0.563 | 0.10 | |
| (Total parasites) | (0.354) | (0.236) | (0.134) | | (0.69) |

The values for total parasites are given in parentheses. (Note that the percentage of the variance accounted for by each factor is conditional on the factors entered earlier in the table.)

RSq. = 55.6%; D.F. =18; $F$ =18.75; $P = \leq 0.001$.

5.4% and 1.3% of the total variance, respectively, compared with only a non-significant contribution of 0.04% made by the total parasites. However when individual parasitic taxa are substituted for total parasites in the multiple regression equation only one, namely *C. metoecus*, the most abundant parasite, is significantly predictive of the condition factor. The fact that the numbers of

*C. metoecus* are significantly positively correlated with condition factor of the trout suggests that this parasite may not adversely affect the condition factor of its host. The following observations also imply that this conclusion can be extended to other parasites. Firstly, in the case of *N. rutili* there was a significant positive relationship between the numbers of this parasite and the condition factor. This conclusion is valid as, unlike the other parasites, *N. rutili* abundance values are independent of host age or season. Secondly, the adipose index was significantly positively correlated with numbers of *D. sagittata*, *C. metoecus*, nematodes and total parasites.

The results for salmon parr are in accord with those for trout. Firstly, linear regression analyses showed that there was a significant positive relationship between the numbers of *N. rutili* and the condition factor of salmon-parr. As explained above for trout, linear regression analyses are only valid for *N. rutili* because numbers of this species are independent of age and season, unlike the other parasite species. Secondly, multiple regression analysis shows that the condition factor of the salmon parr is predicted mainly by season, station and age. These factors account for 78.6%, 18.1%, and 2.4% of the total variance, respectively, compared with only a non-significant contribution of 0.7% made by the total parasites. When individual parasitic taxa are substituted for total parasites in the multiple regression equation only one, namely *N. rutili*, the most abundant parasite, was a significant predictor of the condition factor. As with trout the numbers of the most abundant parasite of the salmon parr were positively correlated with the condition factor. Secondly, the adipose index of the salmon parr was significantly positively correlated with numbers of *N. rutili* and total parasites. It must be concluded therefore that these statistical analyses provide no evidence that the helminth parasites of either the brown trout or salmon parr affect the wellbeing of their hosts as measured by the condition factor and adipose index. To the contrary the results indicate that there are significant positive relationships between parasite abundance and the condition factors and adipose indices of both trout and salmon parr.

Two possible reasons can be advanced to explain these results. Firstly, they may be caused by behavioural differences. It has long been known that salmonid fish populations are strongly hierarchical in their behaviour (Elliott, 1994; Armstrong *et al.*, 1999). As a result it is to be expected that the dominant, more aggressive fish, with higher androgen levels (Pottinger and Carrick, 2000), would tend to occupy the optimal home ranges. It can be postulated that such home ranges would provide the fish with the best refuges from predators and fast currents in times of spate and also the best opportunities for obtaining high-quality food. Although salmonid fish need a variety of habitats in their home ranges including pools, fast reaches, riffles and the sheltered habitats with depositing sediments on the edge of currents, the latter may be ranked as the most important. It was found that these depositing sediments are the preferred microhabitats

of ammocoete larvae (the intermediate hosts of *C. truttae*), *Sphaerium corneum* (the intermediate hosts of *P. simile*), *Lymnaea peregra* or *Pisidium* spp. (the intermediate hosts of *C. metoecus*) and ostracods (the intermediate hosts of *N. rutili*) (Thomas, 1964c). It can therefore be postulated that the dominant trout would tend to eat proportionately more infective larvae than the subordinate trout not only because of their larger size but also because they would tend to spend more time in microhabitats with depositing sediments. Although there is more overlap in the home ranges of salmon parr than brown trout (Armstrong *et al.*, 1999) the same arguments can be applied. It may be suggested therefore that the positive correlation between the condition factor and abundance of *N. rutili* in salmon parr may be due to the dominant, better-conditioned fish spending more time in habitats with depositing sediment favoured by ostracods and *Sialis* larvae which are the intermediate hosts of *N. rutili*.

It might also be postulated that as the dominant, larger trout tend to spend proportionately more time in microhabitats with depositing sediment than their subordinates they would also tend to become more heavily parasitised with monogenetic parasites. The rationale for this argument is that the eggs of *D. sagittata* would tend to aggregate in depositing sediments with detritus. These eggs hatch out at night to produce infective oncomiracidia at a time when the trout are resting (Gannicott and Tinsley, 1997). It would be predicted therefore that the abundance of *D. sagittata* would be positively correlated with size and condition factor of the trout. The results support this hypothesis as the abundance of *D. sagittata* was significantly correlated with the size of trout and salmon parr. However, multiple regression analyses showed that the relationships between the condition factors of the salmonid species and the abundance of this species were non-significant (Tables 22 and 23).

Secondly, the other possibility is that the parasites may help to promote somatic growth of the salmonid fish by providing them with nutritional benefits. As the parasites respire anaerobically the hosts may sequester the amino acids and carboxylic acids, including lactic, succinic and short-chain $C_2$, $C_3$ and $C_4$ acids released by the parasites as end products of their metabolism. Parasites may also benefit the hosts by releasing digestive enzymes, acting as sinks for potentially toxic heavy metals (Sures and Sidall, 1999) and by competitively excluding perhaps potentially more harmful parasites. However, it seems probable that on balance the nutritional disadvantages of parasitism to the host would outweigh the advantages.

There is also evidence that some parasites have evolved mechanisms to enhance the somatic growth of the host. Although this might appear to be to the host's advantage this is not the case as the parasite also inhibits the reproductive growth of the host. As a result the parasites benefit from additional space and energy resulting from the enhanced somatic growth of the host by the more efficient channelling of energy for their own growth and reproduction. A

well-documented example of such cases is provided by a bird schistosome species that promotes somatic growth and inhibit reproductive growth of *Lymnaea stagnalis* (de Jong-Brink, 1995). According to de Jong-Brink (1995) the parasite achieves this by releasing a cercarial factor that causes schistosomin, a cytokine-like factor, to be released by the haematocytes and connective tissue cells of the host's immune system. The schistosomin then binds to cells of the neuro-endocrine regulatory system thus inhibiting the development of the reproductive system of the host. It thus appears that the parasites have evolved survival strategies that enable them to benefit from the stress response that they evoke in their host. According to Eckert (1991) the plerocercoids of *Spirometra mansonoides* use a more direct approach as they apparently enhance the growth of rodents by producing a substance which mimics the action of mammalian growth hormone.

Plerocercoids, such as those of *Ligula*, can result in high parasitisation indices (weight of parasite/weight of parasite + host). According to Hoole (1994), the literature dealing with the effects of ligulosis on fish growth and general condition is inconclusive although the reduction of gonadal development in ligulosed fish is well documented. There is evidence that it is associated with alterations in the cellular composition of the mesoadenohypophysis region of the pituitary gland. However, the biochemical mechanism involved remains to be elucidated. In this connection it would be interesting to ascertain whether the parasite is capable of manipulating the host's immune and neuro-endocrine systems as is the case with schistosomes in *Lymnaea stagnalis*.

In the case of the salmonids in the present case study further research is needed to explain the positive relationships between the abundance of certain parasites and the condition factors and adipose indices of the fish. Two hypotheses need to be tested. The first postulates that the relationship is attributable to socially determined differences in fish behaviour whereas the second postulates that it is due to parasite-induced growth enhancement. Much work clearly remains to be done to elucidate how the parasites of fish and other animals can manipulate the host's immune and neuro-endocrine system for their own benefit. The possibility that parasites may also inhibit reproduction of their fish host as well as promoting growth should also be investigated. Birds have been very well studied in this respect and the negative effects of parasites on a wide range of their fitness components, including timing of reproduction, brood size, offspring size and number of clutches have been recently reviewed (Møller, 1997). There is evidence that fish may also incur more subtle reproductive costs from parasites as the latter may adversely affect their sexual behaviour (McLennan and Shires, 1995). However Hamilton and Poulin (1995) and Stott and Poulin (1996) could find no evidence that parasite load had any influence on male aggression or parental care in male upland bullies, *Gobiomorphus breviceps*. They suggest that these differences are attributable to these fish occupying harsh, unpredictable environments.

## 6.4. Reasons for the Absence of Harmful Effects due to Parasites

The statistical analyses carried out failed to produce any evidence that the parasites had a detrimental effect on either the salmon parr or the trout in the Teifi. This situation is not unique. Thus, Chubb (1973) states that 'most British freshwaters are not troubled with regular parasite problems, although parasites are always present in abundance'. Following an extensive long-term study in North America Lemly and Esch (1985) also concluded that mortality of centrarchid fish due to infection by *Uvulifer ambloplitis* was a rare event occurring only in one of 43 aquatic habitats studied. Other authors have also cited a number of case studies supporting the view that parasite infections are generally relatively harmless to their long-term fish hosts when living under natural conditions (Allison, 1982; Holmes, 1982; Crompton, 1991; Dorocu *et al.*, 1995; Bakke and Harrris, 1998; Scholz, 1999). It can be postulated that these apparently benign relationships between host and parasites in natural ecosystems have evolved because it is disadvantageous for the parasite to kill the definitive host in which it is reproducing. The host may also derive some metabolic and other benefits from benign parasites as mentioned above. This concept can be rationalised by reference to $R_0$, the basic reproductive rate of the parasite which natural selection will tend to maximise. Maximising $R_0$ will involve trade-offs between production of transmission stages (which ideally should be high) and damage to the host (which ideally should be small). $R_0$ will therefore be clearly maximised by the host surviving as long as possible. It can be postulated therefore that as a result of long-term coevolution the virulence of the parasite to its host will become attenuated and that the parasite will become adapted to the immune defence of the host and even use it to its own advantage.

F. Thomas *et al.* (2000) have recently stressed the role of the environment in determining the outcome of host–parasite interactions. They cite some cases where the parasites can be detrimental to host fitness in one environment but beneficial in another and also review other cases where parasitised individuals enjoy a selective advantage over unparasitised conspecifics.

## 6.5. Reasons for some Parasites Causing Detrimental Effects

The following reasons can be advanced to explain why parasites may become more pathogenic and reduce the longevity of their hosts under certain circumstances. Firstly, selective pressure will favour parasites modifying the behaviour of the intermediate host if this results in increasing the probability of it being eaten by the definitive host. In such cases it may be said that the parasite gene is being phenotypically expressed in the host's body and Dawkins (1990) gives many examples of this phenomenon. In this case, the intermediate hosts are acting as vehicles for transmission, rather than for reproduction.

However, timing is critical as development must be completed first. It would be clearly disadvantageous for the parasite to kill its host prematurely. Examples of such larval parasites, which inhabit parenteral organs, include *Diplostomum* spp., metacercariae of *Uvulifer ambloplitis* and the metacestode larvae of *Diphyllobothrium ditremum* and *Triaenophorus crassus*. The former may cause blindness whilst the latter may reduce host activity. However, even in these cases there is evidence that the parasites may not be highly detrimental to the host. Thus, Kennedy (1983) concluded that the empirical evidence suggested that diplostomiasis has little or any significant impact on natural fish populations. Rather surprisingly Rahkonen and Kosti (1997) found that there was a slight, positive correlation between the numbers of *D. ditremum* larvae in muscle and the condition factor for each age group of brown trout examined in Lake Inari, Finland. This was the case despite the presence of severe chronic granulomatous peritonitis associated with the parasites. There was no evidence that heavily parasitised fish were being eliminated from the population. Pukkinen and Valtonen (1999) came to a similar conclusion when studying the effects of *Triaenophorus crassus* larvae on the whitefish *Coregonus laveratus*. These results suggest that long-term coevolution had occurred in these cases.

Secondly, parasites inhabiting tissues of organ systems other than the gut tend to be more pathogenic than gut-inhabiting forms because they directly utilise and damage host tissue thus generating a stronger immune response. It is not surprising therefore that it is parasites living on the eye (*Diplostomum*), in the blood system (*Sanguinicola*), the body cavity and viscera (tapeworm larvae such as *Ligula*, *Schistocephalus* and *Diphyllobothrium*), the swim bladder (*Anguillicola crassus*) and gills (*Gyrodactylus salaris* and sea lice) which occupied pride of place in a recent symposium dealing with parasitic diseases of fish (Pike and Lewis, 1994).

Thirdly, parasites may be pathogenic because of their novelty to their fish hosts. When novel species or genetic strains of parasites are introduced into a new area susceptible endemic hosts may suffer mortality because they have low levels of resistance or immunity to the parasites. Examples of cases where parasites introduced in farmed fish have caused parasite epidemics in wild or farmed fish are given by Fryer (1968), Kennedy and Fitch (1990), Bauer (1991), Williams and Jones (1994), Kennedy (1994) and Hoffman (1998). According to Kennedy (1994) *Anguillicola crassus* is not pathogenic to its coevolved host, *Anguilla japonicum*, in Japan but became pathogenic to *Anguilla anguilla* following its anthropogenic introduction into eel populations in continental Europe and Britain. Similarly, *Gyrodactylus salmonis* appears not to have been pathogenic to salmonids in the Baltic seaboard but following its introduction into Norway by infected fish from Sweden it has caused devastating epidemics in salmon parr populations in at least 37 Norwegian rivers (Mo, 1994). However, changes in the genetic composition

of the fish (Johnsen and Jensen, 1991) and environmental changes in the river systems may have been contributory factors in causing the epidemics (Heggberget and Johnsen, 1982). These examples can be extended to other taxa including birds as Dobson and McCallum (1997) have shown how parasites from bird introductions can place rare, threatened native bird species under increased threat. The ever-increasing dangers of fish parasites, including the cestode *Bothricephalus acheilognathi*, spreading globally due to inadequate control of fish importations have again been stressed recently by Scholz (1999).

In endemic areas where the hosts and parasites have coevolved over long periods newly introduced populations of a susceptible host species may suffer high mortality because they have a lower resistance or immunity to the endemic parasites.

Fourthly, parasite species may become more pathogenic and cause mortality if the host populations are placed in a new environment that is highly favourable to transmission. Examples are provided by fish farms and small, closed systems such as ponds or small lakes. There are numerous reports of epidemics and fish mortalities when host fish are living at high densities under these conditions (Dogiel, 1961; Moravec, 1994; Ronga 1995; Hoffman, 1998; Scholz 1999, Rahkonen *et al.*, 1996). An analogous example is provided by *Trichostrongylus tenuis* which is known to be pathogenic to red grouse in wet moors but is of little importance in dry moors where the survival of the infective larvae is low (Dobson and McCallum, 1997).

There is also some evidence that parasites may have deleterious effects on fish in harsh natural ecosystems. Thus, Pukkinen and Valtonen (1999) claim that back calculations revealed that, amongst the older whitefish, the smaller ones harboured more parasites than the larger ones. Hoffman *et al.* (1986) also found that there was an inverse relationship between the condition factor, packed cell volume, red blood cell count and haemoglobin level and the numbers of cestodes (*Eubothrium salvelini*) in the arctic charr. However, the white blood cell count increased with increasing parasite intensity indicating the development of immunity. These authors concluded that the decline in condition factor might be caused by competition for food rather than by parasites. The environment being highly oligotrophic exacerbated this. Ayala *et al.* (1992) also claim that *Neochinorhynchus cylindratus* in the large-mouth bass, *Micropterus salmoides*, may harm their hosts by lowering protein digestibility and amino acid availability.

Fifthly, parasites can become more harmful to the host as a result of damage to the innate and adaptive arm of the immune system caused by detrimental changes in the environment. Examples are provided by low water temperature (Chubb, 1982; Hardie *et al.*, 1994; LeMorvan *et al.*, 1998; Bernstein *et al.*, 1998), specific pollutants including heavy metals (Dunier, 1996) and ammonia (Hurvitz *et al.*, 1997), general habitat degradation (Bakke and Harrris, 1998)

and stress due to overcrowding or starvation (Waagbo *et al.*, 1994). The immune response may also be suppressed by reproductive activity (Møller, 1997). However, according to Demers and Bayne (1997) although chronic stress is immunosuppressive acute stress may help enhance both cellular and humoral components of innate defences in rainbow trout. It may also be suggested that immunosuppression resulting from lowering in temperature, reproductive activity and changes in diet may be responsible for seasonal changes in parasite numbers.

## 6.6. Conclusions

Despite the fact that some of the helminth parasites encountered damaged the tissues of their salmonid hosts there was no evidence that they caused mortality or that they had a detrimental effect on the growth of their hosts. To the contrary it was found that parasite abundance was significantly correlated with the condition factor and adipose index in some cases. Further research is needed to test the following hypotheses to ascertain the possible mechanisms that might explain these positive relationships. Firstly, they may be due to the hierarchical behavioural patterns of salmonid fish. As a result the larger, dominant fish tend to ingest more infected intermediate hosts because they spend more time in the transmission sites which are the sediment-depositing microhabitats favoured by the intermediate hosts. Secondly, it is postulated that the positive relationships may be due to the parasites manipulating the immune and neuroendocrine system of the hosts to stimulate their somatic growth and inhibit reproduction. It is argued that these apparently benign relationships between the helminth parasites and their salmonid hosts have evolved because it is disadvantageous for the parasite to kill the host on which its biological fitness depends. Further work is also needed to develop a better understanding of the benefits that the host may derive from its parasite. Host–parasite relationships cannot be adequately described by the simple + and – signs. It may be suggested that the apparently benign relationships between parasites and salmonid fish in the Teifi are attributable to the fact that hosts and parasites have coevolved at least since the last ice age. However, the evidence suggests that this delicate balance could be easily upset by anthropogenic influences including the introduction of new genetic strains of either hosts or parasites during stocking or by habitat destruction and pollution. It is probably true to say that most of the major disease epidemics in the world today have been caused by inadvertent human intervention.

The medical and veterinary importance of persistent epidemics in anthropogenically disturbed environments may have caused parasitologists to develop an exaggerated view of the harmful effects caused by parasites. In order to obtain a more balanced view further work to assess the impact of

parasites on host evolution in natural populations is therefore indicated. In this connection it is of interest that a recent study on the impact of blood parasites on bird species did not support a role for parasites in the overall maintenance of genetic variation via frequency-dependent selection (Poulin *et al.*, 2000).

## 7. SEX BIAS IN PARASITISM AND CAUSATIVE MECHANISMS

### 7.1. Introduction

The question of whether there is a sex bias in parasitism is important in view of its relevance to the widely cited Hamilton–Zuk (Hamilton and Zuk, 1982) and immunocompetence (Folstad and Karter, 1992; Zuk 1992; Hillgarth and Wingfield, 1997) hypotheses. The Hamilton–Zuk hypothesis is based on the following assumptions:

1. A set of parasites will adversely affect host fitness and susceptibility to parasites is inheritable.
2. The expression of secondary sexual characters is partly dependent on host condition and therefore on parasite resistance genes. Hamilton and Zuk argued therefore that elaborate male secondary sexual characters have evolved through female choice because they provide 'honest' information about heritable resistance to parasites.
3. Females are able to evolve a discriminatory preference for secondary sexual characters that reveal genetic susceptibility to parasitism.

Some of the main assumptions and predictions based on the immunocompetence and Hamilton–Zuk hypothesis are as follows:

1. Male vertebrates will be more vulnerable to infection than females because testosterone is immunosuppressive.
2. Male animals that need high testosterone levels to produce secondary sexual characters will be more vulnerable to parasitism.
3. Species harbouring harmful parasites over their evolutionary history will be more likely to evolve extravagant sexually dimorphic traits (Schall and Staats, 1997).
4. When species with extravagant sexually dimorphic traits are compared with others with more subdued dimorphic traits the males in the former case will be more heavily parasitised.
5. Infections will reduce testosterone levels thus causing secondary sexual characters to be poorly expressed.

These predictions will be examined sequentially below with reference to the present and related data sets.

## 7.2. An Evaluation of the Hamilton–Zuk and Immunocompetence Hypotheses

*Male vertebrates will be more vulnerable to infection than females*

Many authors have accepted this prediction as being gospel not only on the grounds that testosterone is immunosuppressive but also because competition for mates is more energy demanding and more stressful for males than females (Herbert and Cohen, 1993). For example the following statements are commonly encountered in the literature: 'males of many species are more susceptible than females to infection by parasites' (Klein, 2000a) and 'males generally exhibit reduced immune responses as well as increased intensity and prevalence of infections compared to female conspecifics' (Klein, 2000b). Poulin (1996) has even claimed that helminth parasites are also slightly larger in male hosts than in females as well as being more abundant.

However, the present data do not support the first prediction as there were strong, statistically significant female-biased susceptibilities to helminth infection. Thus, a comparison of the percentage prevalence values for the parasites of male and female trout, using the $\chi^2$ test, shows that the prevalence values for *P. simile* and nematodes are significantly higher in female than in male trout (Table 24). The mean abundance values for the various parasitic taxa and the total parasites were also higher in female trout than the corresponding values for the males in all cases (Table 25, Figure 15). However, these differences were only statistically different in the cases of *P. simile*, nematodes and the total numbers of parasites when the transformed data were analysed using either analyses of variance or the Mann Whitney test. Subsequent comparisons of the intensities of infection in male and female trout of different ages, using Mann Whitney tests, revealed that the abundance values were significantly higher only when the trout were sexually mature and over 3 years old. During the spawning and post-spawning periods (October–December and January–March, respectively) the mean abundance values for *N. rutili* and *P. simile* in sexually mature (3 years +) female trout were significantly higher ($P < 0.05$) than for male trout of the same age.

Multiple regression analyses revealed that the sex of the trout had significant effects on the total number of parasites and the numbers of *P. simile* and *C. metoecus* found ($P < 0.001$, $P < 0.007$and $P < 0.005$, respectively). In all cases the bias was in favour of the female trout. However, the contributions made by the total number of parasites, *P. simile* and *C. metoecus* to the total

*Table 24* Comparisons of the percentage prevalence of the parasites in male (M) and female (F) brown trout using $\chi^2$

| | *D. sagittata* | | *C. farionis* | | *C. metoecus* | | *P. simile* | | *N. rutili* | | Nematodes | | All parasites | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| % Prevalence | 19.26 | 22.63 | 17.96 | 19.61 | 75.00 | 74.96 | 13.96 | 21.86 | 68.33 | 71.11 | 49.42 | 56.98 | 94.81 | 96.14 |
| $\chi^2$ value | 1.979 | | 0.515 | | 0.000 | | 12.374 | | 1.057 | | 6.607 | | 1.177 | |
| *P* value | 0.159 | | 0.473 | | 0.987 | | < 0.001 | | 0.304 | | 0.010 | | 0.278 | |

*Table 25* Comparisons of the abundance of parasites (transformed values ($\log_{10}(1 + x)$) in male (M) and female (F) brown trout using analyses of variance and Mann Whitney tests

| | *D. sagittata* | | *C. farionis* | | *C. metoecus* | | *P. simile* | | *N. rutili* | | Nematodes | | All parasites | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Mean numbers untransformed | 0.363 | 0.510 | 0.381 | 0.458 | 16.70 | 21.30 | 0.454 | 0.706 | 7.46 | 8.20 | 3.28 | 3.79 | 28.65 | 34.89 |
| *F* value | 4.46 | | 0.61 | | 9.10 | | 5.48 | | 1.14 | | 1.57 | | 11.62 | |
| *P* value | 0.035 | | 0.436 | | 0.003 | | 0.019 | | 0.286 | | 0.210 | | 0.001 | |
| Mean numbers transformed | 0.191 | 0.239 | 0.190 | 0.199 | 2.011 | 2.160 | 0.171 | 0.278 | 1.434 | 1.551 | 0.837 | 0.945 | 4.838 | 5.365 |
| *F* value | 3.14 | | 0.12 | | 2.81 | | 11.30 | | 2.70 | | 3.25 | | 9.61 | |
| *P* value | 0.077 | | 0.728 | | 0.094 | | 0.001 | | 0.101 | | 0.072 | | 0.002 | |
| Mann Whitney value | 0.1383 | | 0.589 | | 0.070 | | 0.0003 | | 0.073 | | 0.040 | | 0.0022 | |
| *P* value | > 0.05 | | > 0.05 | | > 0.05 | | < 0.05 | | >0.05 | | < 0.05 | | < 0.05 | |

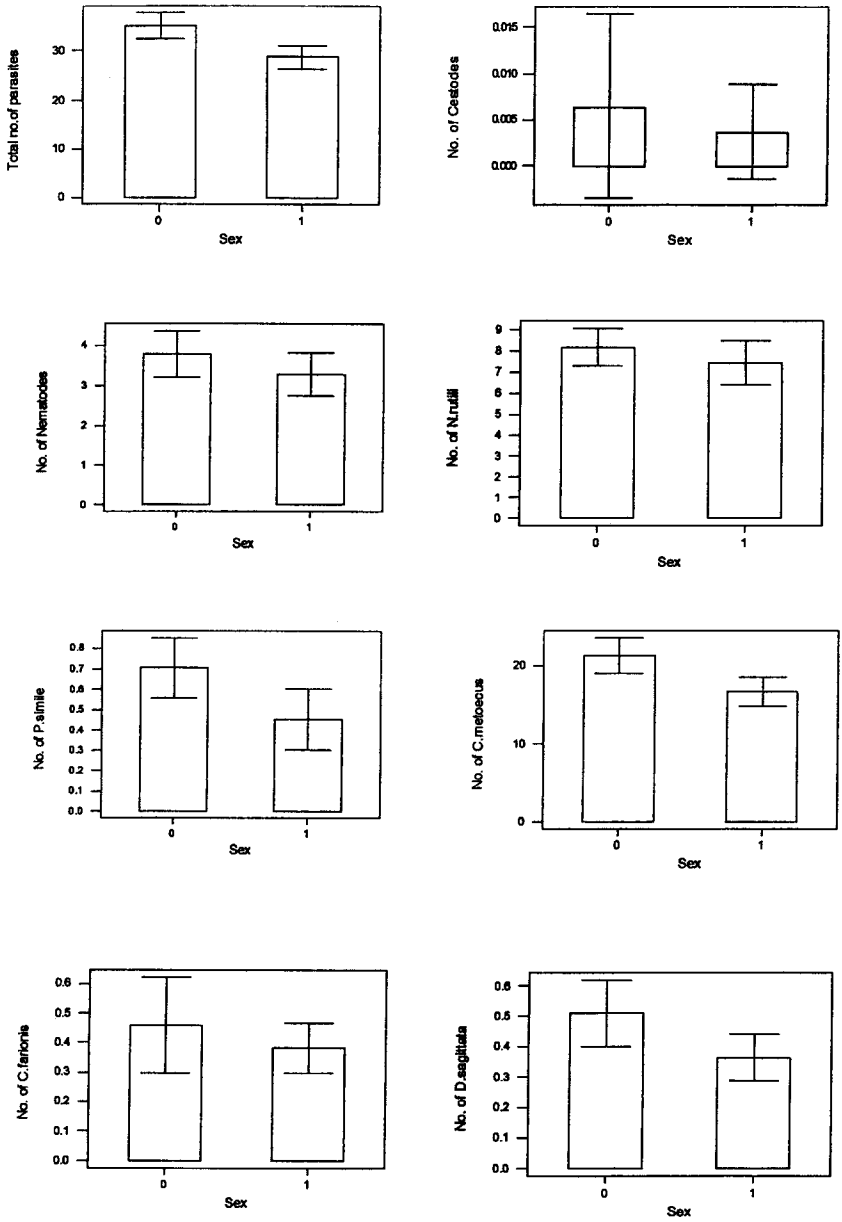


*Figure 15* The mean abundance (± 95% C.L.) of the parasites of male (1) and female (0) brown trout.

*Table 26* Comparisons of mean (± S.D.) of length, weight and condition factor of male and female brown trout

| | Length (cm) | *F* | *P* | Weight (g) | *F* | *P* | Condition factor | *F* | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Male | 18.55 ± 4.47 | 5.94 | 0.015 | 80.69 ± 62.31 | 2.16 | 0.142 | 10.85 ± 0.85 | 8.63 | 0.003 |
| Female | 19.18 ± 4.30 | | | 85.77 ± 55.65 | | | 10.69 ± 0.91 | | |

*Table 27* Comparisons of parasite abundance (transformed values ($\log_{10}(1 + x)$) in male (M) and female (F) salmon parr

| | *D. sagittata* | | *C. farionis* | | *C. metoecus* | | *N. rutili* | | Nematodes | | All parasites | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| Mean (tr.) numbers | 0.024 | 0.016 | 0.129 | 0.124 | 0.348 | 0.207 | 1.693 | 1.809 | 0.211 | 0.159 | 0.830 | 0.851 |
| *F* value | 0.32 | | 0.020 | | 2.96 | | 0.73 | | 0.79 | | 0.13 | |
| *P* value | 0.573 | | 0.888 | | 0.086 | | 0.395 | | 0.373 | | 0.714 | |

variance were very small (0.72%, 4.84% and 0.54%, respectively) compared with other variables such as season, station and age of the trout.

Table 26 shows that although the female trout in the sample are significantly longer than the males and also have a tendency to be heavier, their mean condition factor was significantly less than that of males.

Unlike the trout there were no significant differences between either the prevalence or abundance values for the total numbers of parasites or the numbers of individual parasites found in male and female salmon parr (Table 27, Figure 16). Multiple regression analyses also revealed that the sex of the salmon parr is not a significant predictor of the total numbers of parasites or of the numbers of individual parasite species found ($P$ = 0.440–0.980). As with the trout the contributions made to the total variance by the total number of

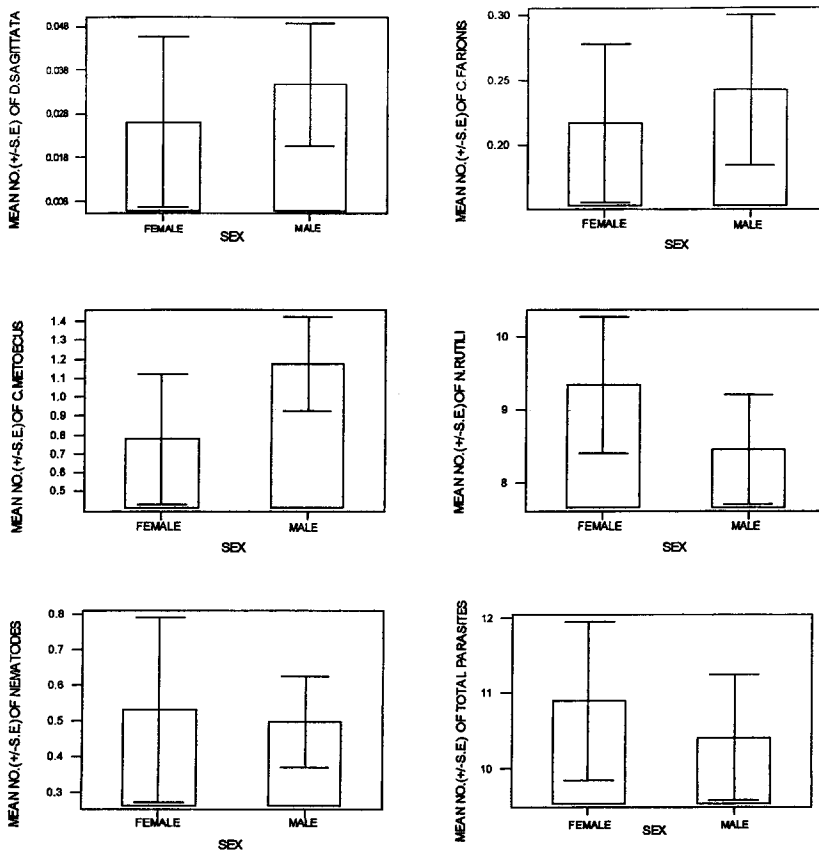


*Figure 16* The mean abundance (± 95% C.L.) of the parasites of male and female salmon parr.

parasites and numbers of individual parasite species were very small (0.005–3.04%) compared with other variables such as season, station and age of the trout.

The results for trout are in accord with the following cases in which female fish were also more heavily parasitised than males: Florida Garfish infected with metacercariae of *Odneriotrema incommodum* (Leigh 1960), *Rutilus rutilus* infected with *Caryophaeides fennica* (Borgström and Halvorsen, 1968), *Leuciscus leuciscus* infected with *Caryophyllaeus laticeps* (Kennnedy, 1968), *Esox lucius* infected with *Triaenophorus nodulosus* (Borgström, 1970), *Salmo salar* in coastal Labrador infected with *Bunodera luciopercae* and *Diplostomum spathaceum* (Hicks and Threlfull, 1973), *Micropterus salmoides* infected with *Neoechinorhynchus cylindratus* (Eure, 1974), *Fundulus heteroclitus* infected with *Eustrongylides* sp. (Hirschfield *et al.*, 1983) and whitefish, *Coregonus laveratus*, infected with *Diphyllobothrium ditremum* (Talonen *et al.*, 2000).

In contrast, male fish were more heavily parasitised than the females in the following cases: brown trout from 5 to 7 years old infected with *Discocotyle sagittata* (Paling, 1965, 1969), *S. salar* infected with *Eubothrium crassum* (Hicks and Threlfull, 1973), sexually mature brown trout infected with a variety of fungal pathogens and ectoparasites (Pickering and Christie, 1980), three species of cichlids infected with helminths (Batra, 1984) and rays infected with *Calicotyle kroyeri* (Williams and Jones, 1994). Reimchen and Nosil (2001) in an extensive 15-year study on sex-biased parasitism in threespine stickleback, *Gasterosteus aculeatus*, also found that the overall parasite prevalence was greater in males than in females. However, the excess did not occur for each species of parasite. Males had higher prevalence of the cestode *Cyathocephalus truncatus* and the trematode *Bunodera* sp. relative to females, while females had higher prevalence of the cestode *Schistocephalus solidus* and nematodes. Sex bias in fish parasites has also been reported to change seasonally. Thus, *Monobothrium ulmeri* occurred only in male *Erimyzon oblongatus* from February to May but by June it was commoner in female fish (Grimes and Miller, 1976).

The absence of sex bias in parasitic infection of fish has also been noted in the stickleback, *Gasterosteus aculeatus* (Bakker and Mundwiler, 1999), *Onchorhynchus mykiss* (Valles Rios and Ruiz Campos, 1992) and in juvenile salmon parr from the Teifi in the present investigation. Haley (1958), Dudzinski and Mykotowycz (1963) and Schalk and Forbes (1997) have also commented on the absence of sex bias in parasitism of juvenile mammals.

Similar conflicting results have been encountered in amphibia and reptiles. Thomas (1965) found that the intensity of infection of *Mesocoelium monodi* was significantly higher in female *Agama* lizards than in the larger males. In contrast, male frogs were found to be more heavily parasitised than females (Hickman, 1960; Lees, 1962). Using data from 33 studies McCurdy *et al.*

(1998) found no overall difference in prevalence of parasites between male and female breeding or non-breeding birds. However, they found that infections by *Homoproteus*, the most common genus of blood parasite, were significantly commoner among breeding females than breeding males. Again, when the analyses were restricted to breeding birds of polygynous species females were more likely to be infected than males. After examining evidence of sex bias in 38 published studies of mammals Schalk and Forbes (1997) found no sex bias in the case of helminth parasites although there was a male bias in parasitism overall and for arthropod parasites in particular. They concluded however that, on average, differences in parasitism between the sexes are small and statistically significant male biases in parasitism are not a general rule. Sheridan *et al.* (2000) came to a similar conclusion with regard to arthropods as these authors state: 'overall male and female arthropods did not differ in prevalence or in intensity of parasite infections'.

This review illustrates the danger of generalising from one example as Wedekind and Jakobsen (1998) attempted when they found a male-biased susceptibility to helminth infection in one species of copepod. They concluded that 'the pattern (male sex bias) seen in many vertebrates can be extended to an invertebrate host that lacks testosterone'. However, from the above evidence it must be concluded that statistically significant male biases in parasitism do not occur as a general rule for either vertebrates or invertebrate hosts.

*Male vertebrates will be more vulnerable to infection because testosterone is immunosuppressive*

Many influential reviewers support this view (Glick, 1986; Grossman, 1990; Slater & Schreck, 1993; Zuk and McKean, 1996; Klein and Nelson, 1999; Klein, 2000a, b) while others claim that oestrogen enhances immunity (Haley, 1958; Dobson, 1961a, b; Lees and Bass, 1960; Hall *et al.*, 1978; Brown, 1994). More recently Duffy *et al.* (2000) have provided further experimental evidence that this hormone suppresses both cell and humoral immunity in a species of songbird. There is also evidence that testosterone may benefit the parasites directly by enhancing their development, growth and survival *in vitro* (Fleming, 1985) and *in vivo* (Harder *et al.*, 1992)

However, the following experimental results are in conflict with the widely accepted view that testosterone is immunosuppressive. Firstly, although testosterone, oestrogen and progesterone may suppress parts of the immune system they may also under certain conditions have a stimulatory effect (Grossman, 1990). Secondly, testosterone, dihydroxytestosterone and oestradiol enhanced cell-mediated immunity through direct action on the immune cells of both male and female Siberian hamsters (Bibio and Nelson, 2001). Thirdly, secondary antibody production in redwing blackbirds was not suppressed by

testosterone either at physiological levels or above normal concentration (Hasselquist *et al.*, 1999). Fourthly, testosterone was found not to suppress existing immunity against *Plasmodium chabaudi* malaria in female mice although it appeared to prevent the development of protective immunity (Wunderlich *et al.*, 1992). Fifthly, Klein and Nelson (1997) concluded that circulating testosterone does not mediate sex differences in immunocompetence or body mass in *Peromyscus californicus*. Sixthly, there was no evidence that a higher androgen concentration in polygynous male voles influenced sex or species differences in immune function (Klein and Nelson, 1998). Seventhly, Hillgarth and Wingfield (1997) pointed out that many sexual characters in birds are not under the control of testosterone and concluded that there is little evidence that circulating levels of testosterone are immunosuppressive in wild birds. Visco (1973) and Hillgarth and Wingfield (1997) also concluded that there is no evidence that increasing testosterone levels can influence parasite intensity. There are also conflicting views regarding the claim that oestrogen is beneficial to parasites as Wang and Belosevic (1994) found that oestradiol increases the susceptibility of goldfish to trypanosomes while Sonnex (1998) has suggested that it may also enhance the pathogenicity of many urogenital microorganisms.

It must be concluded therefore that the evidence that testosterone is immunosuppressive or that sex hormones in general can influence the immune response or parasite burdens is equivocal. The cautionary note 'that it remains to be seen whether natural levels of testosterone or any other steroid can influence parasite intensity in free living wild birds' by Hillgarth and Wingfield (1997) is therefore very opportune and can be extended to all other animal taxa.

Many reasons can be advanced to explain the apparently conflicting effects of testosterone and other sex hormones on the immune mechanisms and the parasites when animals are kept under experimental conditions. These include variation in the nature of the host or parasite genotypes, the dosage rate of the hormone used, interactions between hormones, the handling and maintenance regimens and the physiological state of the animal when treated. Klein *et al.* (1997) have demonstrated that both the genotype and the social environment of voles may influence the immune function. Thus male meadow voles had higher immune responses than females when housed in pairs whereas the converse was observed in the case of the prairie vole. Wedekind (1992, 1994) has also suggested that the parasite genotype may influence the nature of the immune response and the development of secondary sex characters in roach.

The immune function can also be influenced by changing photoperiod (Nelson *et al.*, 1998) and by seasonal changes in general (Nelson and Demas, 1996; Pickering, 1989; Pottinger and Carrick, 2000). Seasonally induced stressors may influence the hypothalamic–pituitary–interrenal axis, resulting in elevated levels of glucocorticoids, inhibition of sex hormones and modulation

of the immune system in trout and other animals (Pickering, 1989; Nelson and Demas, 1996). Thus, Slater and Schreck (1993) have shown that cortisol may act synergistically with testosterone to suppress the development of leucocytes in fish under *in vitro* conditions. According to Brown (1994), however, potent modulators of the immune system such as corticosteroids may act in either a stimulatory or inhibitory manner depending on a number of factors including the concentration used or length of exposure. It must be concluded therefore that much work remains to be done to improve our understanding of the involvement of the endocrine system in influencing the immune mechanism in vertebrates in general and fish in particular.

Despite our limited understanding of the subject the possibility that the endocrine and immune systems may be implicated in influencing the parasite fauna of the salmonids in the River Teifi can be considered. It can be hypothesised that the absence of sex bias in the prevalence and abundance of parasites of salmon parr is attributable to these fish being juveniles. As a result it can be postulated that the endocrine and immune systems would be identical in male and female juvenile parr, unlike adult fish (Schalk and Forbes, 1997). However, this hypothesis requires experimental verification as the majority of the male salmon parr had enlarged precociously developed testes during the breeding season. If androgens were in fact immunosuppressive then a male bias in parasitism of the salmon parr would therefore be expected. The lack of sex bias in parasitism of the salmon parr therefore provides circumstantial evidence that androgens may not be strongly immunosuppressive as claimed by Zuk and McKean (1996), Klein and Nelson (1999) and Klein (2000a, b). The possibility that the parasitic fauna of male and female salmon parr might have been influenced by behavioural differences between the sexes can be excluded, as there were no significant differences between their mean sizes or dietaries.

The possibility that the strong female bias in prevalence and abundance of parasites in mature Teifi trout may be attributable to sexual differences in the endocrine or immune systems may also be considered. Pottinger and Carrick (2000) found that plasma testosterone levels in rainbow trout were higher in female than in male trout from September to January. If testosterone is immunosuppressive, as claimed by Grossman (1990), Zuk and McKean (1996), Klein and Nelson (1999) and Klein (2000a, b), then it follows that this observation might explain the female sex bias in parasitism.

A consideration of life history strategies helps to explain why female trout have higher testosterone levels than their male conspecifics. It is well known that androgens promote dominant social behaviour in vertebrates including fish and mammals and that it is generally the males that are the dominant sex with the higher androgen levels (Munro and Pitcher, 1985). However, in the case of the brown trout females are larger than the males presumably because of the higher energy costs of producing ova compared with sperms. They therefore need to behave aggressively to obtain favourable feeding territories. Although

it is the male trout that demarcate territories during the reproductive season the female trout also has to behave aggressively. Thus, it is the female trout rather than the male that makes the decisions regarding the timing, location and construction of redds. Female trout may construct an average of 5.7 redds during the breeding season and may thus mate with several different males (Barlaup *et al.*, 1994). Furthermore, it is the female trout that initiate mating by ovipositing in redds. They may also have to compete with female conspecifics for the key resource for successful reproduction, namely the optimal microhabitats for redd formation. It is not surprising therefore that they have higher androgen levels than males during the breeding season. There is an interesting similarity here between the trout and birds such as Wilson's and red-necked phalarope. In these bird species the females, which are larger and have brighter plumage than the males, have more testosterone in their ovaries than males have in their testes prior to incubation (Hohn, 1970). However, when the males start to incubate the position is reversed (Hillgarth and Wingfield, 1997).

There is at least one other explanation for female sex bias that also involves hormones. Pottinger and Carrick (2000) also found that the female hormone oestradiol-17β levels were significantly greater in female than in male rainbow trout throughout the study period and that this difference was maximal during the reproductive or post-reproductive period (September and January). Previous studies had shown that oestradiol-17β and testosterone have diametrically opposed effects on stress responsiveness in trout, with the former enhancing, and the latter suppressing the cortisol response to a stressor. As there is evidence that stress hormones, corticosteroids, may reduce immunity to parasitism (Pickering and Pottinger, 1985, 1987) the raised levels of oestradiol-17β in female trout may explain the female sex bias in trout parasitism. Sexual dimorphism in the glucosteroid response to stress, with the female being more responsive than the male, has also been observed in mammals (Aloisi *et al.*, 1994; Spinedi *et al.*, 1994). It may be suggested that the increased sensitivity of the female trout to stress may be due to the fact that it is often the female that makes the decisions regarding the timing and exact location of reproductive activity. It is vitally important therefore that the female should be able to respond sensitively to environmental signals that are unfavourable to reproduction. It is possible however that this response has incurred an associated cost, namely an increased susceptibility to parasitism. A reduction in the autoimmune response might be another trade-off resulting from the suppression of the immune response (Raberg *et al.*, 1998). This response might be particularly beneficial during the reproductive season.

Trout hormones have also been invoked to explain the male bias in prevalence and abundance of ectoparasitic infection reported by Paling (1965) in the case of *D. sagittata* and by Pickering and Christie (1980) for a variety of ectoparasites and fungal pathogens. The period of enhanced susceptibility in

male trout coincided with a marked sexual dimorphism in the skin structure characterised in the male by dermal and epidermal thickening and almost total depletion of mucus-secreting cells (Pickering, 1977; Pottinger and Pickering, 1985a). This kind of sexual dimorphism in skin structure has also been described for a number of other fish species (Pottinger and Pickering, 1985a). These changes in the skin structure were closely correlated with the rising phase of 11-ketotestosterone profile and disappeared during the post-spawning period when gonads regress and circulating androgens return to basal levels (Pottinger and Pickering, 1985a). Pottinger and Pickering (1985b) have shown that 11-ketotestosterone appeared to promote both epidermal and dermal thickening and reduce the numbers of goblet cells whereas testosterone stimulated epidermal thickening. However, as testosterone levels were higher in females during this period (Pottinger and Carrick, 2000) presumably other mechanisms must have been involved in inhibiting the changes described in the skin of male trout from occurring in female trout. As pointed out by Pottinger and Pickering (1985a) the functional significance of demucification is far from clear, particularly in view of the supposed protective role of the mucus layer (Blackstock and Pickering, 1982). They also postulated that the thicker integument would be advantageous to the male trout during the traumatic spawning season. However, it could also be argued that a thicker integument would also be selectively advantageous to the female while redd cutting at this time.

Further work is required to elucidate the causative mechanisms responsible for the above cases of apparent male bias in infection by ectoparasites and for its absence in the present and other investigations carried out on fish parasites. It can be hypothesised that the cases where male bias has been reported may have been due to the oncomiracidia of *D. sagittata* finding the skin of male trout more attractive or easier to penetrate than that of the female trout. Alternatively, the male trout might be more tolerant than female trout following infection. If Paling's (1969) claim that the oncomiracidia of *D. sagittata* do not locate their hosts by using olfactory signals is accepted then we are left with the last two hypotheses.

The conflict between the present results and those of Paling (1965) might be attributable to the increased vulnerability of male fish being restricted over a period when they are mature and sexually active. Thus, Pottinger and Pickering (1985a) found that changes in the skin structure, which were thought to be responsible for increased vulnerability of males to parasitism, were closely correlated with the rising phase of 11-ketotestosterone profile. These changes disappeared during the post-spawning period when gonads regressed and circulating androgens returned to basal levels. Consequently, if the window for sex bias to occur was narrow and failed to overlap the main infection period then no differences in the infection rates of males and females would be expected. However, Paling (1965) was unable to find support for this

hypothesis. The fact that sex bias is restricted to male trout in the 5–7 year age groups appears to rule out hormone involvement as trout become sexually mature when they are 3 or 4 years old.

It may be suggested therefore that the most likely reason for the male sex bias is age-related changes in the behaviour of the two sexes which results in the 5–7-year-old male trout spending more time in microhabitats frequented by oncomiracidia than female trout. Sexual dimorphism and differences between the sexes in behaviour, life history strategies and habitat preferences are well documented for trout (Jonsson, 1989; Elliot, 1989b). Paling (1965, 1969) also invoked behaviour to explain differences in infection rates of trout and charr with *D. sagittata*.

Behavioural differences between the sexes can also be used to explain female biases in parasitism of trout by *P. simile*, *C. metoecus*, nematodes (mainly *C. truttae*), *N. rutili* and total parasites in the present investigation. As female trout tend to be larger than males they would tend to consume more food items and hence more infective larvae. It can also be postulated that female trout would be more aggressive than the males due to their larger size and higher androgen levels (Pottinger and Carrick, 2000). As a result proportionately more females than males might frequent the more favoured, sheltered habitats with depositing sediments, on the edge of currents. These depositing sediments are the preferred microhabitats of ammocoete larvae (the intermediate hosts of *C. truttae*), *Sphaerium corneum* (the intermediate hosts of *P. simile*), *Lymnaea peregra* or *Pisidium* spp. (the intermediate hosts of *C. metoecus*) and ostracods or *Sialis* (the intermediate hosts of *N. rutili*). Female trout might therefore be expected to eat proportionately more infective larvae than male trout. In common with other detritus the eggs of *D. sagittata* would also tend to aggregate in depositing sediments. These eggs hatch out at night to produce infective oncomiracidia at a time when the trout are resting (Gannicott and Tinsley, 1997). If female trout spend proportionately more time than males in these microhabitats then a sex-biased infection rate with *D. sagittata* in favour of female trout would also be expected. This proved to be the case although the differences were not statistically significant ($P > 0.05$).

Other workers have also used gender-related differences in host behaviour to explain sexual differences in infection. Thus Schad (1962) attributed the heavier parasitic infection of male geese, compared with the females, to their larger size. This resulted in them eating more infective larvae. Thomas (1965) explained female sex bias in infections of female *Agama* lizards with *M. monodi* on the grounds that they spent more time than the males in the vicinity of transmission foci. As a result they would tend to eat more infected intermediate hosts.

Behavioural differences have also been invoked to explain male bias in infection by parasites. Thus, male desert toads (*Scaphiopus conchii*) spend more time in the aquatic transmission sites than females and therefore have a

higher probability of becoming infected with the monogenean *Pseudodiplorchis americanus* (Tinsley, 1989). Similarly, male wood mice, *Apodemus sylvaticus*, are considered to have higher infections of ectoparasitic ticks and flukes than females because they cover more ground and eat more of the invertebrate primary host (Langley and Fairley, 1982). Reimchen and Nosil (2001) also considered that the preponderance of *C. truncatus* and *Bunodera* sp. in male sticklebacks and S. *solidus* and nematodes in females was best explained on the basis of differences between the feeding niches of the two sexes rather than by invoking reproductive costs. Thus, the female fish utilise food items from the pelagic zone, where the primary host of *Schistocephalus solidus* tends to occur, to a greater extent than male fish. In contrast, male fish utilise more food items from the benthic zone, where the hosts of *C. truncatus* and *Bunodera* occur, than do female fish.

*Sex differences in immune function will be more pronounced among polygynous than monogamous species*

The arguments for advancing this hypothesis also hinge on the assumption that testosterone is immunosuppressive. It was postulated that because polygynous males need higher testosterone levels than monogamous males their immune mechanisms would be inhibited to a greater extent and they would therefore be more vulnerable to parasitism. However, Klein and Nelson (1997, 1998) found no evidence to support this hypothesis and concluded that higher androgen concentrations in polygynous male voles did not influence sex or species differences in the immune function.

*When species with extravagant sexually dimorphic traits are compared with others with more subdued dimorphic traits the males in the former case will be more heavily parasitised*

This is linked to another related, but untestable, hypothesis which states: 'Species harbouring harmful parasites over their evolutionary history will be more likely to evolve extravagant sexually dimorphic traits'. Both hypotheses are based on the assumptions that testosterone is imunosuppressive and that species with extravagant sexually dimorphic traits would have higher levels of testosterone than species with more subdued dimorphic traits. However, there is little support for the hypothesis involving extant species. Thus, Schall and Staats (1997) could find no association between parasitic load and colour when investigating species of *Anolis* lizards in the Caribbean islands. Likewise, Underhill and KaleitaSummers (1995) found that groups of birds with the highest brightness score had infection rates similar to the dullest group. Both

Hillgarth and Wingfield (1997) and Hamilton and Poulin (1997) conclude that support for this hypothesis is ambiguous and the latter authors state that 'the generality of the Hamilton and Zuk hypothesis in respect to parasite mediated sexual selection across taxa is thrown into doubt by these results'. This conclusion is not surprising as other environmental factors as well as parasitism may influence the sexual dimorphism in plumage colour. Thus Badyaev (1997), for example, found that species occupying lower elevations are more sexually dimorphic in plumage than species at higher elevations.

*Increased parasitic infections will reduce testosterone levels, the expression of secondary sexual characters and selection by the opposite sex*

There are several examples of parasites appearing to reduce the levels of testosterone in the host. These include the trematode *Schistosoma mansoni* in mice (Isserhoff *et al.*, 1986) and mites infecting male chickens (De Vaney *et al.*, 1977). Parasitic infections may also cause corticosteroid levels to increase in rats (Chernin and Morinan, 1985). Although the effects of parasites on steroid levels in brown trout appear not to have been investigated it is likely that their effects would be similar to those induced by environmental stress such as confinement (Pickering, 1989). According to this author most forms of environmental stress activate the hypothalamic–pituitary–interrenal axis of the trout. This causes an elevation of blood cortisol levels followed by a marked suppression of the plasma levels of both testosterone and oestradiol. As a result normal sexual development will be impeded in both sexes and the fish become more susceptible to disease.

The evidence that parasitic infections affect traits under sexual selection is conflicting. Studies that fail to support Hamilton and Zuk's prediction include that of Berg *et al.* (1995) as they found no reduction in two sexually dimorphic features in male sockeye salmon, *Onchorhynchus nerka*, with increased levels of infection with the nematode *Philonema onchorhynchi*. Likewise, Bronseth and Folstad (1997) found a positive relationship between the size of the pectoral fin in male three-spined stickeback and the intensity of prevalent parasites. Nevertheless, these authors argue that as the results show that the large fins are indicative of the individual's ability to tolerate increased parasitic exposure, they provide support for the contention that secondary sexual characters can provide information about the host's ability to tolerate parasitic infection.

In contrast, other results appear to support Hamilton and Zuk's original prediction. Thus, Houde and Torio (1992) found that orange spots were less bright in infected male guppies and as a result they were significantly less attractive to the females than non-infected males. In the case of the Arctic charr, Liljedal *et al.* (1999) found that the intensity of infection by a nematode

species, the density of circulating granulocytes and spleen mass were all negatively associated with ejaculate quality and red spawning coloration of male fish. Bakker and Mundwiler (1999) also found that small pectoral fins in male three-spined stickeback were associated with infection by the parasite *Pomphorhynchus laevis*. Lopez (1998) also claimed that secondary sexual characters were displayed more prominently by resistant male guppies than by non-resistant males. In the case of birds, Hillgarth and Wingfield (1997) state that 'there is increasing empirical evidence that male birds with high mating success and well developed secondary characters have lower parasitic intensity'. However, in 9 of the 13 cases cited by them the results failed to support the hypothesis.

Although tests of this hypothesis have focused on males rather than on females, one study involving female sticklebacks has shown similar trends. Thus, McLennan and Shires (1995) were able to show that the abundances of two helminth species were negatively correlated with the intensity of the female's aggressive response to intruding females and positively correlated with the intensity and duration of the female's courtship.

Comparable studies on birds also appear to support the view that plumage brightness serves as an indicator of condition and as an honest advertisement of resistance to parasitism. Thus male house finches surviving an epidemic had significantly redder plumage than males that did not survive (Nolan *et al.*, 1998). However, when Hamilton and Poulin (1997) reanalysed, 199 separate data sets they concluded that 'as a whole intraspecific correlations between parasite load and male showiness provided very little support for the hypothesis with only the effect of parasites on fish morphology matching the Hamilton and Zuk prediction'.

Unfortunately, no comparable studies have been made to investigate the possible effects of parasitism on secondary sexual characters of brown trout. The trout is sexually dimorphic and secondary sex characters such as the size of the kype and the colour and size of the adipose fin could be readily quantified in the male trout. However, as both males and females have distinctive and prominent coloured patterns it is possible that these could act as signals of health, wellbeing and resistance to parasitism to members of the opposite sex.

## 7.3. Conclusions

Statistical tests including multiple regression analyses have revealed that the sex of the brown trout has a significant influence on the total number of parasites and more specifically on *P. simile*, nematodes, *C. metoecus* and *N. rutili*. In all cases the bias was in favour of the female trout. However, the contributions made by the host sex to the total variance were very small (0.54–4.84%) compared with those made by seasonal change or station. This

scenario can be extended to other taxa as strong sex bias in parasitism is uncommon. Despite this much has been written about sex bias in parasitism and this information has been used as a basis for testing the Hamilton–Zuk and immunocompetence hypotheses. However, on the whole, the present review fails to support predictions 1–5 of the above hypotheses. It was also concluded that statistically significant male biases in parasitism are not a general rule. The linked hypothesis that male bias in parasitism is due to the immunosuppressive action of testosterone also lacks sound experimental support and is therefore invalid. This conclusion helps to explain the lack of support for hypotheses 3 and 4 as they are also based on the assumption that testosterone is immunosuppressive.

The fifth hypothesis, which states that increased parasitic infection will reduce testosterone levels, the expression of secondary sexual characters and selection by the opposite sex is supported by some experimental evidence and therefore has more validity. However, it is not supported by all the experimental results. This is not surprising in view of the fact that parasites vary in their pathogenicity and testosterone levels can also be influenced by a number of other environmental factors. The most likely explanation for the decline in testosterone levels and in the development of secondary sexual characters in parasitised animals is that they are caused by increased corticosteroid levels due to stress. There is evidence that high corticosteroid levels cause a depression of the immune system, a lowering of sex hormone levels and hence result in less well developed secondary sexual characters.

Taken as a whole the arguments of the Hamilton–Zuk hypothesis seem sound. Parasites may adversely affect vertebrate host fitness in general and secondary sexual characters in particular to varying degrees and susceptibility to parasitism will be inherited. However, the predictions used to verify it which are based on the proposition that testosterone is immunosuppressive seem flawed and are highly questionable. Before this hypothesis can be properly assessed it is clearly necessary to have a better understanding of how the endocrine and immune systems are influenced by parasitism in both male and female animals. It is also necessary to know how important parasitism is as a selective force. The statistical analysis carried out on the Teifi trout failed to produce any evidence that the parasites had a detrimental effect on the trout. This situation is not unique. Thus, Chubb (1973) states that 'most British freshwaters are not troubled with regular parasite problems, although parasites are always present in abundance'. Allison (1982) also cites a number of case studies in support of the view that parasite infections are relatively harmless to those animal hosts with which they have a long-term relationship. However, those same parasites may have seriously detrimental effects on newly introduced host species or when the parasites themselves are introduced into new regions. It can be postulated that these apparently benign relationships are a consequence of long-term coevolution.

This concept can be rationalised by reference to $R_0$, the basic reproductive rate of the parasite which natural selection will tend to maximise. Maximising $R_0$ will involve trade-offs between production of transmission stages (which ideally should be high) and damage to the host (which ideally should be small). $R_0$ will therefore be clearly maximised by the host surviving as long as possible. However, in cases where death of the host is an integral part of the transmission cycle or where the transmission efficiency may be enhanced by modifying host behaviour in such a way as to shorten its life the circumstances are different.

Other environmental factors such as seasons (climate) or location may well be more important selective mechanisms in determining the nature of secondary sex characters than parasites. Thus, Badyaev (1997), for example, found that bird species occupying lower elevations are more sexually dimorphic in plumage than species at higher elevation. One other criticism of this hypothesis is that it focuses on the male's secondary sex characters as signals of health and wellbeing. A more balanced approach would also involve assessing the signals used by males to assess the health and wellbeing of females.

It is necessary to focus on particular host species in order to elucidate the causative mechanisms responsible for sex bias in parasitism or the lack of it as in salmon parr. In the case of the Teifi trout, for example, three hypotheses have been advanced to explain the female sex bias in parasitism. These include testosterone immunosuppression, corticosteroid-based immunosuppression and behavioural differences between the sexes. Further endocrinological and immunological research is clearly needed to evaluate the possible involvement of the first two hypotheses. However, on the basis of the evidence presented it would appear that the last two hypotheses have the most credence and could be tested under experimental conditions. Reimchen and Nosil (2001) also favour the ecological hypothesis and state 'that their results, combined with those in the literature suggest that ecological differences between the genders may be a more important component to patterns of parasitic infections in natural populations than currently appreciated'. Niche partitioning between the sexes is widespread in the animal kingdom and may have evolved as a result of the selective pressure imposed by intraspecific competition (Schluter, 1994). It is strongly advocated therefore that further studies should be carried out to test the 'behaviour' hypothesis under natural conditions by comparing microhabitat selection, the feeding niches and the selection of intermediate hosts, in particular, by the two sexes for a wide range of species. However, the possible involvement of immunosuppression in causing sex bias can also be tested under controlled conditions in the laboratory by providing male and female trout of the same age with identical doses of infective larvae. The lack of sex bias in the case of the salmon parr suggests that there are no differences between the sexes in either their feeding niches or immune mechanisms.

Evidence in support of the former hypothesis is given by Thomas (1962). The fact that the majority of the male salmon parr are precocious and have enlarged testes may be cited as circumstantial evidence against the hypothesis that testosterone is immunosuppressive.

## 8. THE HELMINTH FAUNA OF TROUT AND SALMON PARR: COMPARATIVE ASPECTS

At the ecological level the heminth parasites common to both the trout and salmon parr share many spatial and temporal patterns. Thus, *C. metoecus* exhibit summer troughs and winter highs, unlike *N. rutili*, in both fish species. However, the abundance of *C. farionis* does not vary seasonally in the case of salmon parr although it does so in the trout. Likewise *D. sagittata* and *N. rutili* are more abundant in both trout and salmon parr at Station A than at Station B.

As with trout there were significant tendencies for the abundances of *D. sagittata*, *C. metoecus* and *C. farionis*, but not *N. rutili*, to increase with the length of salmon parr. However, unlike the trout, there were no statistically significant relationships between the abundances of any of the parasite species and the weight of the salmon parr. This absence of relationship can be attributed to the much smaller range in weight of the salmon parr compared with trout. With the exception of *N. rutili* and *D. ditremum* the abundances of all the parasite species in trout increased significantly with age. This was also the case with the helminth parasites of salmon parr with the exception of *N. rutili* and *C. farionis*. As with the trout the abundance values of the dominant parasite, *N. rutili*, were significantly positively correlated with both the condition factor and the adipose index. There was no evidence therefore that the helminth parasites had an adverse effect on the wellbeing of either fish species as measured by the condition factor and the adipose index.

However, the helminth communities of salmon parr and the brown trout (Tables 3 and 14; Figure 17) also differ in many important respects. Firstly, although the trout and salmon parr share five species (*D.sagittata*, *C. farionis*, *C. metoecus*, *N. rutili* and *C. ephemeridarum*) the trout has a richer helminth fauna as it is also parasitised by *P. simile*, *D. ditremum*, *R. acus*, *C. truttae* and species of *Capillaria*. Secondly, at the component population level both the Simpson and Shannon Wiener diversity indices and the equitability index are much higher for the trout than the salmon parr. In contrast, the Berger–Parker predominance index is higher for the salmon parr than trout. Thirdly, the Brillouin and maximum Brillouin indices are higher in trout than in salmon parr at both the component and infrapopulation levels. These results are to be expected as species richness, the mean number of parasites per fish and the

maximum number of parasites per fish at the infrapopulation level are much higher for trout than salmon parr. In contrast, more salmon parr were without any parasites (13.3–14.3%) compared with trout (1.0–1.3%). With the exception of *N. rutili*, the dominant parasite in the case of the salmon parr, the abundance values of shared parasite species were much higher for trout. This trend was particularly marked in the case of *D. sagittata.*

Although it can be postulated that these differences are attributable to the salmon parr having a higher level of immunity or resistance than trout it is more probable that they are caused by differences in age distribution and the niches occupied by the two species (Figures 18 and 19). Thus, nearly all of the salmon parr are in the 1 or 2 year age category whereas many of the brown trout are much older. The evidence that parasite abundance increases with age

| | BROWN TROUT | SALMON PARR |
|---|---|---|
| **CHARACTER DISPLACEMENT** | LARGER GAPE; MANDIBLES EXTEND TO POSTERIOR MARGIN OF EYE; BODY STOCKIER; CAUDAL PEDUNCLE THICKERAND CAUDAL FIN LESS INDENTED | SMALLER, MORE ROUNDED GAPE; MANDIBLES EXTEND TO MIDDLE OF EYE; BODY MORE SLENDER; CAUDAL PEDUNCLE NARROWER; CAUDAL FIN MORE INDENTED |
| **NICHE DIVERSIFICATION (HABITAT)** | TEND TO OCCUPY MID-WATER POSITION IN LAMINAR FLOW; TERRITORIES INCLUDE POOLS, BACKWATERS AND SLACK WATERS UNDER BANKS | TEND TO OCCUPY RIFFLE ZONES, CLOSE TO SEDIMENTS; EXCEPT UNDER SPATE OR DROUGHT CONDITIONS AVOID BACKWATERS AND SLACK WATERS UNDER BANKS |
| **NICHE DIVERSIFICATION (FEEDING)** | DIET HAS HIGHER %'S OF *Lymnaea*, *Sphaerium*, *Asellus*, *Gammarus*, and ammocoete LARVAE ASSOCIATED WITH DEPOSITING SEDIMENT, WHICH ACT AS INTERMEDIATE HOSTS | DIET DOMINATED BY RIFFLE AND FAST REACH FAUNA (e.g., species of *Ancylus*, *Brachycentrus*, *Baetis*, *Ephemerella*, *Ecdyonurus* and *Simulium* |
| **PARASITIC FAUNA** | PREVALENCES AND ABUNDANCE VALUES FOR *D.sagittata*, *C.farionis*, *C.metoecus*, *P.simile*, *N.rutili*, *R.acus* and *C.truttae* HIGHER THAN FOR SALMON PARR | *P.simile*, *R.acus* & *C.truttae*, ABSENT; PREVALENCES AND ABUNDANCE VALUES FOR *D.sagittata*, *C.farionis*, *C.metoecus* and *N.rutili* LOWER THAN TROUT BUT THOSE FOR *C.ephemeridarum* HIGHER |

*Figure 17* Summary of the differences in the niche requirements and parasitic fauna of the brown trout and salmon parr.

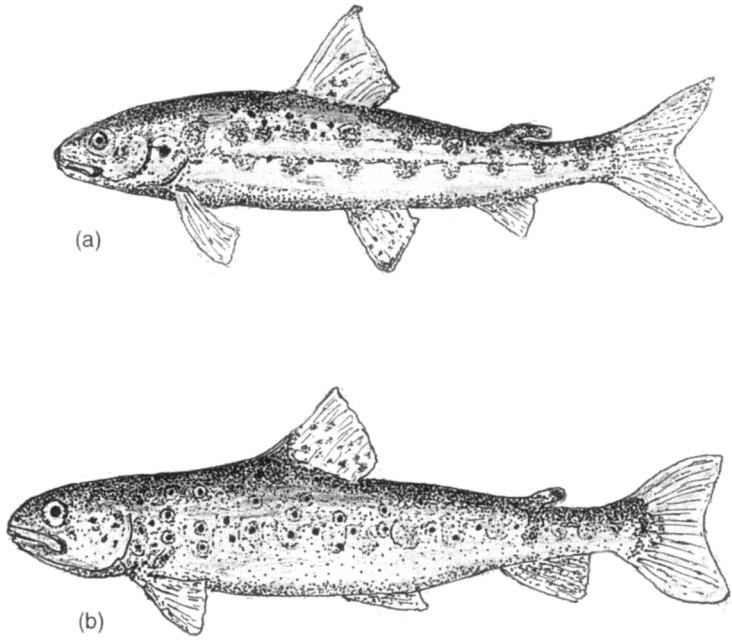


*Figure 18* Differences in the morphological features of the salmon parr (a) and brown trout (b). The major differences include the larger maxillary bone of the trout and hence its wider gape, the more rounded gape of the salmon parr, the more rounded, elongated body and more pronounced forked tail of the salmon parr. These adaptations allow the parr to occupy habitats close to the substrate in riffles and also to enter spaces between small cobbles in winter. The trout anatomy is better adapted for life in laminar flow in midwater.

has already been given. As most helminth parasites are transmitted orally it is more likely however that the differences between the parasitic fauna of the two salmonid species are mainly attributable to differences in habitat selection and dietaries. These in turn are linked to differences in character displacement and niche diversification (Figures 17 and 18).

There is a great deal of cumulative evidence that differences between the community structure of helminth parasites of fish species are determined largely by differences in their feeding behaviour (Dogiel, 1961; Fiorillo and Font, 1996; Carney and Dick, 1999). The latter authors postulate that the feeding niches of perch species and their helminth parasites have remained conservative for millions of years and that as a result parasitic assemblages are predictable and not merely stochastic assemblages. Niche diversification has also been invoked to explain differences in the helminth communities of distinct morphs in populations of lake-inhabiting arctic charr (Curtis *et al.*, 1995;

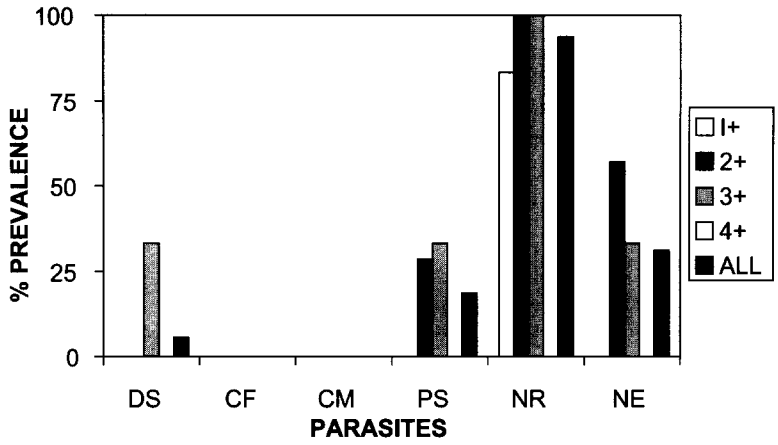


*Figure 19* The percentage prevalence values for the major parasites and total parasites of the brown trout in 1998. DS, *D. sagittata*; CF, *C. farionis*; CM, *C. metoecus*; PS, *P. simile*; NR, *N. rutili*; NE, nematodes.

Knudsen *et al.*, 1997) as well as sex bias in the parasitism of trout and other species like the three-spined stickleback (Reimchen and Nosil, 2001). Changes in the food webs attributable to environmental stress, such as increased acidity (Marcogliese and Cone, 1996), competition for food (Dubois *et al.*, 1996) and cannibalism (Hammar, 2000) have also been invoked to account for differences in the parasitic helminth fauna of freshwater fish. According to Due and Curtis (1995) the absence of the cestoda *Cyathocephalus truncatus*, nematodes of the genus *Cysdicola* and freshwater acanthocephalans from fish in Greenland can be attributed to the absence of Mysidae, Amphipoda, Ephemeroptera and Odonata, which act as intermediate hosts.

The salmon parr and trout are both territorial (Stuart, 1953). However, there is evidence that the home ranges of salmon parr overlap to a greater extent (Armstrong *et al.*, 1999) and that the trout has the competitive advantage (Kalleberg, 1958). The trout tend to occupy midwater positions in laminar flow on the edge of currents when feeding but the larger trout particularly also spend more time in pools, backwaters and in deeper water under banks, which are the transmission zones for helminth parasites, than do the salmon parr. In contrast, feeding salmon parr tend to spend more time resting on the substrate, usually in riffles, with the pectoral fin outstretched, the tail rigidly extended and the dorsal profile slightly concave while waiting to intercept drift. According to Valdimarsson and Metcalfe (1998) salmon parr, unlike trout, become nocturnal during the winter and seek refuge during the day in the interstitial spaces in gravel beds in flowing water. These authors cite evidence that this behavioural pattern has evolved to reduce predation.

Morphological differences such as the larger body size and wider gape of the trout, combined with their wider habitat range, also help to explain why trout have more catholic dietaries than the salmon parr. As a result invertebrates such as *Lymnaea*, *Sphaerium* and *Gammarus*, associated with depositing sediments as well as ammocoete larvae and fish, are commonly encountered in the trout dietary but are rare or absent from that of salmon parr (Thomas, 1962). In contrast, the salmon parr occur predominantly in riffles or fast reaches where their small rounded gape enables them to ingest some of their main food items such as *Ancylus* and *Simulium* by suction (Figure 18). As *Sphaerium*, fish species and ammocoete larvae are the intermediate hosts of *P. simile*, *R. acus* and *C. truttae*, respectively, this helps to explain their absence in salmon parr. The fact that the salmon parr spend less time in microhabitats with low flows and depositing sediments in the transmission sites may also help to explain why the two *Crepidostomum* species and *D. sagittata* are less prevalent or abundant in them than the trout. Ostracods, which are the intermediate hosts of *N. rutili*, commonly occur in the interstitial spaces in gravel beds. The tendency for the salmon parr to occupy these habitats, particularly in winter (Valdimarsson and Metcalfe, 1998), may help to explain why *N. rutili* is the dominant parasite for this fish.

It seems likely that the nocturnal habits of the salmon parr in winter result in a reduced level of feeding and that this may be a factor contributing to this species having a helminth community with a lower species richness and diversity than sympatric brown trout. In contrast, the trout remains a diurnal or crepuscular feeder throughout the year. There is both experimental (Kennedy, 1972) and circumstantial evidence (Dogiel, 1961) that a reduction in feeding rate or starvation of fish hosts will result in a corresponding reduction or loss of the helminth fauna. The fact that the eel is a nocturnal feeder and that it stops feeding during the winter months when the temperature falls below 10 °C (Thomas, 1962) may partly account for the impoverished and isolationist nature of its intestinal helminth community (Kennedy and Hartvigsen, 2000).

## 9. CHANGES IN THE HELMINTH FAUNA AND THEIR HOSTS BETWEEN 1950 AND 1998: CAUSATIVE MECHANISMS AND POLLUTION BIOINDICATORS

### 9.1. Comparison of Prevalence, Abundance and Diversity of Helminth Parasites in 1950 and 1998

A comparison of Figures 10 and 19 reveals that the prevalence values for parasites of trout of various age groups have changed dramatically between 1951 and 1998. Although *C. metoecus* was one of the most prevalent trout parasites

in the 1950s both *C. metoecus* and *C. farionis* were conspicuous by their absence in the 1998 fish sample. With the exception of *N. rutili* the prevalence values for trout parasites in the sample taken in 1998 were less than the corresponding ones in the 1950 sample.

The community structure analysis of brown trout parasites (Table 14) also reveals that major changes had occurred between 1950 and 1998. Thus, species richness had declined from 8 to 5 and at the component population level *N. rutili* had replaced *C. metoecus* as the dominant parasite. The various component population indices, including the Berger–Parker index, Simpson's diversity index, Brillouin's index, and the equitability index and the slope 'a' of the geometric series had all decreased. At the infrapopulation level (Table 14) the mean number of parasite species per fish and the maximum number of parasite species per fish had halved between 1950 and 1998. During 1998 the percentage values for fish with no parasite species or with one or two parasite species only were less than in 1950 whereas the converse was true for the percentage values for fish with three to six parasites only. These results were reflected in the mean Brillouin's indices for all fish and infected fish. The maximum Brillouin's index was also lower in 1998 than in 1950.

## 9.2. Reasons for the Decline in Parasitic Fauna between 1950 and 1998

Several reasons can be advanced to explain why the helminth parasites of trout had declined in prevalence, abundance and diversity from 1950 to 1998. Firstly, the decline is partly due to the catastrophic decline in brown trout density and to a change in the age structure since 1950 (Figure 20). Thus, the trout population in 1998 consisted mainly of juvenile fish in the first and second year and older, mature fish were uncommon. It is possible that the change in age structure is due to a shift from what was predominantly a brown trout life history strategy to that of the migratory sea trout. This change may be regarded as an adaptation to the River Teifi and its tributaries becoming harsher and more erosive in character as a result of changes in land use. Many of the juvenile fish in the 1998 sample may therefore have been sea trout parr and it is noteworthy that trout in the year classes 4–6 were absent from the sample (Figure 20). As the prevalence, abundance and diversity of helminth parasites in fish are positively correlated with age it is possible that the lower values in the 1998 sample may be partly due to the preponderance of juvenile fish.

Another possible reason for the decline in prevalence, abundance and diversity of parasites from 1950 to 1998 is that the intermediate hosts have become less abundant. Preliminary sampling in 1997 had revealed that *S. corneum*, the intermediate hosts of *P. simile* (Thomas, 1957, 1958a, b), which were abundant

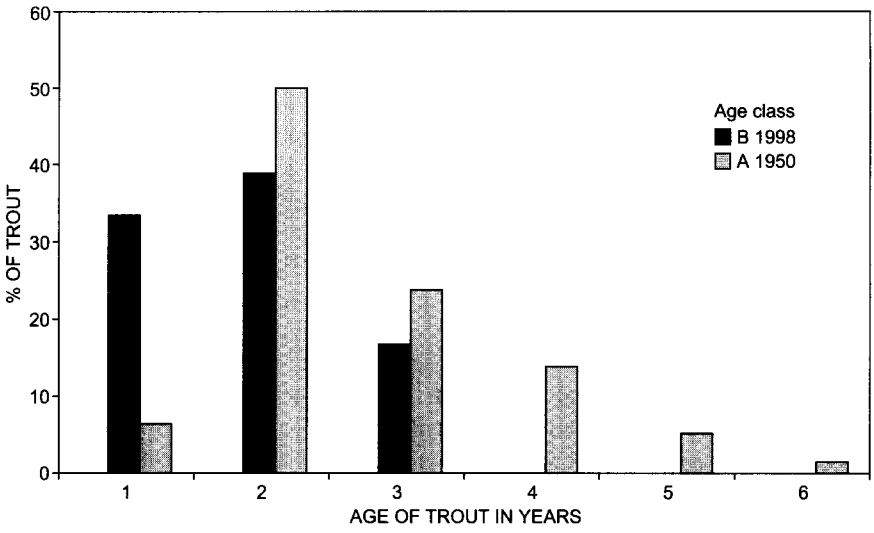


*Figure 20* The percentage of brown trout in various age groups in 1950 and 1998.

in the sediments of sites 1–8 in 1943 (Jones 1943) and also in the 1950s (Thomas, 1956, 1958, 1962, 1964a, b, c), had apparently disappeared. The absence of this species from the fish and invertebrate sampling sites 1–7 on the Teifi was confirmed by qualitative sampling of the fauna at eight sites in 1998 (Table 28, Figure 21). *S. corneum* was only found in depositing sediment in a backwater at site 8 in the vicinity of a small ox-bow lake that may have acted as a refugium. As this species was not found during the course of a very thorough survey of the Teifi in 1981 by Jenkins *et al.* (1984) it would appear that it has been in serious decline in its population density between 1950 and 1981. As *Sphaerium corneum* was identified as the molluscan host of *P. simile* by Thomas (1956, 1958a) the presence of adult *P. simile* in the trout in 1998 was unexpected. It must be concluded therefore that *P. simile* must be using another mollusc, probably species of *Pisidium*, as its intermediate host. It may be suggested that this low level of intermediate host specificity may be a general characteristic of the parasites of salmonid fish. This feature may be attributable to the high levels of uncertainty in the environment in which the fish and their parasites have evolved.

Small numbers of *Pisidium* species, which are potential intermediate hosts of *P. simile* and also *C. farionis* and *C. metoecus*, were patchily distributed at all stations (Table 28). The *Pisidium* species listed in Table 28 and Figure 21 were also recorded by Jenkins *et al.* (1984). Quantitative samples, taken with the cylindrical corer, confirm that both the bivalve and gastropod molluscs, including *Lymnaea peregra*, the intermediate host of *C. metoecus* according to

*Table 28* The numbers of potential intermediate hosts of helminth parasites, sampled by netting, in September 1981 and May 1998

| | Year (station number) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1981 (1 and 2) | 1981 (8) | 98 (1 and 2) | 98 (3) | 98 (4) | 98 (5) | 98 (6) | 98 (7) | 98 (8) |
| **Mollusca** | | | | | | | | | |
| *Potamopyrgus jenkinsi* (Smith) | – | – | – | 465 | 55 | – | 2 | – | 52 |
| *Lymnaea peregra* (Müller) | 1 | 14 | 48 | 27 | 11 | – | 4 | 4 | 1 |
| *Lymnaea palustris* (Müller) | – | – | 1 | – | 1 | – | 2 | – | – |
| *Planorbis carinatus* (Müller) | – | 70 | 9 | 22 | 53 | 3 | 3 | 9 | 6 |
| *P. contortus* (L.) | – | – | – | 60 | 50 | 2 | 3 | 4 | – |
| *Ancylus fluviatilis* (Müller) | – | 288 | – | 1 | 14 | – | 7 | 3 | – |
| *Pisidium casertanum* (Poli) | 5 | 12 | – | – | – | – | – | – | – |
| *P. personatum* (Malm) | – | – | – | – | 1 | – | – | – | – |
| *P. obtusale* (Lamarck) | – | – | – | – | – | – | – | – | – |
| *P. millium* (Held) | – | – | – | 5 | 1 | – | 1 | – | 1 |
| *P. subtruncatum* (Malm) | 4 | 5 | – | 7 | – | 1 | 7 | – | 1 |
| *P. hibernicum* (Westerlund) | 113 | 3 | – | 2 | 4 | – | 4 | – | 1 |
| *P. nitidum* (Jenyns) | 13 | 2 | 7 | 5 | 5 | – | 10 | – | 1 |
| *P. pulchellum* (Jenyns) | – | 1 | – | – | – | – | – | – | – |
| *Pisidium* spp. | 77 | 3 | 9 | – | 1 | – | 22 | – | 1 |
| *Sphaerium corneum* (L.)[a] | – | – | – | – | – | – | – | – | 10 |
| *S. lacustre* (Müller) | – | – | – | – | – | – | – | – | 6 |
| **Crustacea** | | | | | | | | | |
| Ostracoda | – | 7 | 11 | 7 | 12 | 3 | 5 | 9 | 7 |
| Copepoda | 25 | 1 | 45 | – | – | – | – | – | – |
| *Asellus meridianus* (Racovitzc) | 900 | 335 | – | 4 | 4 | 1 | 6 | 1 | 1 |
| *Asellus aquaticus* (L.) | – | – | 1 | 90 | 35 | 45 | 109 | 10 | 6 |
| *Gammarus pulex* (L.) | 2 | 730 | – | – | – | – | – | – | – |
| *Crangonyx pseudogracilis* (Bousfield) | – | – | 1 | – | 5 | 15 | 9 | 2 | 11 |

[a]Abundant in 1943 (Jones, 1943) and in 1950 (Thomas, 1958a, b,1964b, c).

*Note:*
1981 values based on Jenkins *et al.* (1984)

Awachie (1968), were overdispersed and present at very low densities in the depositing sediments (Figures 21 and 22). The occurrence or abundance of other potential intermediate hosts for helminth parasites has also changed since 1950 or 1981. Thus, *Asellus meridianus* has been largely replaced by *A. aquaticus* while *Gammarus pulex*, the most important intermediate host for *C. metoecus* according to Awachie (1968), was absent from all the samples and appears to have been replaced by *Crangonyx pseudogracilis* (Table 28; Figure 23). According to Awachie (1965) *Gammarus pulex* also serves as the intermediate host of acanthocephalan parasites of trout such as *Echinorhynchus*

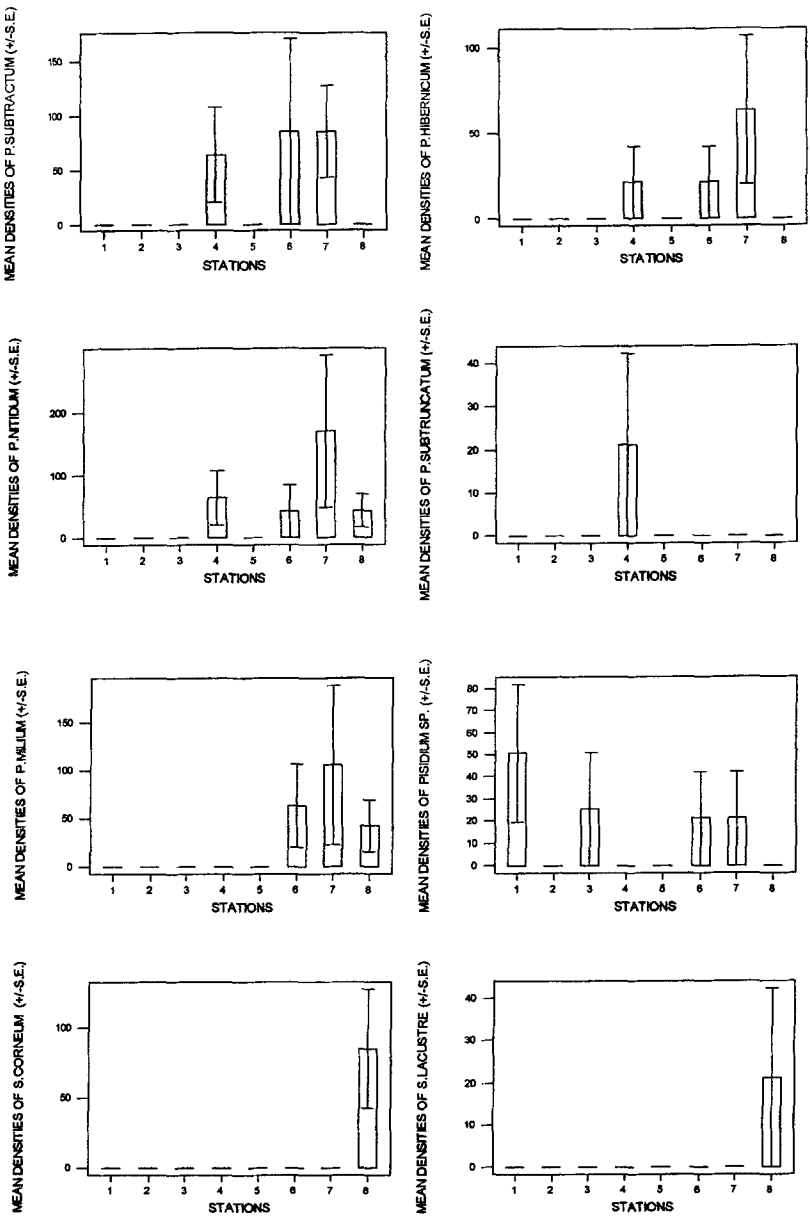


*Figure 21* The mean densities (± S.E.) of the potential sphaeriid hosts of helminth parasites at the various stations in the River Teifi in 1998.

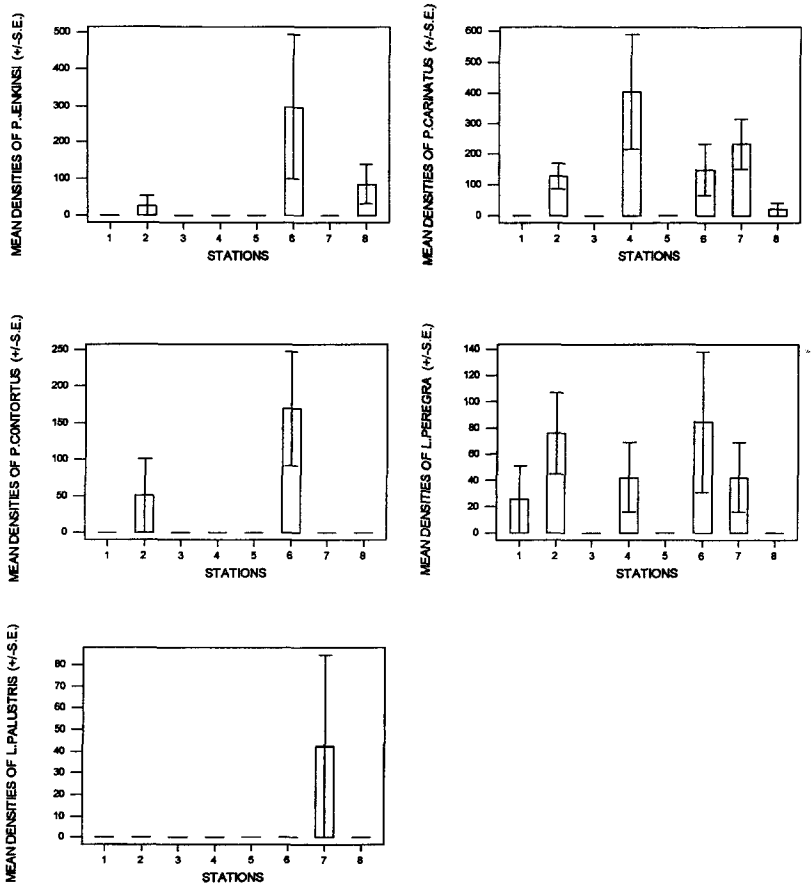


*Figure 22* The mean densities (± S.E.) of the potential gastropod hosts of helminth parasites at the various stations in the River Teifi in 1998.

*truttae*. It is likely that the dearth of suitable *Pisidium* host species and the absence of *Gammarus pulex* may explain the absence of *Crepidostomum* species in the trout collected in 1998. However, ostracods, the intermediate hosts of *N. rutili* (Lassiere, 1988; Dezfuli, 1996), were common in the gravel at all stations although *Sialis lutaria* and the other intermediate host were very patchily distributed (Figure 23). These observations explain the fact that *N. rutili* was the dominant parasite in the trout collected in 1998. Copepods, the intermediate host of *D. ditremum*, were very abundant at Stations 1 and 2 in the Bog area (Table 28). As cormorants are frequent visitors to the Tregaron Bog area conditions would appear to favour the transmission of *D. ditremum*. However, the dearth or absence of older fish contraindicates this.

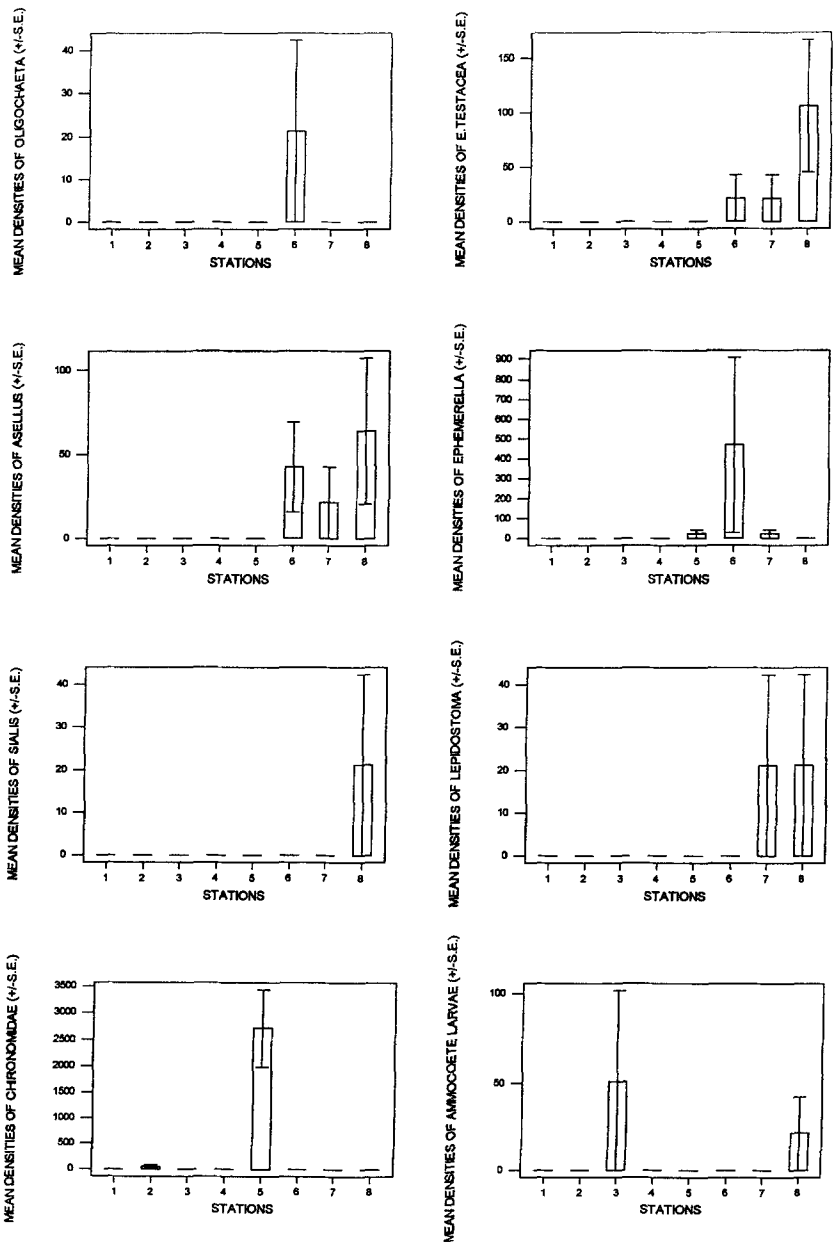


*Figure 23* The mean densities (± S.E.) of other potential hosts of helminth parasites at the various stations in the River Teifi in 1998.

Ammocoete larvae, the intermediate hosts of the nematode *Cucullanus (T.) truttae* (Moravec, 1994), which coexisted with *S. corneum*, were abundant in depositing sediments in the 1950s and featured in the food of the trout at that time (Thomas, 1962). However, by 1998 they had become very rare and highly overdispersed (Figure 23). This observation explains the much lower prevalence and abundance values for *C. truttae* in the trout examined in 1998 compared with 1950 (Figures 10 and 19). *Rhaphiascaris acus* the only other nematode found in 1998 occurred in only one trout. As this nematode has a fish intermediate host the low prevalence value may be attributable to the absence of piscivorous larger fish in the sample.

## 9.3. Elucidation of Environmental Factors that May have Caused the Decline in the Intermediate Hosts

In order to elucidate the possible causes for the declines in parasite diversity and in the numbers of intermediate hosts such as *S. corneum* and other sediment dwellers such as ammocoete larvae and *Gammarus pulex* the physicochemical factors that might be implicated were investigated.

The relatively low range of ammonia, nitrite, nitrate, phosphate and BOD values (Table 1) indicate that neither river is likely to suffer from eutrophication or organic pollution. To the contrary, the nutrient and organic loading may be beneficial in these acid waters as they facilitate the release of bases and hence deacidification during decomposition in the sediments (Davison, 1986, 1987).

Changes in pH associated with changes in the volume of water are more likely to be harmful. The pH values, which varied between 5.5 and 7.7 and 4.8 and 6.9 in the Teifi and Pysgotwr, respectively, were inversely correlated with water level or volume (Table 1). However, there is evidence that in times of heavy spate the pH values often declined to values even lower than those given in Table 1 (Wade, verbal communication). It is well established that under these conditions the mobilisation of heavy metals, such as aluminium, zinc, lead and copper into the labile, ionic form will be favoured (Hutchinson and Sprague, 1986; Howells *et al.* 1990).

The results of analyses carried out on sediment cores in the River Teifi suggest that heavy metal toxicity may be involved in causing the decline in the distribution and abundance of intermediate hosts. Thus, according to the United States Environmental Protection Agency (US EPA) guidelines (Giesy and Hoke, 1990) for sediments the metal concentrations (Figure 24a–d) are indicative of moderate pollution in the case of copper and chromium (> 25 mg/kg dry weight) and of heavy zinc (> 200 mg/kg dry weight) and lead pollution (> 60 mg/kg dry weight). These concentrations also fall within the 'action levels' of 40 and 200 mg/kg for lead and zinc, respectively, according to the equilibrium partitioning approach of Webster and Ridgway (1994). They

are also outside the limits of tolerance (250 and 800 mg/kg for lead and zinc, respectively) set by the Ontario Ministry of the Environment (Giesy and Hoke, 1990).

The levels of metal pollution found in the River Teifi sediments are comparable to those in the sediments of industrially polluted aquatic habitats. Thus, Che and Cheung (1998) found lead and zinc levels of 100–155 and 226–421 mg/kg, respectively, in the Mai Po marshes in Hong Kong whilst Bubb *et al.* (1991) found lead and zinc levels of 100–155 and 226-421 mg/kg, respectively, in the River Yare, Norfolk.

Heavy metals such as lead and zinc are readily accumulated by invertebrates and hence by their fish predators (Woodward *et al.*, 1994). As low pH conditions favour the mobilisation of heavy metals, such as aluminium, zinc and lead, into the labile, ionic forms (Muniz, 1991) it might be expected that the rate of bioaccumulation and hence toxicity would be enhanced by increased acidity. However, the relationship between pH and metal toxicity is not simple. In the case of copper, for example, although the concentration of the $Cu^{2+}$ ion, which is the major toxic species, increases with increase in $H^+$ concentration its toxicity is reduced by competition from $H^+$ at the active sites (O'Sullivan *et al.*, 1989). It is also noteworthy that high $H^+$ concentrations on their own can also be toxic to aquatic organisms as they disturb acid-base homeostasis and ionic regulation (Wood, 1987).

Although nothing specific can be said about the toxic effects of heavy metals in the sediments as the pore water concentrations are unknown some conclusions can be drawn from the chemical analysis of the water column (Table 1). The total hardness values for both the Pysgotwr and the Teifi are very low (2.5–5.1 mg $l^{-1}$ and 6.5–25.5 mg $l^{-1}$, respectively). As a result Table 1 shows that the environmental quality standards (EQS) for aluminium, zinc, lead and copper (100, 8, 4, and 1 μg $l^{-1}$, respectively) are often exceeded in these base-deficient rivers (Department of the Environment and Welsh Office, 1989).

The potential dangers to the biota of base-deficient water bodies from aluminium toxicity are well documented (Turpenny, 1989; Howells *et al.*, 1990; Merret *et al.* 1991; Department of Water Affairs and Forestry, 1996). Toxicity due to aluminium is most likely to occur during episodic, spate conditions when toxic aluminium and $H^+$ concentrations rise by an order of magnitude whilst $Ca^{2+}$ concentrations, which have an ameliorating effect, fall (Reader and Dempsey, 1989). It is most toxic at pH 6.5–5.0 when the hydroxy forms $Al(OH)_2^+$ and $Al(OH)^{2+}$ predominate but within that range the toxicity is independent of pH (Howells *et al.*, 1990).

The pH values fall to 5.5 and 4.8 in the Teifi and Pysgotwr, respectively, under spate conditions (Table1). It is probable that under these conditions the aluminium concentrations (Table 1) will exceed the target water quality range (5 μg $l^{-1}$), the chronic effect value (10 μg $l^{-1}$) and the acute effect value (100 μg

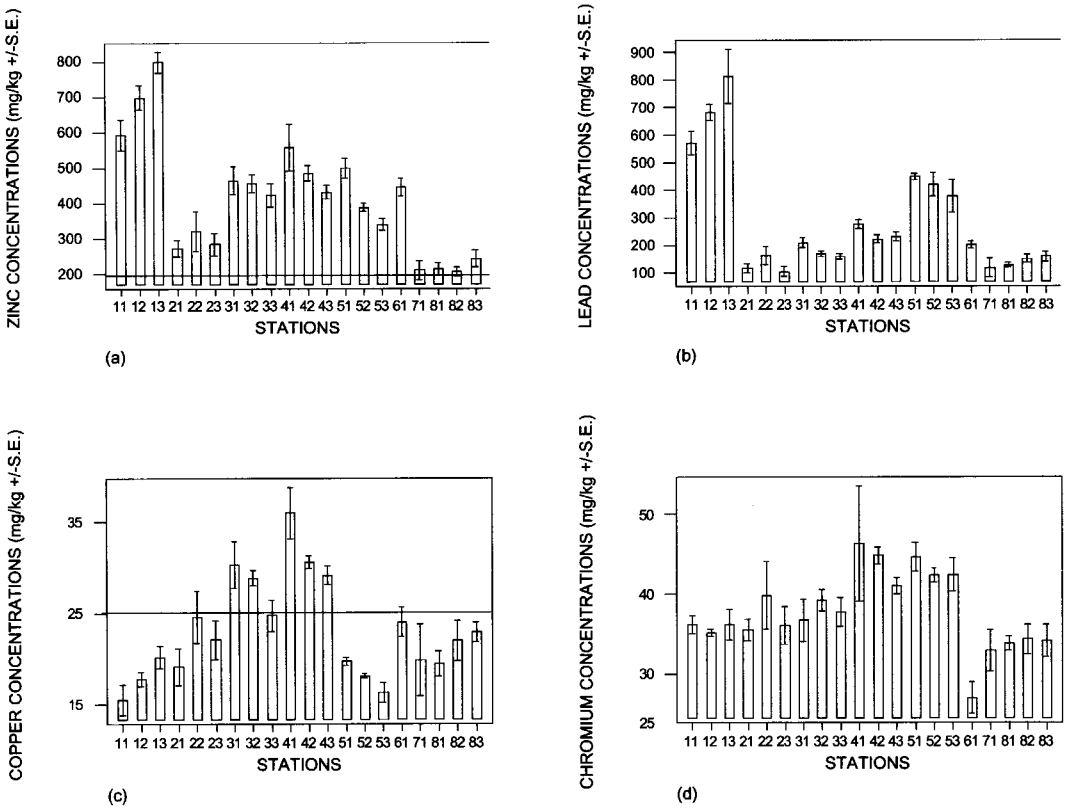


*Figure 24* The mean concentrations of zinc (a), lead (b), copper (c) and chromium (d) in mg/kg ± S.E. in the sediments at the various stations on the Teifi. Key to numbers on the x-axis: the statios (number 1–8) is indicated by the first number; for the second number, 1 refers to the first 0–5 cm of sediment; 2 refers to the sediment at a depth of 5-10 cm; and 3 refers to the sediment at a depth of 5–10 cm. The horizontal lines indicate critical threshold values (see text).

$l^{-1}$) specified by South Africa Water Quality Guidelines (Department of Water Affairs and Forestry, 1996). According to Bodet *et al.* (1988) when the pH is between 4.0 and 5.6 aluminium at concentrations of 100 μg $l^{-1}$ is acutely toxic to fish. The toxic effects peak at pH 5.2–5.6 which are within the ranges for the Teifi and Pysgotwr. Toxicity values given by Turpenny (1989) for rainbow trout are even lower (40 μg $l^{-1}$ $LC_{50}$ 24 h) and sublethal effects in the form of reduced growth rates have been reported for brown trout when the aluminium concentrations were 20 μg $l^{-1}$ (Kelly, 1988). Invertebrates vary in their susceptibility to aluminium toxicity with members of the Cladocera and Ephemeroptera being most vulnerable and the Plecoptera and Sphaeriidae being most resistant (Merret *et al.*, 1991; Wren and Stephenson, 1991). In the case of the Sphaeriidae the apparent aluminium resistance under natural conditions may be due to the bacterial generation of alkalinity in the depositing sediments where they live (J. G. Jones, 1985; Thomas and Love, unpublished data).

In view of the above considerations it seems possible that high aluminium concentrations in time of spate in the base-deficient rivers investigated may have contributed to the decline in both the brown trout and their helminth parasites. However, further research is required to test this hypothesis as inorganic and organic ligands such as humic substances may act as complexing agents and modify aluminium toxicity (Bodet *et al.*, 1988). There is evidence that humic substances (HS) may reduce the toxicity of aluminium to rainbow trout and other fish and that the survival rates of invertebrates, which can ingest HS–metal complexes, may also be enhanced up to a critical threshold (Thomas, 1997). As both the Pysgotwr and the Teifi are dystrophic and therefore rich in humic substances this may help to explain why the fauna appears not to have suffered even more from heavy metal pollution although the brown trout have disappeared from the Pysgotwr.

Other heavy metals, which may have contributed to the decline of the fauna in the Teifi, are zinc, lead and copper which occur at concentrations of 11–109, 2–5 and 1.7–5.0 μg $l^{-1}$, respectively. The corresponding values for the Pysgotwr, which are 2–18, 2 and 1–2 μg $l^{-1}$, respectively are much lower reflecting the lower level of mining activity in this catchment. In contrast, zinc, lead and silver were being mined in the Esgairmwyn, Cwm-mawr and Abbey Consols mines in the upper catchment of the Teifi in the nineteenth century. Carpenter (1926) found that the effluents from the Esgairmwyn mine were causing rapid deterioration in the fauna of the Marchnant, a headwater of the Teifi, and stressed that there was a grave danger of eventual injury to the main river. As pointed out by Jones (1940, 1958) persistence is one of the main characteristics of heavy metal pollution and the problem has remained to the present day. Thus, although much of the main river quality was classed as Grade 1A in 1980 (National Water Quality Survey) some tributaries and the main river upstream of Tregaron were designated Grade 2 because of elevated

concentrations of zinc originating from the derelict mine workings in the upper catchment. More recently, Hall (1998) found that the environmental quality standards (EQS) for zinc, lead or copper were breached on 13 occasions in Nant Lluest, a small tributary of the Teifi, near Strata Florida Abbey, both downstream and upstream of the input from Cwm-mawr mine (SN 736 673). Although the invertebrate fauna appeared unaffected during the brief survey period some of the salmonid fish showed evidence of black-tailing attributable to metal poisoning. In view of Hall's findings it is not surprising to find that the concentrations of zinc, lead and copper in the River Teifi water column (Table 1) often exceed the EQS set by the Environment Agency. These are 8, 4 and 1 $\mu g\ l^{-1}$, respectively, for soft waters with hardness < 50 $mg\ l^{-1}$. As with aluminium the availability of these metals to aquatic organisms increases with decreasing water hardness, pH, alkalinity and calcium concentrations. Their availability therefore tends to increase during episodic spate events (Jones, 1943, 1958; Kelly, 1988). However, although their toxicity increases with decreasing water hardness the relationship is not linear as the efficiency of the carrier-mediated transport system decreases with falling pH because of competition between the metal ions and the $H^+$ ion (Wren and Stephenson, 1991; Gerhardt, 1993; O'Sullivan *et al.*, 1989).

As was the case with the River Ystwyth during its recovery phase (Jones, 1940) the concentration of zinc exceeds that of lead indicating that zinc is more persistent and therefore more likely to be a danger to the aquatic community. This danger is exacerbated by the fact that although zinc is less toxic than copper it has a lower tendency to adsorb. As a result a greater proportion is likely to exist in solution.

Although there is a great deal of toxicological data available for zinc and other metals evaluation is often hampered because of differences in physicochemical conditions (e.g., temperature, water hardness, calcium concentrations and water flow), times, genotype and physiological state of the assay animals. According to the data presented by Kelly (1988) the $LC_{50}$ 21 d zinc values for salmon parr under physicochemical conditions resembling those for the Teifi ranged from 0.34 to 1.45 $mg\ l^{-1}$ of zinc. These compare with values of 0.6-4.76 $mg\ l^{-1}$ $LC_{50}$ 48h for rainbow trout. Both values are above the concentration ranges found in the Teifi. However, according to Jones (1962) zinc may be fatal to the ova and alevins of rainbow trout at concentrations of 10–20 $\mu g\ l^{-1}$ which are within the range of concentrations found in the Teifi.

The majority of the $LC_{50}$ values obtained in toxicity tests for zinc carried out on aquatic invertebrates cited by Kelly (1988) are also generally well above the zinc concentrations found in the Teifi, the exceptions being *Tubifex* and *Daphnia* spp. which appear to be highly susceptible to zinc. Free-living trematode larvae also appear to be vulnerable to zinc. Thus, the cercarial encystment in the case of *Notocotylus attenuatus* and the survival time and infectivity of *Echinoparyphium recurvatum* cercariae were impaired at zinc concentrations

of 4–10 μg $l^{-1}$ and 0.01–10.0 μg $l^{-1}$, respectively (Evans, 1982a, b). More recently it has been shown that the survival of *Schistosoma mansoni* miracidia and cercariae of *Diplostomum spathaceum* are impaired when zinc concentrations exceed 100 μg $l^{-1}$ (Morley *et al.*, 2001a, b). In the case of the latter species it was shown that increasing hardness and decreasing temperature (Morley *et al.*, 2001b) reduced toxicity. Observations by Jones (1940, 1949,1958,1962) in zinc-polluted Welsh rivers showing that oligochaete worms, leeches, molluscs and crustacea are more susceptible to zinc than lithophilous insects are in accord with toxicological data (Kelly, 1988).

The concentrations of lead in the Teifi water column (2–5.4 μg $l^{-1}$) are low compared with more heavily polluted rivers such as the Derwent (65 μg $l^{-1}$; Kelly, 1988) and the Ystwyth (50 μg $l^{-1}$; Jones, 1940). These relatively low concentrations are attributable to the fact that lead pollution is less persistent than that caused by zinc (Jones, 1940). Furthermore, most of the soluble lead tends to be removed by the sediments or by suspended particulate organic and inorganic matter such as hydrous oxides, clays and humic substances. However, although the soluble lead concentrations in the Teifi may appear to be low they fall within the TQR (target quality range) (0.2 μg $l^{-1}$), the CEV (chronic effect value) (0.5 μg $l^{-1}$) and the AEV (acute effect value) (4.0 μg $l^{-1}$) set by the South African Water Quality Guidelines for water with hardness less than 60 mg $l^{-1}$ calcium carbonate (Department of Water Affairs and Forestry, 1996).

It is difficult however to draw any firm conclusions from the above values as the toxicology of lead is complicated by the following considerations. Thus, it can exist in several oxidation states (0, I, II and IV) and also form complexes with organic and inorganic ligands. Lead speciation and hence its biovailability can be influenced by a number of physicochemical factors including pH. Under acid conditions it is the lower oxidation state, Pb (II), that is the most stable under normal oxidising conditions and its solubility is controlled by the availability of the sulphate ion. According to Gerhard (1993) and Wren and Stephenson (1991) there is evidence that lowering pH increases lead uptake by invertebrates and the effect is variable and dependent on species. Furthermore, there is uncertainty regarding how aquatic animals sequester lead. Invertebrate lead levels appear to be largely influenced by sediment lead concentrations and surface adsorption may account for a large proportion of lead in benthic invertebrates (Wren and Stephenson, 1991). When absorbed on mucus covering gills it may induce coagulation and death by suffocation. Although lead bioaccumulates in individual organisms there is no evidence that it biomagnifies in the aquatic food chain (Wren and Stephenson, 1991).

However, it would appear from the acute toxicological data for lead, summarised by Kelly (1988), that the $LC_{50}$ for salmonid fish and aquatic invertebrates are often orders of magnitude higher than the ambient lead concentrations in the Teifi. On the whole therefore the present results would

appear to support the conclusions of Wren and Stephenson (1991) that the concentrations at which lead is acutely toxic to aquatic organisms are generally much higher than ambient concentrations.

A word of caution is needed here however as some taxa such as *Gammarus* species are particularly vulnerable to lead toxicity. Thus, the $LC_{50}$ 28 d value for *Gammarus pseudolimnaeus* was 0.0284 mg $l^{-1}$. In contrast, *Crangonyx pseudogracilis*, which has an $LC_{50}$ 48 h of 43.8 mg $l^{-1}$ for lead, is much more tolerant, which is in common with molluscs such as *Lymnaea* and *Pisidium* species (Wren and Stephenson, 1991). As lead may also induce harmful sub-lethal effects it is possible that these observations on lead toxicity under acidic spate conditions may help to explain the replacement of *Gammarus pulex* by *Crangonyx* in the upper Teifi. Further work on the sub-lethal effects of lead toxicity is indicated particularly as under aerobic conditions methylation of lead can occur. There is evidence that organometallic lead species are often more toxic than inorganic lead species (Kelly, 1988).

Copper is one of the most toxic of the heavy metals. According to South African Water Quality Guidelines the TQR, the CEV and AEV values for copper in soft waters with hardness less than 60 mg $CaCO_3$ are less than 0.3, 0.53 and 1.6 μg $l^{-1}$, respectively (Department of Water Affairs and Forestry, 1996). As the copper concentrations in the water column of the Teifi exceed these values copper toxicity is a potential problem particularly during acidic episodes in times of spate when speciation to the toxic $Cu^{2+}$ is favoured. However, the toxicity of copper is inhibited by the presence of humic acids, which are abundant in these dystrophic waters, and also by amino acids, suspended solids, $H^+$ and other metals. The acute toxicity levels for copper summarised by Kelly (1988) are however more than an order of magnitude higher than the South African guidelines. Thus, the $LC_{50}$ 96 h values for copper, in the case of adult and juvenile rainbow trout in soft, acidic, continuously flowing water were 28.9 μg $l^{-1}$ and 17–38 μg $l^{-1}$, respectively. The metox range for zinc, copper and lead given by Turpenny (1989) as a fraction of the predicted $LC_{50}$ 48 h values for salmonid fish are even higher ranging from 0.26 to 0.49 mg $l^{-1}$. Molluscs are particularly susceptible to copper toxicity and as in the case of salmonids susceptibility declines with maturity. Thus, the $LC_{50}$ 96 h for juvenile *Potamopyrgus jenkinsi* was 0.054 mg $l^{-1}$ compared with 0.079 mg $l^{-1}$ for adults (Kelly, 1988). Evans (1982a, b) also found that the larvae of digenetic trematodes as well as their hosts were adversely affected by sub-lethal concentrations of copper. Thus, a copper concentration of 0.01 μg $l^{-1}$ caused a reduction of snail activity and shedding of *Notocotylus attenuatus* cercariae (Evans, 1982a) while a copper concentration of 0.01–10 μg $l^{-1}$ reduced the survival time and infectivity of *Echinoparyphium recurvatum* (Evans, 1982b).

This review indicates that heavy metal toxicity, involving aluminium, zinc, lead and copper, is potentially a cause for concern for the upper Teifi whereas

aluminium alone seems to be a problem for the upper Towy. It is very important therefore that further research should be undertaken in the field to ascertain the variation in the range of concentration of the toxic metal species encountered in the water column and in the pore water at different seasons and during episodic spate events. This work should be followed up with toxicological studies, under simulated natural conditions, to ascertain possible sub-lethal and acute effects of the heavy metals on salmonid fish and well-selected representatives of the invertebrate fauna. Attention should be given to the possibility of synergistic and ontogenetic effects involving the metals and pyrethroids and also that the fauna may have become habituated to metal toxicity. The development of resistance to metal toxicity is a well-known phenomenon. Thus, Mason (1996) cites the example of *Asellus* having an $LC_{50}$ 48 h of 3.50 mg $l^{-1}$ for lead in contaminated water compared with 0.28 mg $l^{-1}$ in the control river.

The pyrethroids, cypermethrin and flumethrin, derived from sheep dipping (Armstrong and Phillips, 1998) were also present in measurable concentrations of up to 11.7 and 19.6 µg/kg, respectively, in the sediment pore water at Stations 2, 5 and 7 (Figure 25a, b). However, as these pesticides bind readily to minerals and humic substances their bioavailability is reduced except to sediment-feeding organisms. Cypermethrin in the water column is very toxic to the majority of freshwater organisms including mayfly larvae (24 h $LC_{50}$ 0.6 µg $l^{-1}$; Stephenson, 1982), *Gammarus pulex* (24 h $LC_{50}$ 0.009 µg $l^{-1}$; Stephenson, 1980) and fish (96 h $LC_{50}$ 1.2 µg $l^{-1}$ for brown trout; Stephenson, 1982). Molluscs are however less susceptible to cypermethrin as the 96 h $LC_{50}$ for *Lymnaea acuminata* is 0.36 mg $l^{-1}$ ( Singh and Agarwal, 1986). During the time of the present investigation the concentrations of cypermethrin and flumethrin in the water column were less than 0.002 µg $l^{-1}$. However, the Welsh Environment Agency found that 21% of the sites investigated in Welsh rivers during 1997 failed to meet the environmental quality standards for cypermethrin (Environment Agency, Wales, 1998). It is therefore evident that the invertebrate and fish communities in the Teifi, and other rivers where sheep farming is the dominant form of agriculture, are under serious threat from episodic pollution by pyrethroids derived from sheep dipping.

The other underlying anthropogenic reasons for the possible decline in the brown trout populations and their parasites in the Teifi are summarised in Table 29. The environmental factors that are implicated interact synergistically to the detriment of the aquatic environment. The ecological threat posed by acid deposition has been well reviewed by a number of people (Howells and Morris, 1989; Mason, 1992). Unfortunately, the hard, base-deficient, rocks and acidic soils have made the Teifi and the Tywi catchments particularly susceptible to acidification. This tendency has been further exacerbated by changes in land use. These have included afforestation of the catchments of some of the major tributaries with coniferous trees and the loss of hill farms. There is evidence that the pinnate leaves of coniferous trees facilitate the

trapping of acidic aerosols and that their roots further deplete the soil water of base cations already in short supply (Howells and Morris, 1989). Furthermore, the land now occupied by coniferous forests is no longer subject to regular liming as was the case when it was farmed. Detailed evidence that afforestation with coniferous trees has resulted in increased stream acidity followed by other detrimental changes which have eventually caused fish mortality is given by Stoner (1985) and Howells and Morris (1989).

Changes in land use including land drainage leading to the loss of wetland combined with canalisation of parts of the main river and many of the tributaries have caused the river to become more erosive in character. This has

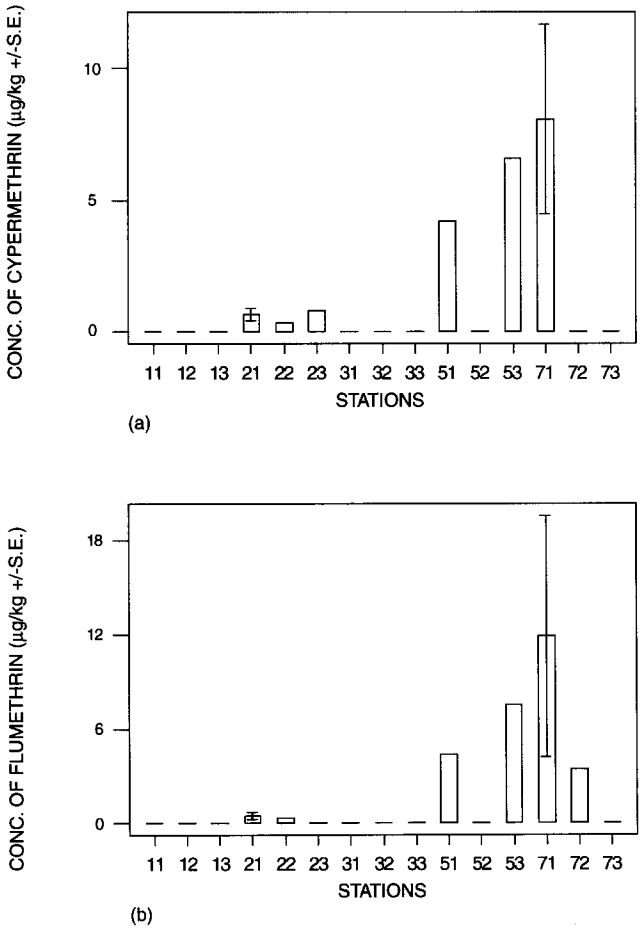


*Figure 25* The mean concentrations of cypermethrin (a) and flumethrin (b) in µg/kg ± S.E. in the sediments at the various stations on the Teifi.

*Table 29* Major factors possibly responsible for decline in brown trout populations and in the component and infra–population indices of the parasite between 1950 and 1998

| Major environmental factors | Consequences |
|---|---|
| **Atmospheric pollution** | |
| Increased acid deposition | Episodic reduction of pH < 5.0 leading to increased mobilisation of toxic heavy metals including Zn, Pb and Al |
| **Engineering perturbations** | |
| Lead mining (up to mid-19th century) | Release of Zn, Pb and other heavy metal ions into catchment |
| Increase in water abstraction | Reduction of flow; particularly serious in dry summers |
| Land drainage; loss of wetland | River becomes more erosive |
| Canalisation of river channels | Loss of habitats and substrate heterogeneity; favours switch from brown trout to migratory trout life history strategy |
| **Changes in agricultural practices** | |
| Reduction in deciduous woodland | Reduction of base cation input into river |
| Loss of hill farms; reduction in liming | Reduction of base cation input into river |
| Afforestation of upper catchments with coniferous trees | Reduction of base cation input into river<br>Increased susceptibility to acid deposition effects |
| Intensification of sheep farming | Land erosion (including river–banks), overgrazing and destruction of protective riparian vegetation such as alder and willow cause increased inputs of Zn and Pb from mine wastes and pyrethroids from sheep dips |
| **Angling management** | |
| Increase in fishing pressure | Decrease in fish density and particularly in proportion of fish in older age classes |
| **Agricultural,domestic pollution** | |
| Episodic releases of farm or domestic organic effluent | May cause $O_2$ depletion and nutrient loading.<br>Latter less harmful and organic effluent possibly beneficial in acidic base-deficient waters |

resulted in a reduction in habitats described as 'dead zones' (Hildrew and Ormerod, 1995) where depositing organic and fine sediment collect. Such microhabitats can be divided into first-order refugia, which include pools, backwaters and slackwater under banks, and second-order refugia that include spaces underneath tree roots and stones. These are vitally important components of lotic ecosystems because they act as refugia for macrophytes, key invertebrates in the food chain, including those that act as intermediate hosts for the helminth parasites of fish, and vertebrates such as ammocoete larvae and salmonid fish.

There is also evidence that the community of microorganisms and invertebrates associated with depositing sediments play a key role in the deacidification of both the sediment pore water and the overlying water. The deacidification process involves alkalinity production by microbes using $NO_3$, $SO_4$, Fe(III), Mn (IV) and various carbon compounds as terminal electron acceptors (J. G. Jones, 1985; Schindler 1986). This mutalistic process is enhanced by the presence of tubiculous chironomid larvae and tubificid worms which cause bioturbation (Pelegri and Blackburn, 1995; Thomas and Love, unpublished data).

Another major factor in causing the decline of the brown trout and their parasites has been the intensification of sheep farming. Between 1950 and 1996 the total sheep population in the UK doubled from 20.4 to 41.5 million (Armstrong and Phillips, 1998). Sheep farming is particularly important in Wales and Scotland as 50–60% of holdings carry sheep. Unfortunately, the sheep can be very harmful to lotic habitats as they destroy and damage valuable riparian trees, such as alder and willow, and cause erosion in the catchment in general and to the banks in particular. As a result the input of allochthonous organic matter is reduced, the banks are destabilised and invertebrates and salmonid fish are deprived of important refugia including those provided by undercut banks and tree roots. Such 'dead zones' are important as they facilitate the retention of silt and organic matter which allow sediments to become anoxic and base formation to occur.

It is possible also that increased erosion resulting from overgrazing by sheep may facilitate the release of heavy metals from soils particularly in the upper catchment of the Teifi where the metalliferous Esgairmwyn, Cwm-mawr and Abbey Consols mines are located. Mine tailings are still present in these mines and as pointed out by Jones (1940, 1958, 1962) one of the outstanding features of pollution by heavy metals is its persistence. There is also some circumstantial evidence that sheep grazing may have contributed to acidification of streams and lakes in Norway as intensification of sheep grazing at higher levels coincided with increased acidification (Mason, 1992).

## 9.4. Helminth Parasites and other Aquatic Organisms as Pollution Bioindicators

As a result of ever-increasing levels of anthropogenic environmental pollution it has become obligatory to monitor physicochemical parameters to evaluate water quality on a routine basis and also when pollution incidents are suspected. However, because of their episodic or transitory nature, pollution incidents may easily be overlooked when using such methods. It has therefore been stressed by many aquatic biologists including Hynes (1960) and Mason (1996) that the physicochemical investigations must be complemented by

using bioindicators under both field and laboratory conditions. Biomonitoring also has the added advantages of making it possible to identify the pollution source and of predicting the possible consequences to the community of the type of pollution involved.

There has been much discussion regarding the kinds of organisms that might be used as bioindicators of pollution. It may be suggested that the following criteria should be met when selecting bioindicators:

1. It should be possible to predict the kind of aquatic community that one might expect in a non-polluted aquatic environment from the relevant physicochemical data and then use these data as a basis for comparison with polluted habitats.
2. The biomonitoring method should be robust and have general application in a range of habitats.
3. The selected organisms should be highly responsive to the particular pollutant or the type of pollution being investigated.
4. The eco-physiological, biochemical and behavioural responses shown by the organisms to the pollutant should be understood.
5. The bioindicators selected should be readily accessible and identifiable.
6. It should be possible to apply the method without causing undue environmental damage.
7. The biotic index used should be cost effective with respect to the resources available.

Many parasitologists including Khan and Thulin (1991), Poulin (1992), MacKenzie *et al.* (1995), Yeomans *et al.* (1996), Kennedy (1997), Chubb (1997) and Landsberg *et al.* (1998) have discussed the possibility of using fish parasites as bioindicators of pollution. Unfortunately, although fish parasites, in common with other aquatic organisms, may respond to pollution, the arguments presented below show that they are not ideal bioindicator species.

As pointed out by Kennedy (1997) fish parasites fail to satisfy even the first of the above criteria. Thus, Hartvigsen and Kennedy (1993) state that 'the predictive value of their studies have foundered on the erratic and unpredictable occurrence of many fish helminth species'. This situation led Kennedy (1978a, b, 1981, 1985) and Kennedy *et al.* (1986) to argue that helminth communities in freshwater fish are fundamentally stochastic assemblages, their composition being dependent on chance introductions of parasites into localities and on chance colonisation and extinction events. These arguments are supported by Esch *et al.* (1986) and Dobson (1990).

While it is to be expected that the free-living stages of the parasites would be highly susceptible to pollutants this does not mean, unfortunately, that they are ideal candidates for biomonitoring for the following reasons. Firstly, parasites are renowned for their exceptionally high rates of reproduction. As

reproduction may be spread over a long period it may well not be seriously affected by pollution, as this is often episodic in nature. Furthermore, once the endoparasites have entered their intermediate or definitive hosts they receive a measure of protection from the direct effects of environmental pollution. Although fish ectoparasites will potentially be in greater danger than endoparasites from exposure to pollutants mucus secreted by the host will provide them with some protection. In addition the high level of mobility of the host fish enables them to move away from heavily polluted areas. As a result it is possible to envisage metapopulations at different stages of development surviving in spatially discontinuous habitats despite pollution. In the case of digenetic trematodes the quantification of the suprapopulation, which includes all developmental phases of a parasite at a particular time and place, is an extremely difficult task, particularly in cases where all the definitive and intermediate hosts have yet to be identified. The life history strategies adopted by helminth parasites help to explain their evolutionary success. Unfortunately, as this tends to makes them less responsive to pollution than free-living organisms their use as bioindicators is further contraindicated.

Unlike free-living organisms relatively little is known about the eco-physiological, biochemical and behavioural responses shown by the various stage in the life cycle of the parasites to the pollutants. This is mainly due to the short life span of the free-living stages and the difficulty of culturing the parasitic stages. As a result it is difficult to predict how the various phenotypes in the life cycle might respond to particular pollutants.

The use of fish parasites as bioindicators of pollution also fails to satisfy the fourth criterion, namely that the bioindicators selected should be readily accessible, widely distributed and identifiable. Unlike free-living invertebrates, fish hosts such as trout and hence their parasites tend to be restricted to the upper reaches of rivers and are replaced by coarse fish in the lower reaches. As fish parasites generally exhibit overdispersed distributional patterns it is necessary to sample a large number of fish in order to obtain reliable data. Identification may also be difficult as sibling species such as *C. farionis* and *C. metoecus* may coexist as adults. Larval stages generally present even greater problems with identification.

Unfortunately, the sampling of fish, such as trout, in order to obtain parasitological data to monitor pollution is unacceptable as like other salmonid species they are under threat of extinction. Furthermore the gathering of such data would be very time consuming and not cost effective with respect to resource availability. It must be concluded therefore that as none of the above criteria are met the possibility of using fish parasites for biomonitoring pollution is hard to justify.

It is not surprising therefore that other biotic indices, such as the Saprobien index (Mason 1996), the BMWP indices (Mason, 1996) and those based on the River Invertebrate Prediction and Classification System (RIVPACS) (Wright *et*

*al.*, 1993) which meet the above criteria are preferred. All these indices were developed to monitor the effects of organic pollution, which has become an ever-increasing problem due to rising human population, farming intensification and the disposal of human sewage into river systems. The Saprobien index recognises four stages in the oxidation of organic matter: polysaprobic, α-mesosaprobic, β-mesosaprobic and oligosaprobic. It is taxonomically demanding because it makes use of 2000 taxa which are individually graded on a 1–8 scale, based on increasing tolerance to organic pollution, and 1–4 on the basis of relative abundance. Although widely used in continental Europe it has received little support in Britain or America. It is not surprising therefore that it has been superseded by the BMWP and RIVPACS indices for biomonitoring organic pollution in lotic ecosystems. As shown by Metcalfe (1989) these indices and other related forms have evolved from the Trent biotic index.

Table 30 shows that all the scores for Station 1 in the Teifi bog area are lower than at other stations further downstream. However, the scores for Station 1 although relatively low are still indicative of good water quality whereas those for the other stations are exceptionally high, particularly those where the BMWP and average score per taxon (ASPT) scores exceed 200 and 6.0, respectively. There are two possible explanations for the relatively low biotic indices for the bog area site. Firstly, they may be attributable to the toxic effects of heavy metals, including zinc and lead, which have been shown to be present at higher concentrations in the sediments in the bog area than further downstream. Secondly, the lower values in the bog area may be the result of lower habitat diversity compared with the downstream sites.

Despite the fact that the scores for Station 1 are within acceptable Environment Agency standards the lower indices and the higher levels of heavy metals in the sediments compared with downstream stations are clearly causes for concern. In view of this it may be suggested that the Environment Agency should focus more on both chemical and biomonitoring of sediments in areas which are susceptible to pollution by heavy metal or organic toxicants. The existing methods used by the Environment Agency rely heavily on monitoring

*Table 30* The number of aquatic invertebrate families encountered, the Biological Monitoring Working Party (BMWP) score and the average score per taxon (ASPT) obtained for samples taken at the various stations in the upper Teifi in May, 1998

| | Station number | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 6 | 7 | 8 |
| Number of families | 21 | 37 | 33 | 26 | 29 | 32 | 32 |
| BMWP score | 121 | 230 | 223 | 161 | 178 | 190 | 213 |
| ASPT score | 5.8 | 6.2 | 6.8 | 6.2 | 6.1 | 5.9 | 6.7 |

potentially toxic substances in the water column and on the use of biotic indices based on BMWP, ASPT and RIVPACS scores. As heavy metals and organic toxicants tend to accumulate in sediments (Thomas, 1997) their importance may be seriously underestimated when only the water column is monitored. The biotic indices currently used by the Environment Agency are also inappropriate for monitoring the effects of metal pollution as they were designed to identify cases of organic pollution. Consequently, they give a great deal of weight to oxygen-dependent, rheophilous, riffle-inhabiting organisms whereas sediment dwellers, which are likely to encounter higher concentrations of heavy metals, are virtually ignored. It is suggested therefore that the Environment Agency should routinely monitor sediments for heavy metals and organic toxicants and also develop biomonitoring methods that give greater weight to sediment-dwelling organisms known to be susceptible to heavy metal pollution. These include oligochaete worms, chironomid larvae, amphipods, bivalve molluscs and ammoecete larvae (Gerhardt 1993; Phipp *set al.*, 1995).

## 9.5. Fish Parasites as Sources of Information on Pollution

As stated above it is difficult to use parasites as bioindicators of pollution because of the complexity and variability of their interrelationships with the environment. Despite these limitations various parasitologists have suggested that they may have some informational value as bioindicators of the following forms of pollution.

*Heavy metal pollution.* As heavy metals often bioaccumulate at higher concentrations in the parasites than in their fish hosts several authors (Riggs and Esch, 1987; Sures *et al.*, 1994; Chubb, 1997; Landsberg *et al.*, 1998) have advocated their use as sensitive biomarkers.

*Acidification.* Cone *et al.* (1993) found that the parasite communities in the American eel, *Anguilla rostrata*, inhabiting acidic water bodies with pH values of 4.5–5.0 were characterised by the absence of digenetic trematodes, lower species richness and fewer multiple infections compared with those in less acidic, limed waters. Subsequent studies by Marcogliese and Cone (1996) on the helminth parasites of eels show that component community diversity, as measured by species richness, Shannon–Wiener index and Hill's number, decreased when the pH was less than 5.4. These authors argued therefore that the parasitic helminth fauna reflects differences in the food webs in these contrasting habitats and conclude that parasitic assemblages may be good indicators of environmental stress. These findings are in accord with those in the present investigation as the parasitic community of the trout from the Pysgotwr, the most acidic river, had the lowest species diversity indices.

*Eutrophication.* In the initial stages an increase in eutrophication of lentic waters favours the growth of phytoplankton populations and hence those of

organisms along the food chain which benefit from this including zooplankton, planktivorous fish and piscivorous birds. It is not surprising therefore that Kennedy *et al.* (1994), Kennedy (1995) and Yeomans *et al.* (1996) found that increased levels of eutrophication were positively correlated with population increases in fish parasites such as *Ligula* and trichodinids, respectively. One might also predict that communities of fish parasites would become more species rich and diverse with eutrophication increasing up to a certain level and then declining as the water bodies become hypertrophied.

*Organic pollution.* This may take the form of increased loading with waste organic matter of human or animal origin or organic pesticides. The former harm the environment by oxygen depletion whereas the latter act as toxicants. Yeomans *et al.* (1996) and Landsberg *et al.* (1998) discuss the possibility of using trichodinids as indicators of enhanced organic loading. However, as pointed out by MacKenzie *et al.* (1995) many of the studies associating changes in fish parasites with the effects of specific pollutants have been speculative and inconclusive.

Many authors, including MacKenzie *et al.* (1995), have suggested that fish parasites would be ideal bioindicators of specific pollutants, as they would be expected to be highly sensitive. However, it has also been suggested (Kuperman, 1993) that the monogenetic fluke, *Diplozoon paradoxum*, and the cestode *Caryophyllaeus laticeps* are suitable bioindicators of pollution by chemical products of the coal tar industry in Russia because of their insensitivity to the toxicants. As a result the parasites are found to be more abundant in the polluted zones. However, this author does not appear to have considered the possibility that this phenomenon might be attributable to the fish immunity being lowered by the toxicants. There are many conflicting reports regarding the effects of pollution on disease prevalence and mortality. Some of these indicate that pollution results in an increase in disease prevalence and mortality whereas others come to the opposite conclusion (Williams and Jones, 1994; MacKenzie *et al.*, 1995; Landsberg *et al.*, 1998). It is not therefore possible to generalise and further work on specific cases is needed before any firm conclusions can be drawn. The dissemination of pathogenic parasites by the stocking and dumping of fish may also be regarded as another form of pollution (Kennedy, 1975; Williams and Jones, 1994).

## 10. INTERACTIONS BETWEEN SPECIES OF HELMINTH PARASITES IN FISH

### 10.1. Introduction

The question of whether interspecies competition may be involved in regulating population densities (Figure 1) and influencing the course of evolution

remains a controversial subject in ecology in general and in helminthology in particular (Rohde, 1998; Vidal-Martinez and Kennedy, 2000). In order to resolve this question it is necessary to define the term clearly. Interspecies competition is best defined as any interaction between two or more populations, which adversely affects their growth and survival. This definition is based on the effects caused by the interactions and does not incorporate the causative mechanisms initially as this can lead to confusion. These effects may be caused either by a 'free-for-all', exploitative ('scramble') competition for food or as a result of contesting space, with its associated resources. The various strategies used for contesting space fall under the heading of interference competition and include threat, direct physical aggression, territorial marking with scents and the release of antagonistic, allelopathic substances or antibiotics that may repel or harm other species. Parasites may also manipulate the host to harm other members of the parasite community indirectly by stimulating cross-immunity to which they remain partially or completely resistant. At the intraspecies level this phenomenon is known as concomitant immunity.

Evidence that interspecies competition is occurring in nature may be direct or circumstantial and can be classified under the following headings:

a. Direct evidence. This is based on the observation that the biological fitness of both species is adversely affected when they are coexisting. Usually this criterion can only be satisfied by experimentation.
b. Circumstantial evidence.
    1. Niche diversification including resource partitioning in space or time, habitat segregation or interactive site selection when the species coexist.
    2. Character displacement or morphological differences particularly in those anatomical features concerned with feeding.
    3. Competitive exclusion of a species by another in the same feeding guild.
    4. Extension of a species range following the expansion of its feeding niche.

The evidence of competition among helminth parasites of fish is reviewed below using these criteria.

## 10.2. Evidence of Interspecies Competition

### *10.2.1. Direct evidence*

In a pioneering study Holmes (1961, 1962) demonstrated experimentally that interspecies competition occurred between *Hymenolepis diminuta* and *Moniliformis moniliformis* (= *dubius*) in rats. Since then similar studies have confirmed that interspecies competition also occurs between other helminth

species under experimental conditions (Mapes and Coop, 1971; Silver *et al.*, 1980; Holland, 1984; Bates and Kennedy, 1990). The latter authors studied interactions between the acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in experimentally infected rainbow trout and found that the establishment of both species was unaffected by the presence of the other species. However, it was found that after establishment had occurred *A. anguillae* was numerically disadvantaged by the presence of *P. laevis* whereas the converse was not the case. This type of extreme, asymmetrical interaction should therefore be classed as ammensalism rather than competition. According to Bates and Kennedy (1990) the most tenable explanation for this phenomenon is that it is caused by an exclusion zone around individuals of *P. laevis*, possibly mediated by the host, in which the survival of *A. anguillae* is reduced. They therefore suggested that in rivers where *P. laevis* dominates fish infracommunities, interspecies competition, or ammensalism, might make it impossible for *A. anguillae* to become established. This suggestion can be extrapolated to other acanthocephala species as Kennedy (1985, 1992) showed that examples of co-occurrence of two or more acanthocephala species within freshwater localities in Britain were far fewer than expected.

### *10.2.2. Circumstantial evidence indicating interspecies competition*

In order to evaluate this evidence it is necessary to consider the concept of the niche. The term niche is best considered as a multidimensional space, determined by a large number of physical and biotic variables, within which species exist (Hutchinson, 1957). Each dimension represents a factor that influences the biological fitness of the species. In this context it is best not to make a distinction between parameters which benefit the organism directly, such as food resources, and those which may harm it such as allelopathic compounds or predators. However, the parameters that are given most attention in parasite ecology are the species, age and sex of the host, microhabitats, time and nutrient requirements. Microhabitat selection may be based on long-term, genetically fixed behavioural patterns (habitat segregation) or on short-term, interactive site selection induced by the presence of a potential competitor. The circumstantial evidence indicating that interspecies competition may be occurring among fish parasite communities is discussed below.

### *10.2.3. Habitat segregation*

Although 12 species of helminth parasites occurred in the trout only six, namely *D. sagittata, C. farionis, C. metoecus, P. simile, N. rutili and C. truttae* were commonly encountered and the present analysis is restricted to them. Two of

these, *D. sagittata* and *P. simile*, which occur on the gills and in the urinary bladder, respectively, are completely isolated from the gut-inhabiting species and cannot therefore interact with them directly. It is also unlikely that cross-immunity will influence the survival of these spatially segregated species.

Competitive interactions are however possible between the four gut-inhabiting species. Although these are restricted to the region behind the stomach there is evidence that they have habitat preferences which are probably based on genetically fixed behavioural patterns (Figure 26). Thus, both *C. metoecus* and *C. trutta* occurred predominantly in the pyloric caeca region of the intestine, while *C. farionis* and *N. rutili* favoured the post-pyloric region. As the available surface area of the fish intestines is relatively small the mean densities of helminth parasites per square metre are relatively high. These were estimated to be 10 000, 10 000, 14 000 and 16 000 for 1-, 2-, 3- and 4-year-old trout, respectively. These values compare with maximum densities of 54 000, 46 000, 77 000 and 61 000 for 1-, 2-, 3- and 4-year-old trout, respectively. It is noteworthy that the parasite densities per square metre vary little with age despite the fact that the mean parasite abundance values (19.6, 24.4, 48.3 and 56.6 for 1-, 2-, 3- and 4-year-old fish, respectively) increase markedly with the age of the fish. This observation is attributable to the fact that the surface area of the gut increases as the fish grows. The fact that density changes are minimal with age suggests that space may be a limiting factor. These high densities, which are indicative of a resource-rich habitat where pre-digested food is continuously available, exceed those of free-living, benthic

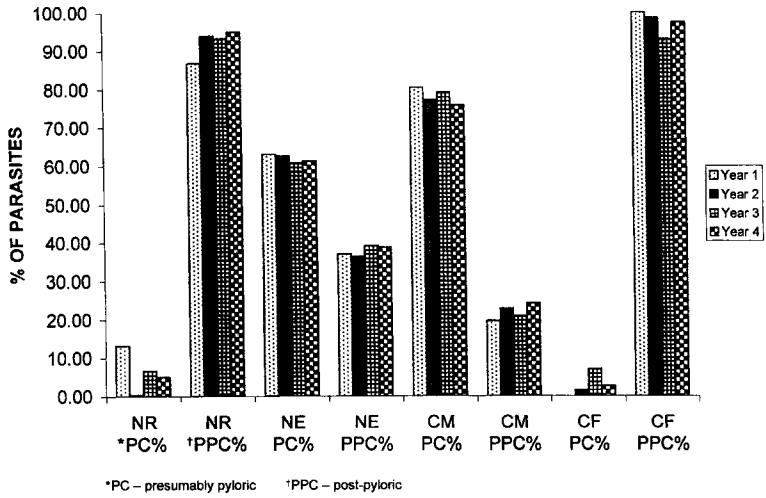


*Figure 26* The distribution patterns in percentages of the major parasites (key as in Figure 19) in the pyloric (PC) and post-pyloric (PPC) region of the trout gut.

invertebrates, such as those in the River Wye (500–22 000 per $m^2$ according to Edwards and Brooker, 1982).

According to Read (1956) and Smyth (1962) the pyloric caeca region immediately posterior to the pyloric sphincter is the most favourable microhabitat for the gut parasites of salmonid fish. It is relatively calm and as the pancreas opens into it it is rich in digestive enzymes and highly nutritive food. In contrast the contents of the post-pyloric caeca region are subject to greater movement and have a lower nutrient content. These changes along the length of the intestine resemble those described for other teleost fish with pyloric caecae such as the green sunfish (*Lepomis cyanellus*) (Richardson and Nickol, 1999). These authors found that protein, free amino acid, lipid and carbohydrate concentrations, pH and aminopeptidase activity were all higher in the caecae than in the intestinal region and suggest that this was the reason for the caecae of *L. cyanellus* being the preferred habitat of the acanthocephalan *Leptorhynchoides thecatus*. According to Kennedy (1996) the pyloric caeca is also the preferred site for *Eubothrium crassum* in the brown trout and rapid growth of this parasite only occurs in this region of the gut.

In the present investigation it is mainly *C. metoecus* and the nematode *C. truttae* which occupy what appears to be the optimal region in the gut for parasites, namely the pyloric caeca region (Figure 26). As these species are not congeneric the extent of niche overlap will be less than for congeneric species such as *C. metoecus* and *C. farionis*. Thus, *C. metoecus* and *C. truttae* occupy different microhabitats with the former species moving freely in the pyloric lumen whereas the latter is generally attached to the mucosa by its peribuccal teeth. It is therefore possible that both *C. metoecus* and *C. truttae* will have a competitive advantage over *N. rutili* and *C. farionis,* as the latter tend to occupy the less favourable post-pyloric region of the gut. To what extent these distributional patterns are due to innate behavioural patterns or to short-term behavioural responses due to the presence of potential competitors remains to be elucidated experimentally. However, Chappell (1969) has demonstrated active site selection in the cases of *Proteocephalus filicollis* and *N. rutili* in the stickleback. It appears that the former species has a competitive advantage over the latter as when the two species coexist the favoured anterior location in the gut is occupied by *P. fillicolis* whereas *N. rutili* attaches more posteriorly. In contrast, in single-species infections the distribution becomes much more widespread in the gut.

### *10.2.4. Associations between different pairs of helminth species in infracommunities*

This aspect of parasite community structure has received a great deal of attention (Anderson and Valtonen, 1990; Lotz and Font, 1991, 1994; Poulin, 2001a).

$\chi^2$ tests were carried out on the infrapopulations of the trout collected in the present investigation to ascertain whether there were statistically significant tendencies for parasite species to coexist or not. The results (Table 31), show that in nearly all cases the associations are significantly positive ($P < 0.05$). The only exceptions to this rule are provided by *N. rutili/C. metoecus* and *N. rutili/C. farionis* as in these cases the associations are negative although the $\chi^2$ value is only statistically significant ($P < 0.05$) in the former case. Rohde (1991) also found when investigating monogenetic parasites in marine fish that 35 of the statistically significant associations found were positive compared with two negatives. However, care is necessary in evaluating such data as a high proportion of rare parasite species, with a low prevalence in the component community, can produce an excess of spurious negative associations, whereas a large proportion of common species can lead to an excess of positive associations (Lotz and Font, 1994).

When the trout were segregated into age groups the *N. rutili/C. farionis* associations were found to be significantly negative ($P < 0.05$) in the case of 4-year-old trout. The associations between *C. metoecus/C. truttae* were also found to be significantly negative ($P < 0.05$) in the case of 3-year-old trout (Thomas, 1964a).

Regression analyses, using transformed numbers of trout parasites, also indicate that with the exception of *N. rutili/C. farionis* and *N. rutili/C. metoecus*, the pairs of parasite species tested exhibit statistically significant positive associations (Table 32). Neither of the above negative associations was statistically significant ($P > 0.05$).

Results obtained from $\chi^2$ tests, using $2 \times 2$ contingency tables, on infrapopulations of parasites in salmon parr of all ages resemble those for trout as with the exception of *D. sagittata/C. farionis* all the associations were positive (Table 33). However, only the positive associations involving the nematodes and the other parasite species and *N. rutili/C. farionis* are statistically significant

*Table 31* The $\chi^2$ results and $P$ values of $2 \times 2$ contingency tests for the parasites of the brown trout

| | *D. sagittata* | | *C. farionis* | | *C. metoecus* | | *P. simile* | | *N. rutili* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parasites | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| *D. sagittata* | | | | | | | | | | |
| *C. farionis* | +4.89 | 0.027 | | | | | | | | |
| *C. metoecus* | +17.65 | 0.000 | +34.33 | 0.059 | | | | | | |
| *P. simile* | +12.20 | 0.000 | +3.35 | 0.067 | +31.28 | 0.000 | | | | |
| *N. rutili* | +23.47 | 0.000 | –0.913 | 0.339 | –5.451 | 0.020 | +47.8 | 0.000 | – | |
| Nematodes | +40.18 | 0.000 | +5.76 | 0.016 | +0.400 | 0.529 | +47.63 | 0.000 | +263.39 | 0.000 |

*Table 32* The regression coefficients (*C*), *F* and *P* values, based on transformed values, as measures of positive (+) or negative (–) associations between the parasites of brown trout

| | *D. sagittata* | | | *C. farionis* | | | *C. metoecus* | | | *P. simile* | | | *N. rutili* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C* | *F* | *P* | *C* | *F* | *P* | *C* | *F* | *P* | *C* | *F* | *P* | *C* | *F* | *P* | |
| *D. sagittata* | | | | | | | | | | | | | | | |
| *C. farionis* | +0.083 | 7.69 | 0.004 | | | | | | | | | | | | |
| *C. metoecus* | +0.065 | 56.11 | 0.000 | +0.062 | 52.6 | 0.000 | | | | | | | | | |
| *P. simile* | +0.131 | 28.28 | 0.000 | +0.053 | 4.79 | 0.029 | +0.503 | 39.15 | 0.000 | | | | | | |
| *N. rutili* | +0.051 | 20.91 | 0.000 | –0.001 | 0.02 | 0.892 | –0.040 | 1.19 | 0.276 | +0.075 | 33.39 | 0.000 | | | |
| Nematodes | +0.098 | 57.77 | 0.000 | +0.046 | 13.05 | 0.000 | +0.089 | 4.20 | 0.041 | +0.095 | 38.55 | 0.000 | +0.422 | 218.3 | 0.000 |

($P < 0.05$). Regression analyses using transformed numbers of salmon parr parasites also show statistically significant positive relationships between *C. truttae/D. sagittata*, *C. truttae/C. farionis* and *C. truttae/N. rutili*. In addition *C. farionis/C. metoecus* are significantly positively associated ($P = 0.008$) whereas *C. metoecus/C. truttae* are significantly negatively associated ($P < 0.024$) (Table 34).

Regression analysis was also applied to analyse the nature of the associations between *C. metoecus*, *C. farionis*, *C. truttae* and *N. rutili* in the pyloric and post-pyloric regions of the trout intestines. The trout were segregated into 11 blocks on the basis of age and season (Thomas, 1964b). The results from $\chi^2$ tests (Table 35) show the associations between *C. metoecus/C. farionis*, *C. metoecus/N. rutili* and *C. metoecus/C. truttae* in the pyloric caeca region were significantly negative except for one positive case involving *N. rutili* and *C. metoecus*. The only other significant positive relationship was that involving *N. rutili/C. truttae* in the pyloric region. In contrast, all the other statistically significant associations involving *C. metoecus/N. rutili*, *C. farionis/N. rutili* and *C. farionis/C. truttae* in the post-pyloric region were negative.

*Table 33* The $\chi^2$ results and *P* values of 2 × 2 contingency tests for the parasites of the salmon parr.

| | *D. sagittata* | | *C. farionis* | | *C. metoecus* | | *N. rutili* | |
|---|---|---|---|---|---|---|---|---|
| Parasite | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| *D. sagittata* | | | | | | | | |
| *C. farionis* | –0.013 | 0.908 | | | | | | |
| *C. metoecus* | +0.186 | 0.667 | +3.574 | 0.059 | | | | |
| *N. rutili* | +0.232 | 0.630 | +4.044 | 0.044 | +1.785 | 0.181 | | |
| Nematodes | +11.329 | 0.001 | +6.974 | 0.008 | +10.523 | 0.001 | +5.080 | 0.024 |

*Table 34* The regression coefficients (*C*) and the *P* values, based on transformed values, as measures of positive (+) and negative (–) associations between parasites of salmon parr

| | *D. sagitatta* | | *C. farionis* | | *C. metoecus* | | *N. rutili* | |
|---|---|---|---|---|---|---|---|---|
| | *C* | *P* | *C* | *P* | *C* | *P* | *C* | *P* |
| *D. sagitatta* | | | | | | | | |
| *C. farionis* | –0.022 | 0.703 | | | | | | |
| *C. metoecus* | –0.001 | 0.921 | +0.079 | 0.008 | | | | |
| *N. rutili* | +0.007 | 0.278 | +0.030 | 0.094 | +0.050 | 0.160 | | |
| Nematodes | +0.049 | 0.001 | +0.091 | 0.029 | –0.186 | 0.024 | +0.487 | 0.000 |

A consideration of the life history strategies can help to explain the large number of significant positive associations between the parasites. As explained earlier the transmission sites for all the helminth species tend to be located in microhabitats where the currents are relatively low and where the sediments are depositing. The low current speed is favourable for oncomiracidial transmission and the intermediate hosts of the other helminth parasites live in depositing sediments. This helps to explain why the monogenetic trematode, *D. sagittata* tends to be positively associated with the other species. Overlapping transmission sites may also explain other cases of positive associations between helminth parasites in salmonid fish including *N. rutili* and *C. farionis* (Dorocu *et al.*, 1995). However it is important to bear in mind that positive associations do not rule out the possibility that competition may be occurring between the coexisting parasites.

When all the trout are taken into account the only parasites which were significantly negatively associated were the most abundant parasites, namely *C. metoecus* and *N. rutili*. In other cases when the entire gut is taken into account the significant negative associations involve parasites which tend to occupy the same region of the gut such as *C. metoecus/C. truttae* and *N. rutili/C. farionis* in the trout and *C. metoecus/C. truttae* in the salmon parr. On the other hand when the pyloric and post-pyloric regions of the gut are considered separately significant negative associations are also found between parasites which may tend to coexist in the same region of the gut or not. When taken together these results strongly suggest the existence of interspecies competition between the helminth parasites in the gut of salmonid fish.

Other negative associations involving helminth parasites which have been reported include tapeworms and acanthocephalids in the gut of *Coregonus albula*, the monogenean *Octobothrium merlangi* and the copepod *Clavella*

*Table 35* A summary of the results of regression analyses carried out on the numbers of parasites (log (1 + *x*) transformed) of the brown trout located in the pyloric region and post-pyloric region of the intestine

| | Pyloric caeca region | | | Post-pyloric caeca region | | |
|---|---|---|---|---|---|---|
| | C.M. | C.F. | N.R. | C.M | C.F. | N.R. |
| C.M. | | | | | | |
| C.F. | – | | | | | |
| N.R. | – – + | 0 | | – | – – – | |
| C.T. | – | 0 | + | 0 | – – | 0 |

The trout were segregated on the basis of age and season into 11 blocks. +, –, indicate the number of statistically significant negative and positive correlations respectively.
0, no significant correlations.
C.M. = *C. metoecus*, C F. = *C. farionis*, N.R. = *N. rutili*, C.T. = *C. truttae*).

*devastatrix* in *Gadus merlangi* and the monogenean *Ancyrocephalus vanbenedeni* and the copepod *Ergasilus nannus* in *Mugil saliens* (Dogiel, 1961). What appear to be clear examples of competitive exclusion have also been reported in the case of parasites in four species of chimaerid ratfish. Each of these species contains two different species of the cestodarian genus *Gyrocotyle* at the component population level (Whitfield, 1979). However, at the infrapopulation level only one parasite species is generally found as the smaller species is competitively excluded by its larger cousin (Whitfield, 1979). More recent examples of negative associations between helminth parasites of fish include the congeneric monogenean species *Protopolystoma fissilis* and *P. simplicis* in the urinary bladder of the toad, *Xenopus wittei* (Jackson *et al.*, 1998), and *Neoechinorhynchus golvani* and *Spirocamallanus rebecae* in the cichlid fish, *Cichlasoma synspilum*, in southeastern Mexico (Vidal-Martinez and Kennedy, 2000) and *E.crassum* and *C. metoecus* in the brown trout (Kennedy and Hartvigsen, 2000). These results indicating habitat segregation provide strong circumstantial evidence that interspecific competition between helminth parasites may be a common occurrence.

### *10.2.5. Evidence based on community structure: null hypothesis based on the concept of nestedness*

The analysis of the degree of order of species assemblages in terms of nested subsets has received a great deal of interest over the last decade. In the context of parasite communities a nested pattern occurs when parasite species found in depauperate infracommunities represent non-random subsets of progressively richer ones. This implies that the distribution of different parasite species among host individuals is not mutually independent (Poulin and Valtonen, 2001). To complicate the picture there is also evidence of antinested patterns. This corresponds to situations in which parasite populations are always absent from infracommunities richer than the depauperate communities in which they occur (Poulin and Guégan, 2000). The latter authors found that antinestedness was as common as nestedness and that there was a continuum from one to the other.

It has been argued that as nestedness is a departure from randomness it is indicative of structuring processes at work and that one of these might be interspecies competition. Poulin (2001a) has however stressed that although this might be the case it does not reveal the causative mechanism. In fact Poulin and Valtonen (2001) have shown that variation in fish size and its effect on parasite accumulation are sufficient to generate nestedness. They concluded that as nested patterns are more a consequence of the way parasites were accumulated by the host rather than of the ecological processes acting among the parasite species, they have limited use in parasite community studies. These processes are very different from those that generate nestedness in free-living

communities. According to Tokeshi (1999) nested distribution in free-living communities is more likely to be seen in a set of species with complete niche partitioning with respect to microhabitats, possibly indicating past but not present competition. The case for using nested patterns in parasite community studies is futher weakened by the questionable validity of statistical methods used to test for deviations from random expectations (Jonsson, 2001). Poulin and Valtonen (2001) found that nestedness was not a common pattern when using the rather conservative Bonferroni correction.

### *10.2.6. Evidence based on community structure: saturation models of parasite communities*

Dove (1999) proposed an index of interactivity based on the nonlinear relationship between the number of hosts examined and the estimated component community richness. The number of recorded parasite species increases with the number of hosts examined until an asymptote corresponding to the true component community richness is reached. By fitting a growth curve to the observed data, Dove (1999) was able to create two parameters, the asymptote and the gradient of the curve before the asymptote. This gradient, which is related to the mean infracommunity richness, becomes steeper as it approaches the actual component community richness. He therefore suggested that the product of the two terms could provide a measure of the degree of interactivity in parasite communities with high values indicating interactive communities. However, according to Poulin (2001a, b) Dove's (1999) index requires further testing before it can be accepted.

The cumulative relationships between infracommunity and component community richness have also been investigated by a number of people including Srivastava (1999). There are two possible scenarios. In the first case the relationship is linear suggesting that the number of species in the infracommunity is generally proportional to the number available in the component community and that species interactions have a negligible effect. The curvilinear relationship in the second case suggests that the infracommunity richness becomes increasingly independent of the component community richness as the latter increases. This result suggests that species saturation is taking place in the infracommunities and that species interaction may be occurring. Poulin (1998) and Kennedy and Guégan (1996) provide examples of cases conforming to the first and second scenarios, respectively. However, both Rohde (1998) and Srivastava (1999) have pointed out that curvilinear relationships can result from processes other than competition. Experimental verification is therefore necessary before it can be concluded that interspecies competition is occurring.

## 10.3. Mechanisms of Competitive Interactions

The strength of exploitative competition at the interspecies level will be nullified to some extent by character displacement, niche diversification, climate and population regulation by intraspecific competition, predation and hyperparasitism. However, before it can be fully assessed it will be necessary to obtain more detailed information regarding the nutritional requirements of the parasites at the biochemical level.

One interesting feature of the numerical or functional responses of adult helminth parasites to coexistence is that many are asymmetrical in nature. Typically one helminth species incurs severe reduction in numbers whilst the other species is unaffected (Silver *et al.*, 1980; Dash, 1981; Holland, 1984; Bates and Kennedy, 1990; Poulin, 2001a). Such results strongly suggest interference competition rather than exploitative competition. Interference competition has also been invoked to explain why the presence of a *Gyrocotyle* species in particular species of chimaerid ratfish results in the virtual exclusion of other congeneric species (Williams *et al.*, 1987). More recently, it has also been suggested that the high prevalence, uniform distribution and the presence of exactly one male and one female of the nematode, *Zonothrix columbianus*, in many of the individual beetle (*Tropisterus columbianus*) hosts is attributable to interference competition (Adamson *et al.*, 1992). Strong asymmetrical numerical responses are also the norm in the case of larval digenetic species in molluscs with the dominant larvae causing substantial reductions in the abundance of the subordinate species (Kuris and Lafferty, 1994).

The nature of the chemicals involved in interference competition remains to be elucidated. It is possible that toxic excretory products or allelopathic substances might be implicated in the semi-closed habitats where the helminth parasites live. Lyons (1978) suggested that the excretion of ethyl alcohol by the acanthocephalan *Moniliformis dubius* might be implicated in the displacement of *Hymenolepis* species in rats. It has also been suggested that the excretion of $H^+$ by cestode parasites may give their transport systems an advantage over those of their hosts and other parasite species (Mettrick, 1975). Further work is needed to ascertain whether helminth parasites also secrete more specific killer factors as demonstrated in the case of bacteria, protozoa, plants and tadpoles (Thomas *et al.*, 1975). In the case of larval digenetic trematodes the exclusion mechanism involved is usually predation (Sousa, 1992).

One of the predictions from the general premise of niche theory is that if the food supply is invariably superabundant selective pressures favour increased specificity, specialisation towards a narrower niche breadth and an increase in the strength of interference responses in order to maintain spatial requirements. This would favour coexistence and niche packing. Sukhdeo and Sukhdeo (1994) have shown that a particular glycine-conjugated bile salt acid of host origin triggers specific site selection by a nematode species. It can also

be postulated that selective pressures would favour diversification of active transporters in coexisting sibling helminth species. This hypothesis receives some support from the work of Uglem (1972) on congeneric acanthocephala in the large-scaled suckerfish, *Catastomus macrocheilus*.

In some cases interference reaches the extreme form of predation. An example is provided by *Phyllodistomum folium* preying on *Myxidium lieberkuhni* in the urinary bladder of the pike, *Esox lucius* (Dogiel, 1961). As a result these parasites tend not to coexist.

As pointed out by Dobson (1985) parasites may also make use of the host's immune mechanism to exclude other species. An example is provided by the development of host-generated exclusion zones around the acanthocephalan *P. laevis* in the eel gut that prevents settlement by *A. anguillae* (Bates and Kennedy, 1990). The monogenean *Dactylogyrus vastator* also induces cellular reactions in the gills of its hosts, manifested by the production of copious mucus and epithelial hyperplasia, which prevent settlement by other species (Holmes, 1973). There are also other examples of cross-immunity being effective against congeneric helminth parasites (Hyneman, 1962).

## 10.4. Mechanisms which Reduce the Severity of Exploitative Competition

### *10.4.1. Character displacement and diversification of food niche*

The mouthparts of the two parasites *C. metoecus* and *C. truttae* that predominate in the pyloric region of trout are very different. In the case of *C. metoecus* the mouth, which is surrounded by a globular oral sucker, first leads into a short pre-pharynx followed by the rounded muscular pharynx, oesophagus and bifurcate gut. The nutritional habits of *C. metoecus* appear not to have been studied in detail but it is likely that in common with digenean gut browsers they ingest host epithelium, food debris and mucus containing nutrients. However, it is likely that as in other digenetic trematodes (Whitfield, 1979), this food source will be supplemented by transintegumental transport of soluble food items via the syncytial tegumentary cytoplasm. In the case of gut-dwelling strigeoids like *Apatemon* it has been shown that the adhesive organs are implicated in both digestive and absorptive processes (Whitfield, 1979). The feeding mechanisms of *C. truttae* are very different from those of *C. metoecus* as it has armature for tissue penetration. The mouth is surrounded by a narrow cuticular flange, which is armed at its base by a row of numerous small teeth. The muscular oesophagus is expanded at the anterior end to form a large pseudobuccal capsule, the inner surface of which is divided by sutures into several sclerotinised plates. The oesophagus is also expanded posteriorly before opening into the tubular, straight gut. Within the host the head end of the

parasite becomes attached to the mucosa of the intestine or pyloric caecum which is then perforated by the peribuccal teeth. According to Moravec (1994) this parasite feeds on host tissue including blood.

The two predominant parasites in the post-pyloric region of the intestine, *N. rutili* and *C. farionis*, exhibit a rather similar pattern of resource partitioning and character displacement to the two dominant parasites in the pyloric region. Thus, the anatomy of *C. farionis* is very similar to that of *C. metoecus* with which it was once confused (Thomas, 1958b). However, *C. farionis* tends to be larger and has a relatively larger ventral sucker than *C. metoecus*. It is possible that these differences have evolved as adaptations to survive in the less stable environment in the posterior intestine. Nevertheless, it is to be expected that its feeding niche will be very similar to that of the congeneric species.

Like *C. truttae*, *N. rutili* penetrates the mucosa with its proboscis causing the tissues in close proximity to it to become hypertrophied. As with cestodes the Acanthocephala lack an alimentary canal. As a result food in the form of dissolved organic matter is sequestered transintegumentally through infoldings called the pore canals in the syncytial hypodermis. These are mainly located in the metasomal region, which protrudes into the gut lumen, rather than in the presoma or proboscis region.

### *10.4.2. Temporal diversification*

Time, viewed as a parasite resource gradient, may also reduce any interspecies competition. As a result competition between *N. rutili* and *C. truttae*, on the one hand, and *C. metoecus* and *C. farionis*, on the other, would virtually cease in the warmer months of the year when the numbers of the latter two species decline. A more complete temporal segregation has also been reported for congeneric nematodes, *Cucullanus heterochrous* and *C. minutus*, which develop in the intestine of the flounder, *Platichthyes flesus*, at different times of the year (MacKenzie and Gibson, 1970).

### *10.4.3. Intraspecific interactions*

So far as nutrient resources are concerned niche overlap is greater between individuals of the same species than between individuals of different species. As a result it is to be expected that exploitative competition would be much stronger at the intraspecies than at the interspecies level. Competition models based on the logistic equation show that this difference may allow species to coexist (Miller, 1967). Although Brown (1986) has provided experimental evidence that the establishment and survival of *Pomphorhynchus laevis* in *Onchorhychus mykiss* is density dependent, opinion seems divided regarding

the importance of intraspecific competition among parasitic helminths of fish in nature. According to Bates and Kennedy (1990) however caution is needed in interpretation where thresholds are cited as the only evidence for intraspecific competition. Density dependence regulation due to space limitation has however been invoked to explain the declines in numbers of *L. thecatus* in the green sunfish (Uganski and Nickol, 1982; Ewald and Nickol, 1989) and also in *E. crassum* in the brown trout during establishment (Kennedy, 1996). In contrast, the survival of *L. thecatus* in the large-mouth bass, *Micropterus salmoides*, and *E. crassum* in the brown trout, following establishment, is said to be density independent (Leadabrand and Nickol, 1993; Kennedy, 1996). Although Esch and Fernandes (1993) argue that the mortality rate of most parasites in fish under natural conditions is density independent more quantitative studies are required to validate this claim. Recent studies by Morand *et al.* (1999) on ectoparasites of marine fish appear to support the so-called aggregation model of coexistence which predicts that interspecies interactions are weaker than intraspecies interactions thus facilitating coexistence.

### *10.4.4. Predation, hyperparasitism and climate*

Predation or hyperparasitism may regulate populations in a density-dependent manner. Although climatic factors act in a density-independent manner they may greatly influence the outcome of competition. Thus, Park (1954) showed that when temperature and humidity were varied the outcome of competition between *Tribolium* populations became probabilistic rather than deterministic.

## 10.5. Evolutionary Aspects of Interspecies Competition

There are conflicing views regarding the importance of interspecies interactions in shaping communities of helminth parasites in fish. Kennedy *et al.* (1986) suggested, on the basis of field surveys, that interspecies competition has been a far greater structuring force in the diverse, species-rich helminth communities in the gut of birds and mammals than in the comparatively poor communities of freshwater fish. However, it appears that there are exceptions to this rule as Kennedy (1985, 1992) claims that even within the species-poor, isolationist communities in the eel intestines interspecies competition may occur between acanthocephala species causing microhabitat width to change when parasite species coexist. More recently several authors also appear to be challenging the notion that interspecies interactions are not important determinants of community structure of gut-inhabiting helminth parasites in fish. Thus, Kennedy (1985, 1992) and Vidal-Martinez and Kennedy (2000) postulate that competitive interactions may be potential determinants of community

structure even in low-diversity helminth infracommunities found in temperate zone and tropical fish, though it is not yet clear to what extent this potential is realised in nature. Kennedy and Hartvigsen (2000) also cite evidence based on community structure ($ICR_{max.}$ = maximum number of species per host and CCR = total number of species per sample) suggesting that even the relatively species-poor helminth communities in the intestines of eel and trout are saturated and suggestive of interspecies interaction. Poulin (1999) also states that dominant parasites may be important in shaping parasite communities either by direct competition or by acting on the host phenotype causing it to become a less suitable habitat. The negative associations and evidence of saturation cited in the present review provide support for the above arguments and strongly suggest that interspecies competition may be a major selective mechanism in the case of gut-inhabiting helminth parasites.

However, when Rohde (1991) and Morand *et al.* (1999) considered the monogenetic gill parasites of marine fish they came to the opposite conclusion, namely that interspecies competition was of no great importance. The evidence cited by them in support of this view includes the preponderance of positive associations, the high prevalence of unoccupied niches or non-saturation, and the insignificant effects of potentially competing species on microhabitats and infection intensities. These conclusions imply that monogenetic trematodes have a low probability of locating their hosts and that their population densities may be determined mainly by transmission success.

It may be suggested therefore that the contrasting views regarding the importance of interspecific competition as a regulatory mechanism in monogenetic and digenetic trematodes may be attributable to differences in their life history strategies. Thus, unlike monogenetic trematodes the digenea have evolved mechanisms that allow them to use intermediate hosts in the food chain to increase the probability of transmission. As a result this makes their populations more susceptible to saturation and hence regulation by both interspecies and intraspecies competition.

Rohde (1991) also considers that intraspecific competition may occasionally occur within populations of monogenea as evidenced by the expansion of microhabitats when infection densities of monogeneans are high. He argues however that the reinforcement of reproductive barriers has been one of the main mechanisms driving the evolution of microhabitat restriction, or niche segregation, in monogenea. However, this argument is not exclusive and other selective advantages linked to microhabitat restriction such as resource partitioning and character displacement cannot be ignored.

Various workers including Schad (1963a, b, 1966) and Petter (1966) have suggested that microhabitat segregation and character displacement in coexisting helminth parasites have arisen as a result of interspecies competition. However, as there is a general consensus that speciation occurs when populations are allopatric, it seems unlikely that interspecies competition could be the

primary cause for the evolution of congeneric, sibling species. Nevertheless, it is possible that interspecies competition may cause further divergences between the species after they became sympatric.

The present review supports one of the generalisations made by Miller (1967) that interspecies competition tends to be asymmetrical. This author also produced evidence that superior competitors tend to be larger, have a more restricted spatial distribution and have stronger interference mechanisms than the less effective competitor. It would be of interest to ascertain whether these generalisations can be applied to helminth parasites. In the case of parasites it is necessary to consider the possibilities that interference may be mediated via allelopathic chemicals as well as the immune mechanisms of the host. Further immunological and biochemical research is therefore required to ascertain the nature and magnitude of the exploitative and interference mechanism which helminth parasites may exploit during either intra- or interspecies competition.

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# Biology of the Schistosome Genus *Trichobilharzia*

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## ABSTRACT

*Trichobilharzia* is the largest genus within the family Schistosomatidae, covering over 40 species of avian parasites. To clarify the existing confusion in the systematics of the genus, we recommend combining knowledge of life cycles and developmental stages, snail/bird hosts, cytogenetical and molecular data together with morphological criteria for the characterization of particular species. The high specificity of *Trichobilharzia* for the intermediate host is a likely reflection of the ability to avoid the internal defence of specific snails. The spectrum of final hosts (birds) seems to be much wider. The infection of birds – trichobilharziasis – may lead to considerable tissue injuries, caused by eggs of the parasite or migration of immature/mature worms through the body. Most *Trichobilharzia* (visceral species) migrate through the viscera of the host, but nasal species display a neurotropic mode of migration. Due to a low specificity of penetrating cercariae, mammals (including humans) can be attacked. This leads to cercarial dermatitis, predominantly in sensitized hosts. Experimental infections indicate that *Trichobilharzia* never mature in an incompatible (mammalian) host. However, not all cercariae and schistosomula are necessarily trapped and eliminated in the skin, and parasites may migrate throughout the viscera and the nervous system of mammals. These findings suggest that the pathogenicity of *Trichobilharzia* may have been underestimated in the past and health risks associated with trichobilharziasis need to be studied further.

## 1. INTRODUCTION

Schistosomes are evolutionarily successful parasitic flatworms that are unusual among flukes because they are dioecious and inhabit the circulatory system of vertebrates. The parasites are best known as human pathogens in tropical and subtropical countries (over 200 million people suffering from schistosomiasis;

WHO estimation). A recent discovery revealed that they parasitize not only warm-blooded vertebrates (mammals and birds), but also reptiles, i.e. crocodiles (*Griphobilharzia*; Platt *et al.*, 1991).

Compared to mammalian schistosomes, represented by the well-known genus *Schistosoma*, the genera that infect birds may seem to be of less importance. However, as parasites of birds, the members of *Trichobilharzia* (the largest genus of the family Schistosomatidae) cause trichobilharziasis of ducks and geese (Wojcinski *et al.*, 1987; Graczyk *et al.*, 1993), a disease comparable with schistosomiasis in humans. From a medical viewpoint, these parasites are the causative agent of cercarial dermatitis in humans, an inflammatory reaction of the skin which is now considered as an emerging disease (de Gentile *et al.*, 1996). Moreover, schistosomula of *Trichobilharzia* spp. have been repeatedly reported from the lungs and other organs of mammals, including nervous tissue in the case of *T. regenti*. The obligatory migration of the latter parasite through the central nervous system of birds and mammals is occasionally associated with neuromotor disorders. Clearly, such properties justify continued attention towards these rather underestimated infections.

The last review of *Trichobilharzia* was published nearly 20 years ago (Blair and Islam, 1983). This paper provides updated information on the genus, including descriptions of new species, their molecular characterization, discoveries in parasite behaviour (host-finding), penetration of the host skin, immune evasion by the parasite, parasite migration and associated pathology in vertebrate hosts, and new data on outbreaks of cercarial dermatitis.

## 2. LIFE CYCLES AND HOSTS

### 2.1. General Life Cycle

The members of *Trichobilharzia* have a two-host life cycle similar to that of the related genus *Schistosoma*, with water snails and birds as the intermediate and final hosts, respectively (Figure 1). Whereas the cercarial larvae of *Trichobilharzia* spp. released from water snails are encountered relatively frequently in connection with outbreaks of cercarial dermatitis, reports describing adult worms are rare. This is partly due to the fact that *Trichobilharzia* adults possess a thin, threadlike body (20–100 μm diameter) and live hidden inside the host. The adult worms can be recovered only by means of a complicated examination of birds. Consequently, reports on these flukes from birds are limited and data on the life cycles are known mainly from experimental infections of both the intermediate and the final hosts.

The cycle starts with the eggs. The mature and fertilized female (depending on the species, located either in the visceral organs or in the nasal mucosa of

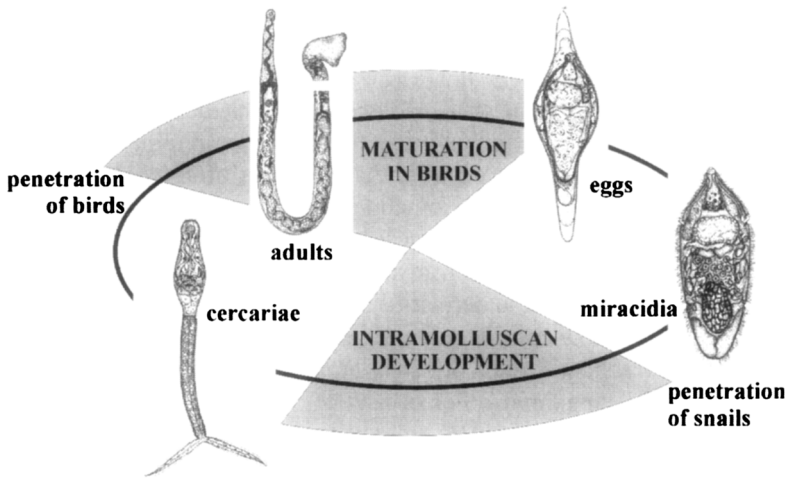


*Figure 1* The life cycle of *Trichobilharzia* sp. The miracidium leaves the egg (either in water or in the nasal host tissues) and infects an appropriate intermediate snail host. Inside, the parasite multiplies asexually. The resulting life stage, cercariae, emerges from the snail and searches for the final host – a bird. Once the bird is found, the cercariae penetrate the skin and develop into schistosomula. The parasite then migrates to a preferred location within the host, where sexual maturation, mating and egg laying take place.

the host) contains a single egg. A similar situation with only one uterine egg is well known from other schistosomes, e.g. *S. mansoni* (see Loker, 1983). Usually, the egg of visceral *Trichobilharzia* is deposited in the circulation (capillaries) of the final host and needs to pass through different tissues in order to reach the lumen of the intestine and, subsequently, leave the host with faeces. In the nasal species of *Trichobilharzia*, eggs are deposited in the nasal mucosa. The eggs mature within the host and the released eggs contain fully developed miracidia that are able to hatch. As an exception, miracidia of nasal schistosomes, e.g. *T. regenti*, hatch from the eggs within the host tissues and await water contact (Horák *et al.*, 1998a). Similar behaviour can be expected from other nasal schistosomes and indeed, miracidia of *T. arcuata* hatch in contact with either water or physiological saline (Islam, 1986b). Not all larvae reach the outside environment. Some eggs are trapped in the tissues, where they trigger host reactions (granulomas) and are destroyed.

Once in the water, the miracidium leaves the egg and searches for the intermediate host, i.e. a water snail. The life span of miracidia of *T. szidati* is about 20 h at 20 °C (Neuhaus, 1952a). The species of *Trichobilharzia* usually have a narrow specificity towards the intermediate host, using only one (or closely related) snail species for larval development. Upon finding a proper molluscan host, the miracidium penetrates and develops into a mother sporocyst in the

head–foot region of the snail. The latter stage produces daughter sporocysts which leave the head–foot region and migrate to the hepatopancreas where further development with subsequent release of cercariae continues. The prepatent period within the snails is variable (about 3–10 weeks) and depends on several factors, including temperature. Details on snail-finding and intramolluscan development are provided in section 4.

It is hypothesized that a set of specialized glands serves for the passage of cercariae through the snail tissues (for *T. szidati* see Neuhaus, 1952a) in order to leave the snail. Once in the water, the cercariae need to find and infect a proper final host quickly because of their short life span. If a vertebrate host is found the cercariae penetrate the skin, transform to schistosomula and start to migrate to the preferred location for egg laying. Depending on the parasite species, two different routes are employed to reach these places: migration via the blood circulation or passage through the nervous tissues (peripheral nerves and the central nervous system). The first route is typical for visceral species (e.g. *T. franki, T. elvae*); the second one is used by the nasal schistosome *T. regenti*. Detailed information regarding host-finding, penetration, migration and associated pathologies is presented in sections 5 and 6.

## 2.2. Experimental Life Cycles and *In Vitro* Cultivations

As mentioned above, cercariae of *Trichobilharzia* spp. are frequently encountered in the environment. However, this stage lacks distinguishing characters and the morphological characterization of other developmental stages (primarily adults) is necessary for species diagnosis. Fortunately, parasite life cycles can be realized experimentally if a proper final host (bird) can be selected. Frequently, birds of the family Anatidae represent a suitable host. Birds can be infected percutaneously (by exposing the feet to cercariae) or, in some cases, by applying cercariae perorally (Macy *et al.*, 1955). With compatible intermediate (snail) and final (bird) hosts available in the laboratory, the whole life cycle can be maintained experimentally for many years (Meuleman *et al.*, 1984b). The feasibility of maintaining parasite life cycles can be restricted by (1) laws intended to protect animals, which down-regulate or prohibit the breeding of experimental ducks and other birds and (2) failure of long-term maintenance of snails in the laboratory due to unknown reasons. As far as *Trichobilharzia* spp. from Europe is concerned, host snails such as *Lymnaea stagnalis* and *Stagnicola* spp., and recently also *Radix peregra/R. ovata*, are easily kept in the laboratory. By comparison, the maintenance of *R. auricularia* and other snails (physid and small planorbid species) is more difficult.

There are several possible ways to simplify the experimental cycle and to replace certain phases of the snail–parasite co-existence. Largely, these approaches aim to avoid undesirable properties of the molluscan host that

may hamper the parasite cycle and the study of the parasite stages. To avoid an unsuccessful penetration of snails by parasites, the miracidia can be injected directly into the snails (*T. ocellata* into *L. stagnalis*); no harmful effects on the host and parasite have been recorded (Meuleman *et al.*, 1984a). The subsequent stages – mother and daughter sporocysts – can be recovered from snails (Amen and Meuleman, 1992). Moreover, miracidia can transform to mother sporocysts and develop further under *in vitro* conditions either in complex culture media (Mellink and van den Bovenkamp, 1985) supporting a long-term cultivation, or in simplified media enabling a short-term incubation and allowing analysis of parasite surface/excretory/secretory molecules (Schallig *et al.*, 1990). Recent experiments showed that tissue cultures of Bge cells (*Biomphalaria glabrata* embryonic cells) or even a lowered oxygen pressure can support complete larval development of *Schistosoma mansoni in vitro* (for a review see Coustau and Yoshino, 2000; Bixler *et al.*, 2001). These approaches may also facilitate cultivation of avian schistosomes.

Two approaches, based on former experiments with the human parasite *S. mansoni* (Clegg, 1965; Samuelson and Stein, 1989; Smyth, 1990), were applied in the *in vitro* development of bird schistosomes from cercariae to schistosomula and adults. Cercariae with mechanically detached tails were incubated in phosphate buffer (300 mOsm, 39 °C) for 5 h to characterize ultrastructural and biochemical changes in the tegument during penetration and cercaria–schistosomulum transformation (Horák *et al.*, 1998b). A nutrient-rich medium based on Earle's saline was used for long-term cultivation of *T. ocellata* to the organogeny stage. The medium contained lactalbumen hydrolysate and duck/chicken serum together with homologous erythrocytes. Although the worms grew and digested erythrocytes, they did not differentiate to the gametogeny stage (Howell and Bourns, 1974).

## 3. SYSTEMATICS OF THE GENUS *TRICHOBILHARZIA*

### 3.1. Introductory Notes

A precise determination of species within the genus *Trichobilharzia* is valuable not only from the biological viewpoint (e.g. to document differences in forms and developmental strategies of parasites within vertebrates) but also as a tool to predict health risks. Cercarial dermatitis, caused by cercariae of *Trichobilharzia*, is considered to be an emerging disease of humans (de Gentile *et al.*, 1996). Clearly, the medical and veterinary importance of *Trichobilharzia* spp. increases considerably with the new insights regarding parasite distribution (outbreaks of cercarial dermatitis), life cycles (penetration of both specific and non-specific vertebrate hosts) and pathogenicity (clinical symptoms and

pathologies caused by penetrating and migrating worms). Cercarial dermatitis is sometimes associated with subsequent generalized symptoms. It cannot be excluded that such symptoms are species-related, depending on parasite (species-specific) antigens, survival, and migration within vertebrates. Although limited data are available on migration of particular species of visceral *Trichobilharzia* and clinical consequences, it is evident that nasal schistosomes (*T. regenti*) invade the nervous tissues of birds and mammals and may cause neuromotor disorders (including paralysis).

However, the identification of *Trichobilharzia* species is very difficult work, complicated by the existence of synonyms, incomplete descriptions or incorrect parasite identifications (e.g. liberal and incorrect use of the name *T. ocellata* – see below). In their thorough review of the taxonomy, Blair and Islam (1983) noted that several synonyms and incomplete descriptions occur among the 40 species of *Trichobilharzia*. Historically, this may in part be due to the poor description of the type species, *T. ocellata*. Although the cercariae were identified by La Valette in 1855, and the corresponding adults by Brumpt in 1931, there is still no consensus on what *T. ocellata* (and *Cercaria ocellata*) actually is (Odening, 1996; see below). At present, there is no doubt that the species composition within the genus needs major revision.

## 3.2. Historical Overview

*Trichobilharzia* was described for the first time at the larval (cercarial) stage by La Valette (1855) who characterized *Cercaria ocellata* from *Lymnaea stagnalis* collected near Berlin in Germany. Subsequently, the cercariae were reported by several other authors (e.g. Ssinitzin, 1910), even from other intermediate hosts, e.g. from *Radix ovata* (see Mathias, 1930; Wesenberg-Lund, 1934), or from multiple lymnaeid, physid, planorbid and hydrobid/bithynid snails (Kilias and Frick, 1964; van den Broek, 1965). The adult worms were found by Skrjabin and Zakharov (1920) in Russia in the visceral blood vessels of a duck. The schistosome was named as *T. kossarewi* and a new genus, *Trichobilharzia*, was established. In 1931, Brumpt performed experimental infections with *C. ocellata* and obtained adult worms which were considered to be identical to those of *T. kossarewi* Skrjabin and Zakharov, 1920. Due to the law of priority, the type species was renamed *T. ocellata*. Since then, however, doubts concerning the description of *T. ocellata* from different intermediate hosts (lymnaeids, or even other snail families) have been raised. The cercariae that emerge from different lymnaeids may vary in size and behaviour. Thus, *C. ocellata* probably represents a collective term for several species (see Szidat, 1942 for review).

It should be noted that the description of the type species, *T. ocellata*, is confusing for several reasons. (1) *C. ocellata* La Valette, 1855, as originally described shows features common to all *Trichobilharzia* cercariae. The only

specific marker is that the larvae were isolated from *L. stagnalis* in Germany. (2) The description of *T. kossarewi* Skrjabin and Zakharov, 1920 (later renamed *T. ocellata* by Brumpt, 1931) was based on one male worm, but the presence of a gynaecophoric canal was not observed (or mentioned) by the authors. McMullen and Beaver (1945) in their description of *T. ocellata* suggest that Skrjabin and Zakharov (1920) had been wrong to omit this anatomical feature. (3) Finally, Brumpt's (1931) description of *T. ocellata* (uniting *C. ocellata* sensu La Valette (1855) and *T. kossarewi* sensu Skrjabin and Zakharov (1920) under the name *T. ocellata* (La Valette, 1855) Brumpt, 1931) lacks the most important characters of the species. There is no information in the text on acetabulum, canalis gynaecophorus, seminal vesicles, male genital papilla, intestinal bifurcation and reunion etc. The drawing of the miracidium does not contain several important diagnostic features. Also, the text mentions that compared with *L. stagnalis,* the miracidia of *T. ocellata* exhibited higher affinity towards *Planorbarius corneus, Planorbis rotundatus* and *Stagnicola palustris*. The attraction to planorbid snails over *L. stagnalis* is in strong contrast to recent findings (e.g. Kalbe *et al.*, 1997). Since then, several authors have tried to clarify the situation, e.g. by redescribing locally occurring *T. ocellata* (McMullen and Beaver, 1945; Chikami, 1961). These (re)descriptions of *T. ocellata* may deal with the original *T. ocellata*, but they may also characterize other species. For example, McMullen and Beaver (1945) synonymized *T. elvae* with *T. ocellata*; however, their main reason for doing this was the shape of eggs, an insufficient marker (see section 3.4.1).

It remains up to current authors to decide which is the most relevant description of *T. ocellata*, if any. In his excellent review, Odening (1996) proposed two choices. (1) The best description of *T. ocellata* can be selected (e.g. *T. ocellata* sensu McMullen and Beaver (1945); *T. ocellata* sensu Chikami (1961); *T. szidati* sensu Neuhaus (1952a) as the most junior 'synonym') and considered to describe the true *T. ocellata* found originally by La Valette (1855) and Brumpt (1931). (2) The view should be adopted that *T. ocellata* represents a complex of species. In the latter case, some new species will continuously be separated from this complex, as may be deduced from the recent descriptions of European species (*T. franki* Müller and Kimmig, 1994; *T. regenti* Horák *et al.*, 1998a; *T. salmanticensis* Simon-Martin and Simon-Vicente, 1999).

Aside from confusion regarding the type species, additional inconsistencies exist within the genus of *Trichobilharzia*. Two new genera were named in the past: *Pseudobilharziella* Ejsmont, 1929 is a junior synonym of *Trichobilharzia* (Bykhovskaya-Pavlovskaya and Ryzhikov, 1958; Blair and Islam, 1983). *Jilinobilharzia* has been separated from *Trichobilharzia* (see reviews of Blair and Islam, 1983; Khalil, 2002) and includes the former *T. yokogawai* (Oiso, 1927) McMullen and Beaver, 1945 and *T. brantae* Farr and Blankemeyer, 1956. Finally, *T. tatianae* Spasskaja, 1954 apparently does not belong to the genus *Trichobilharzia* (see Blair and Islam, 1983).

## 3.3. Diagnosis of the Genus *Trichobilharzia* (based on Farley, 1971; Blair and Islam, 1983; Khalil, 2002)

Among the members of the family Schistosomatidae (also comprising *Schistosomatium, Heterobilharzia, Schistosoma, Bivitellobilharzia, Austrobilharzia, Orientobilharzia, Ornithobilharzia, Macrobilharzia, Griphobilharzia, Bilharziella, Jilinobilharzia, Gigantobilharzia* and *Dendritobilharzia*), the genus *Trichobilharzia* is the largest in terms of number of species. In the adult stage, males and females are similar and threadlike. Oral and ventral suckers are present in both sexes and the posterior end of the body is spatulate. The gynaecophoric canal is short and does not extend to the posterior end of the body. Testes are numerous, occupying space posterior to the gynaecophoric canal on either side of the common caecum. The elongate seminal vesicle is localized in the anterior part of the body, between the acetabulum and gynaecophoric canal. The males possess small cirrus and their genital pore is at the anterior end of the gynaecophoric canal. An elongate and coiled ovary occurs in the anterior part of the female body. The seminal receptacle is well formed and lies immediately posterior to the ovary. The ootype is anterior to the ovary with the uterus opening immediately posterior to the acetabulum. Adults are parasites of birds.

The position and validity of the genus among other schistosomes is well supported by molecular data. Phylogenetic analyses based on partial sequences of 28S rDNA place the genus of *Trichobilharzia* in the clade of bird schistosomes (Snyder and Loker, 2000).

## 3.4. Main Diagnostic Criteria; Traditional and New Approaches in Species Diagnosis

Traditionally, the morphology of particular developmental stages is used as a diagnostic criterion. Arguably, the adult morphology is the most valuable characteristic for species identification (Blair and Islam, 1983). Additionally, eggs, miracidia and cercariae can also provide diagnostic characteristics. However, different species may exhibit very similar morphological features, and other life cycle data (intermediate host-specificity, larval behaviour, site of final location within birds) may also be required. Therefore, any new species description should contain not only the characters of adults, but also of other developmental stages (eggs, miracidia, cercariae), data on specificity towards intermediate and final hosts, organ specificity within birds and, facultatively, behaviour of miracidia and cercariae. Additional techniques may highlight further differences between species, e.g. chaetotaxy (characterization of surface papillae of miracidia and cercariae by staining with silver nitrate) and karyology (characterization of chromosomes in the mitotic metaphase plate).

Recently, molecular taxonomy has proved to be a powerful tool for diagnosis; the rDNA of four species has been sequenced (see section 3.4.8.).

The following overview provides descriptions of the main morphological structures of particular developmental stages and those that are important for species identification are discussed. In addition, current knowledge regarding intermediate hosts, location within birds and karyological and molecular characterization is summarized.

### *3.4.1. Eggs*

The eggs (Figure 2) can be spindle-shaped (*T. parocellata*, *T. franki*), crescentic (*T. ocellata*, *T. arcuata*) or even ovoid (*T. corvi*, *T. filiformis*). Usually, they have a projection/small hook at one end. At the time of release from the bird, the eggs already contain a miracidium. In certain cases, the miracidium hatches from the egg within the bird host (*T. regenti*; see Horák *et al.*, 1998a). Some species produce similar eggs (e.g. *T. salmanticensis* and *T. parocellata*). Thus, although the size and shape of eggs are important criteria, they cannot be the reason for synonymization (used for *T. elvae* and *T. ocellata* by McMullen and Beaver, 1945) or species determination (used for *T. ocellata* by Sluiters, 1983).

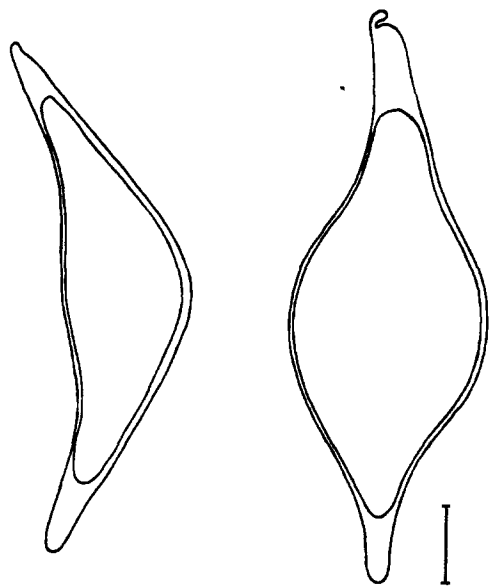

*Figure 2* Shape of the eggs of *Trichobilharzia ocellata* (left) and *T. franki* (right). Scale bar = 30 μm. Reproduced with permission from Kock (2000).

Nevertheless, eggs represent a valuable tool for quick identification of species with different egg morphology that occur sympatrically within the same bird host (e.g. visceral duck schistosomes of *T. ocellata* and *T. franki*).

### *3.4.2. Miracidia*

These pyriform larvae (Figure 3) are covered by a mosaic epithelium, composed of intercellular ridges and ciliated plates. The latter are usually arranged in four tiers of 6:9:4:3 = 22 cells. This pattern may differ in some species (e.g. 6:7:5:4 = 22 in *T. corvi*; see Y. Ito, 1960, cited by Blair and Islam, 1983). Two lateral horns are located between the first and second tier. The anterior papilla (terebratorium) contains terminal or lateral openings of the penetration glands. Generally, two gland stuctures are visible: the apical gland (one or two cells) and the lateral gland (two cells). The posterior part of the body is filled

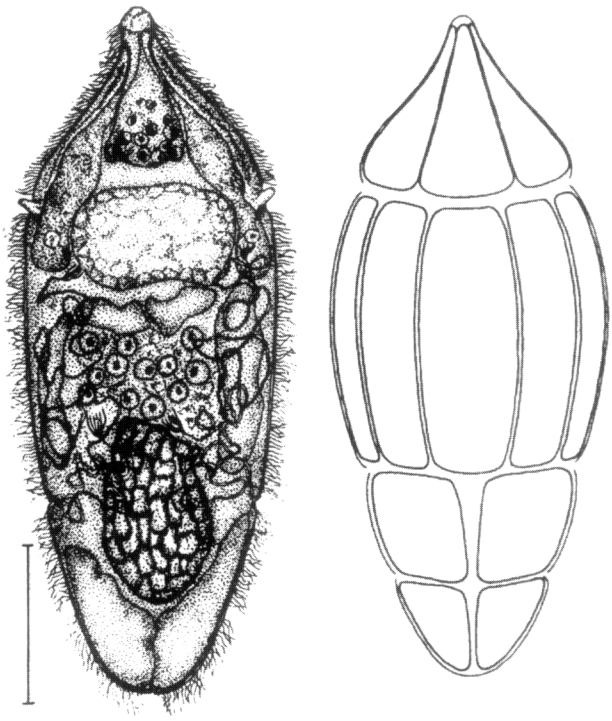

*Figure 3* Miracidium of *Trichobilharzia regenti*. General morphology (left) and the arrangement of ciliated plates (right). Scale bar = 25 μm. Reproduced with permission from Horák *et al.* (1998a).

with clusters of germ cells (these multiply and differentiate to produce daughter sporocysts). Particular species possess different numbers of these germ cell clusters, e.g. one in *T. regenti*, two in *T. ocellata* from Japan, three in *T. corvi*.

Some authors doubt the usefulness of miracidial morphology for species identification, mainly because only a few structures are present to help determination. Usually, the arrangement of ciliated plates, morphology of penetration glands and number of clusters of germ cells were used for distinguishing species. Contrary to the report on heterogeneity in the pattern of ciliated plates in *T. indica* (see Baugh, 1978), we feel that the first two characters mentioned above may represent valid criteria. However, these cannot be used as a stand-alone marker in the absence of additional information concerning other stages or the life cycle. Although considered valid in the past, the value of the number of groups of germ cells has been diminished by the observation that a single mass of germinal cells divided into three groups due to contraction of miracidia of *T. salmanticensis* (see Simon-Martin and Simon-Vicente, 1999).

### *3.4.3. Sporocysts*

The sporocyst stage is usually not mentioned in descriptions of species of *Trichobilharzia* (an exception is, for example, *T. australis*; see Islam, 1986a). This is based on the fact that no distinguishing features are present and morphology does not help in diagnosis. For some species (*T. szidati*; see Neuhaus, 1952a) spination of the anterior end of daughter sporocysts is mentioned. Because of insufficient data and the absence of special structures, we regard sporocysts to be of low value for species identification.

### *3.4.4. Cercariae*

The apharyngate ocellate furcocercariae (Figure 4) were the first stage of *Trichobilharzia* that was described (*C. ocellata* La Valette, 1855). They are composed of the body and tail with furcae. The surface tegument contains spines. Typically, two pigmented eye spots can be found in the first half of the body; their ultrastructure has been studied by van de Roemer and Haas (1984). The anterior end is occupied by a head organ, which contains ducts and openings of the penetration glands. The cercariae have five pairs of penetration glands: two pairs are located dorsally over the acetabulum and three pairs further towards the distal part of the body. The glands differ in their content. Additional to the main penetration glands there are also some other, smaller glands present in the cercarial body (Neuhaus, 1952a). The intestine is

inconspicuous. The excretory system is typically composed of 2[3+3+(1)] = 14 flame cells; ( ) indicates the flame cell on one side of the tail, [ ] represents the pattern of cells on one side of the cercaria = body and tail (the excretory system is symmetrical along the longitudinal axis, see Figure 4). Different patterns occur in some species: (2[3+4+(1)]=16 in *T. arcuata* (see Islam, 1986b) and *T. australis* (see Blair and Islam, 1983), (2[3+2+(1)]=12 in *T. corvi* (see J. Ito, 1960, cited by Blair and Islam, 1983). Moreover, several descriptions list two ciliary clusters within the excretory ducts (*T. szidati, T. regenti, T. salmanticensis*; see Kolářová and Horák, 1996; Horák *et al.*, 1998a; Simon-Martin and Simon-Vicente, 1999).

Unfortunately, the cercariae of different species closely resemble each other and they cannot usually be used for species identification. Their size cannot be used for diagnosis. Some morphological structures may indicate, but do not prove, the taxonomic position of certain species, e.g. the number and

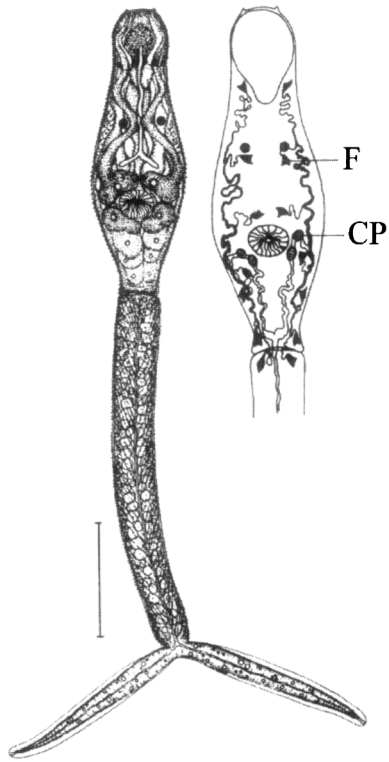


*Figure 4* Cercaria of *Trichobilharzia regenti*. General morphology (left) and excretory system with flame cells (right). F, flame cell; CP, ciliary patches within excretory ducts. Scale bar = 100 μm. Reproduced with permission from Horák *et al.* (1998a).

organization of flame cells in the body. Because several species of *Trichobilharzia* have similar patterns of flame cell organization, other markers have been sought. The distribution of surface sensory papillae (chaetotaxy) has been considered a valuable tool in distinguishing species of cercariae (e.g. Richard, 1971; Bayssade-Dufour and Ow-Yang, 1975; Kolářová and Horák, 1996). These papillae can be observed by staining cercariae with silver nitrate or examining the worms under the scanning electron microscope (Kock and Böckeler, 1998). Chaetotaxy can be used to distinguish certain species with typical papillae patterns, but the value of this method is limited because (1) the distribution of papillae is known for a small number of species only (2) minor differences in papillae positions occur frequently, thus challenging the specificity of this marker and (3) the method requires experienced examiners and many cercariae need to be assessed (usually not all papillae typical of the species are visible in one specimen). Two recent papers clearly demonstrated that chaetotaxy in *Trichobilharzia* studies can be problematic: Kock and Böckeler (1998) did not find any difference when comparing sensory papillae of *T. ocellata* and *T. franki*; and, surprisingly, Gay *et al.* (1999) were able to identify *T. franki* from *R. auricularia* and *T. ocellata* from *L. stagnalis*, and the pattern of *T. franki* was not identical with that reported by Kock and Böckeler (1998).

Evidently, either description of new species or determination of known species based exclusively on cercarial morphology should be avoided. Also, older data should be evaluated carefully, e.g. the findings of *T. ocellata* and *T. szidati* from *Radix peregra* or *R. auricularia* are probably not valid (see sections 3.2 and 3.4.6).

### *3.4.5. Adults*

The isolation of adults from different host organs is a complicated matter and requires attention due to the worm size (usually, the specimens measure between 5 and 10 mm in length and 20–100 μm in diameter) and intimate contact with the host tissues. It is rare to obtain a whole worm intact; frequently only fragments of adults can be recovered. The measurements of the body length/width and of inner organs are generally of limited value for species determination. The worms are able to contract or relax their body such that the diameter (and corresponding length) of body parts may be substantially modified and the shape deformed.

The adults (Figure 5) represent the stage that is most important in species diagnosis (Blair and Islam, 1983). Both sexes have a filiform body of almost uniform width. The body can be covered by tubercles or spines. Usually, the posterior end is spatulate. The oral opening is situated subterminally and surrounded by an oral sucker. The head part of the body has several long sensory

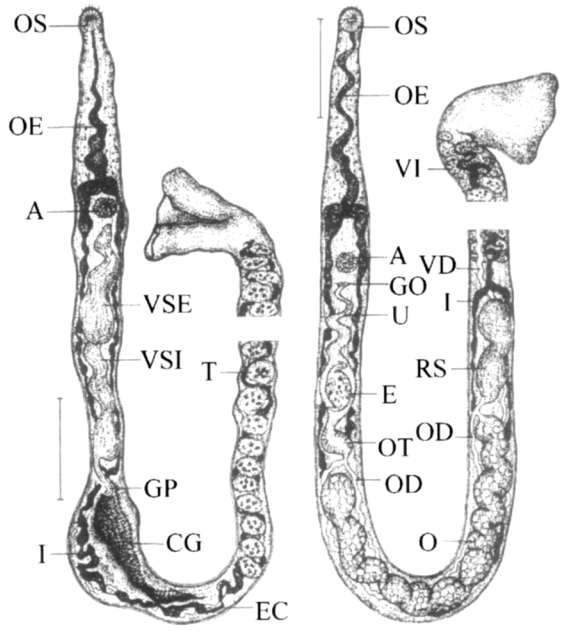


*Figure 5* Anterior and posterior end of a male (left) and a female (right) of *Trichobilharzia regenti*. A, acetabulum; CG, canalis gynaecophorus; E, egg; EC, excretory duct; GO, genital opening; GP, genital papilla; I, intestine; O, ovary; OD, oviduct; OE, oesophagus; OS, oral sucker; OT, ootype; RS, receptaculum seminis; T, testes; U, uterus; VD, vitelloduct; VI, vitellaria; VSE, vesicula seminalis externa; VSI, vesicula seminalis interna. Scale bar = 100 μm. Reproduced with permission from Horák *et al.* (1998a).

cilia. The acetabulum lies very close to the anterior end and it is covered by spines. In males, there is a short canalis gynaecophorus, which is usually decorated with spines. As an exception to the rule, the canalis may be used for diagnosis, as it is unusually long in *T. anatina* (see Fain, 1955, 1956b). The digestive tract lacks a pharynx, and a long oesophagus terminates just before the acetabulum where intestinal bifurcation occurs. The place of reunion of two intestinal branches provides an important taxonomic marker; in some species the reunion is located near the anterior end of the seminal vesicle of males (*T. szidati, T. elvae, T. corvi*), whereas it is situated posterior to the seminal vesicle in *T. brevis* and *T. australis*. The united caecum runs in curves to the end of the body. The reproduction apparatus of males is composed of numerous testes that lie just behind the canalis gynaecophorus and fill up the body to the posterior end. The number of testes varies in mature males; however, a minimum–maximum range can aid in species identification. The ducts leading forward from the testes terminate in the seminal vesicle, located between the

acetabulum and canalis gynaecophorus; the genital papilla is located at the beginning of the canalis gynaecophorus. In females, the posterior part of the body is filled with vitellaria; towards the anterior seminal receptacle, the ovary, ootype and uterus occur. The genital opening is situated just behind the acetabulum. Only one egg is found within the uterus.

The following features seem most appropriate for species identification: type of surface tegumental structures (spination, tubercles), spination and length of canalis gynaecophorus in relation to the body, place of caecal reunion, position of male genital papilla, number of testes (minimum–maximum range) and proportions (size and shape) of internal organs. The measurements of individual bodies and organs (length and width) may be considered informative but cannot be used as independent criteria. These features are easily influenced by at least two factors: frequently occurring contraction/relaxation of the body parts (including shrinkage caused by fixatives) and intraspecific variability in the size of worms (either within the same host organism or in different final hosts).

### *3.4.6. Spectrum of intermediate hosts*

Larval stages of *Trichobilharzia* spp. develop in freshwater pulmonate snails of two families: Lymnaeidae (all over the world) and Physidae (mostly in North America). At present, the use of a pleurocerid snail, *Semisulcospira libertina*, by *T. corvi* (see J. Ito, 1960, cited by Blair and Islam, 1983) is the only exception known. It is believed that particular species of *Trichobilharzia* have rather narrow specificities towards their intermediate hosts, parasitizing either only one snail species or several, closely related species (Blair and Islam, 1983). For instance, *T. franki* develops exclusively in *R. auricularia* (see Müller and Kimmig, 1994), *T. regenti* in *R. peregra* and *R. ovata* (see Horák *et al.*, 1998a) and so on. Two important aspects of intermediate host specificity should be noted: (1) some species of *Trichobilharzia* may share one snail species (e.g. *L. stagnalis*) as intermediate host (Szidat, 1942; Neuhaus, 1952b; Farley 1971; Blair and Islam, 1983); and (2) laboratory experiments clearly showed that miracidia of some specific, well-defined species (e.g. *T. franki*) prefer *R. auricularia* as intermediate host. However, such behavioural studies showed that, to some degree, *T. franki* is also attracted to other, closely related snail species such as *R. ovata.* Moreover, the behavioural preference for particular host species correlates with the success of parasite development in the snails; after exposure to the parasites, *T. franki* developed in 100% of *R. auricularia* and 10% of *R. ovata* (see Kock, 2001).

The larval stages of '*Trichobilharzia* spp.' or '*Trichobilharzia ocellata*' have also been reported from snails of the family Planorbidae (e.g. *Anisus* sp. with *T. ocellata* reported by Beer and German, 1994), Physidae and

Hydrobiidae/Bithyniidae (Kilias and Frick, 1964; van den Broek, 1965). However, caution is warranted; either *Trichobilharzia* spp. with unknown life cycles (and probably not *T. ocellata*) may develop within these snails or the cercariae belong to another genus (e.g. *Gigantobilharzia* spp.; see Dönges, 1965).

The systematic relationships of the intermediate hosts (snails) are also full of confusions and remain to be resolved. Currently, the designations at the genus and species levels are in flux. Unfortunately, this further complicates the study of host specificity of species of *Trichobilharzia*. Concerning the family of lymnaeid snails, one approach is that all species belong to the genus of *Lymnaea,* whereas another approach recognizes several valid genera (e.g. *Radix, Stagnicola, Galba*). This second view, supported by molecular data (Bargues *et al.*, 2001), is adopted for this review. Morphologically, the species are distinguished by the shape and size of the shell. However, this can hardly separate closely related forms such as *R. ovata* and *R. peregra*. Additional morphological features such as the colour of the mantle (*Radix* species), surface sculptures of the shell (*Radix* and *Stagnicola* species; Jackiewicz and Koralewska-Batura, 1995), and the examination of the penis and internal reproductive organs (Jackiewicz, 1988) can be used to help diagnosis at the species level. Recently, molecular techniques have been applied to investigate the phylogeny of lymnaeid snails. Comparative analysis of 18S rDNA sequences (Bargues and Mas-Coma, 1997; Bargues *et al.*, 1997) yielded three clades, composed of *Lymnaea-Stagnicola, Radix* and *Galba* within the lymnaeids. More recently, a review of European lymnaeids (Bargues *et al.*, 2001), which also considered an extensive analysis of ITS-2 sequences, confirmed the grouping into these three clades, while showing heterogeneity within the genus of *Radix*. Based on support from the molecular data, several other names were introduced in addition to *R. auricularia, R. ovata* and *R. peregra*. Interestingly, the molecular analysis suggested that snails previously determined to be *R. peregra* and *R. ovata* (using morphological features) and that serve as the intermediate hosts of *T. regenti* (see Horák *et al.*, 1998a), belong to two other separate species: *R. labiata* and *R. lagotis*. Unfortunately, it seems difficult to distinguish these newly announced species from the morphological viewpoint, and their routine diagnosis in malacology and parasitology is questionable. Taken together, a heterogeneity in susceptibility within certain snail populations for infection by *Trichobilharzia* spp. (and other trematodes) may be due to the occurrence of cryptic (sub)species of snails, classified by traditional methods as belonging to one species. However, ITS-2 data do not distinguish between resistant and susceptible individuals of the same snail species (Bargues *et al.*, 2001), and probably other genetically linked factors play a role in host–parasite compatibility.

### *3.4.7. Spectrum of bird hosts and final location*

It seems that the spectrum of final hosts (suitable birds) is broader than that of intermediate hosts. This can partly be due to the fact that cercariae of *Trichobilharzia* possess an ability to penetrate practically any warm-blooded animal (Haas and van de Roemer, 1998). Yet unknown factors (the immune response might be one of them) allow the subsequent development and maturation of *Trichobilharzia* parasites in birds, but not in mammals. Because larval parasite stages, i.e. cercariae, released from snails have been encountered frequently, these were used to experimentally infect birds. From the successful development of, for example, *T. physellae* in canaries, mallards and pigeons (McMullen and Beaver, 1945) and *T. corvi* in chickens (J. Ito, 1960, cited by Blair and Islam, 1983), it is clear that species of *Trichobilharzia* do not specifically depend on water birds, as might be deduced from their aquatic intermediate snail hosts. This fact has been confirmed by examining naturally infected birds. Concerning the spectrum of bird orders, besides Anseriformes, some species of *Trichobilharzia* have been found in Podicipediformes (*T. aureliani*; see Fain, 1956b), Ciconiiformes (*T. rodhaini*; see Fain, 1955), Coraciiformes (*T. cerylei*; see Fain, 1956b) and Passeriformes (e.g. *T. corvi*; see Yamaguti, 1941).

Once inside the final host, the parasites mature and lay eggs in a specific site. Generally, the species of *Trichobilharzia* can be divided into two main groups: visceral schistosomes (e.g. *T. brevis, T. stagnicolae, T. szidati, T. franki*) and nasal schistosomes (e.g. *T. arcuata, T. australis, T. regenti*). Whereas the nasal schistosomes exclusively inhabit the nasal soft tissues, the visceral parasites may inhabit different sites within the body. This preference is not particularly strict and parasites can usually be found in blood vessels of the intestine (*T. franki*; see Müller and Kimmig, 1994), cloaca (*T. kegonsensis*; see Brackett, 1942), liver (*T. franki*; see Müller and Kimmig, 1994), and in portal (*T. querquedulae*; see McLeod, 1937) and mesenterial (*T. maegraithi*; see Kruatrachue *et al.*, 1968) vessels. Some species (*T. szidati, T. stagnicolae, T. elvae* and *T. physellae*) have been discovered within the tissues of the intestinal wall (including muscles) and associated vessels (McMullen and Beaver, 1945; Neuhaus, 1952a).

### *3.4.8. Cytogenetical and molecular analyses*

To date, karyological studies and rDNA sequencing have been applied to *Trichobilharzia* for species characterization. So far, karyotypes from nine species (isolates) have been characterized, i.e. *T. physellae* (Short and Menzel, 1960), *T. stagnicolae* A (Short and Menzel, 1960), *T. stagnicolae* B (Short and Menzel, 1960), *Trichobilharzia* sp. 1 (Barshene *et al.*, 1989),

*Trichobilharzia* sp. 2 (Barshene *et al.*, 1989), *T. szidati* (Barshene and Staniavichiute, 1993), *T. szidati* (Špakulová *et al.*, 1996), *T. franki* (Špakulová *et al.*, 1997) and *T. regenti* (Špakulová *et al.*, 2001). Špakulová *et al.* (1997) have reviewed the variability in chromosome patterns for the genus *Trichobilharzia.* Generally, the species show similar patterns of chromosomes in metaphase plates, having $2n = 16$ with 14 autosomes and 2 gonosomes. The sex-determining mechanism is ZZ in males and ZW in females. Only *T. stagnicolae* B (Short and Menzel, 1960) was found to possess $2n = 18$. The fifth autosome pair is usually satellited (Špakulová *et al.*, 1997). The main differences between species can be observed in the sex chromosomes. Characterization of *T. regenti*, the only nasal schistosome among these otherwise visceral schistosomes, disclosed the presence of one or two supernumerary B chromosomes in more than 60% of the analyzed cells from male and female sporocysts (Špakulová *et al.*, 2001), the mode of formation of which is unclear. At present, karyological mapping should be considered as a supplementary diagnostic tool, as it cannot be used as a stand-alone method for species identification. This is based on the limited number of species/isolates of *Trichobilharzia* that were karyologically characterized. Moreover, the older descriptions do not contain information on sex chromosomes (Short and Menzel, 1960; Barshene *et al.*, 1989; Barshene and Staniavichiute, 1993) or satellites (Barshene *et al.*, 1989; Barshene and Staniavichiute, 1993). However, re-examination of the older descriptions and characterization of additional species of *Trichobilharzia* may allow future use of karyotypes for determination of species.

Recently, the validity of species and phylogenetic relationships have been tested by sequencing rDNA. This approach was initiated by the study of evolutionary relationships among the Schistosomatidae (Snyder and Loker, 2000) in which partial sequences of the 28S rRNA gene were obtained and used in phylogenetic analyses. A clearly defined position of the genus *Trichobilharzia* (*T. ocellata* as the representative of the genus; strain isolated in Germany from *L. stagnalis*) within the clade of avian schistosomes was confirmed. Consecutive studies, involving sequence data derived from the internal transcribed spacer regions 1 and 2 (ITS-1, ITS-2), reported on interspecific differences and the positions of particular species within the genus. To date, these studies have been limited to a small number of European isolates of *Trichobilharzia* (*T. ocellata*, *T. szidati*, *T. franki*, *T. regenti*, *Trichobilharzia* sp.). Nevertheless, two important conclusions can be drawn from these preliminary data. (1) The European strains of *T. ocellata* and *T. szidati* isolated from *L. stagnalis* (and also from *Stagnicola palustris*; see Kock, 2000) show sequence identity of >99% and belong to one clade (perhaps species) (Kock, 2000; J. Rudolfová, J. Dvořák and P. Horák, unpublished data). With this approach it should be possible to resolve the important dilemma of the composition of the genus *Trichobilharzia* (see also Odening, 1996; Kock, 2000). Should *T. szidati*

*Table 1* Overview of species with known developmental stages and life cycles

| *Trichobilharzia* species | Distribution | Natural and/or experimental bird hosts | Footnote |
|---|---|---|---|
| **Nasal species using lymnaeid snails:** | | | |
| *T. arcuata* Islam, 1986 | Australia | Anseriformes, Columbiformes | |
| *T. australis* Blair and Islam, 1983 | Australia | Anseriformes | |
| *T. regenti* Horák *et al.*, 1998 | Europe | Anseriformes | 1b,c |
| **Visceral species using lymnaeid snails:** | | | |
| *T. alaskensis* Harkema *et al.*, 1957 | N. America | Anseriformes | 2,3 |
| *T. brevis* Basch, 1966 | Asia | Anseriformes | |
| *T. elvae* (Miller, 1923) McMullen and Beaver, 1945 | N. America | Anseriformes, Passeriformes | |
| *T. franki* Müller and Kimmig, 1994 | Europe | Anseriformes | 1b,c |
| *T. jequitibaensis* Leite *et al.*, 1978 | S. America | Anseriformes | |
| *T. jianensis* Liu *et al.*, 1977 | Asia | Anseriformes | 2,4 |
| *T. limnaeae* Yamaguti, 1971 | Asia | Anseriformes | 2 |
| *T. maegraithi* Kruatrachue *et al.*, 1968 | Asia | Anseriformes | |
| *T. ocellata* (LaValette, 1855) Brumpt, 1931 | Europe, N. America, Asia | Anseriformes | 1a,b |
| *T. paoi* (Kung *et al.*, 1960) Tang and Tang, 1962 | Asia | Anseriformes | 2,4 |
| *T. parocellata* (Johnston and Simpson, 1939) Islam and Copeman, 1980 | Australia | Anseriformes | |
| *T. salmanticensis* Simon-Martin and Simon-Vicente, 1999 | Europe | Anseriformes | |
| *T. stagnicolae* (Talbot, 1936) McMullen and Beaver, 1945 | N. America | Passeriformes | |
| *T. szidati* Neuhaus, 1952 | Europe | Anseriformes | 1b,c |
| **Visceral species using physid snails:** | | | |
| *T. adamsi* Edwards and Jansch, 1955 | N. America | Anseriformes | |
| *T. cameroni* Wu, 1953 | N. America | Anseriformes, Passeriformes | |
| *T. jequitibaensis* Leite *et al.*, 1978 | S. America | Anseriformes | |
| *T. oregonensis* (Macfarlane and Macy, 1946) Macy and Moore, 1953 | N. America | Anseriformes | 2,3 |
| *T. physellae* (Talbot, 1936) McMullen and Beaver, 1945 | N. America | Anseriformes, Columbiformes, Passeriformes | |

*Table 1 continued*

| *Trichobilharzia* species | Distribution | Natural and/or experimental bird hosts | Footnote |
|---|---|---|---|
| *T. querquedulae* (McLeod, 1937) McMullen and Beaver, 1945 | N. America | Anseriformes | |
| **Visceral species using pleurocerid snails:** | | | |
| *T. corvi* (Yamaguti, 1941) McMullen and Beaver, 1945 | Asia | Passeriformes, Galliformes | |

Synonymy of the following species has been questioned:
*T. querquedulae – T. physellae – T. limnaeae*
(*T. kossarewi*) – *T. ocellata – T. szidati – T. elvae* (see section 3.2.). Because of the confusion about what is meant by *T. ocellata*, the species was described also from other snail families, e.g. Physidae, Planorbidae and Hydrobiidae/Bithyniidae (genus Bithynia) (Kilias and Frick, 1964; van den Broek, 1965). In fact, these findings most probably represent other species of *Trichobilharzia* or even other genera of bird schistosomes.

[1] The species is characterized by rDNA sequences already (Snyder and Loker, 2000[a]; Kock, 2000[b]; Dvořák *et al.*, 2002[c]).

[2] The original description was not available to the authors; the information is based on Blair and Islam (1983).

[3] The original description was not available to the authors; the information is based on Farley (1971).

[4] The original description was not available to the authors, the information is based on Simon-Martin and Simon-Vicente (1999).

be synonymized with *T. ocellata* (*T. szidati* as a junior synonym), or should *T. ocellata* remain as a complex of species – *species inquirendae* (due to historical uncertainty as to what *T. ocellata* is)? To find a relevant answer is important because so-called *T. ocellata* is held in several laboratories across the world. (2) *Trichobilharzia* sp., *T. regenti* and *T. franki* isolated from *Radix* species (*R. ovata, R. peregra, R. auricularia*) form one clade, based on either ITS-1 or ITS-2 sequence data (Kock, 2000; Dvořák *et al.*, 2002). It can be hypothesized that the close relationship between these species is reflected in the sharing of one genus of snails (*Radix*) as the intermediate host. The different location within the final host (nasal tissue for *T. regenti* and visceral vessels for *T. franki*) is probably of subsidiary importance within the framework of rDNA analysis.

## 3.5. Final Notes on Systematics

It is vital that all new descriptions of *Trichobilharzia* species should be as complete as possible (from the viewpoint of morphology, life cycles, host specificity and molecular analysis). New descriptions should also be based on up-to-date information regarding the latest taxonomic situation within the genus of *Trichobilharzia*. Two recent examples demonstrate some of the

pitfalls that ultimately may further contribute to the general taxonomic confusion. (1) *T. salmanticensis* (Simon-Martin and Simon-Vicente, 1999), although described from Spain, was compared and diagnosed with respect to the Asian/South American species of the genus (*T. paoi, T. maegraithi, T. jianensis, T. jequitibaensis*). Based on the key available from Blair and Islam (1983), these were the most closely related forms. However, since the publication of the review by Blair and Islam (1983), additional European members of the genus (*T. franki* and *T. regenti*) have been described. Unfortunately, these new and probably highly relevant species of *Trichobilharzia* have not been included in their analysis. (2) In a molecular study, Picard and Jousson (2001) isolated cercariae of *Trichobilharzia* spp. from *R. ovata* and *R. auricularia* and characterized their ITS-1 sequences. They also obtained sequences from one adult *Trichobilharzia* worm recovered from the nasal cavity of a duck. All sequences were entered into the GenBank database (accession codes AJ312041–AJ312049; February 2002) with *T. ocellata* as the source organism. However, recent experiments (Müller and Kimmig, 1994; Kalbe *et al.*, 1997; Horák *et al.*, 1998a; Simon-Martin and Simon-Vicente, 1999; Kock, 2000, 2001) demonstrated a narrow intermediate host specificity of particular species of *Trichobilharzia*. This implies that the parasites isolated from European *R. ovata* and *R. auricularia* are likely to be *T. regenti/T. salmanticensis* and *T. franki*, respectively. Moreover, the nasal *Trichobilharzia* does not definitely belong to *T. ocellata*. In conclusion, assigning these sequences to *T. ocellata* may be less then accurate, and it can confuse future use of the GenBank data for phylogenetic studies.

### 3.6. Overview of Species

Ideally, in full agreement with the suggestions of Blair and Islam (1983) and Müller and Kimmig (1994), future descriptions of any *Trichobilharzia* must combine data on morphology of the adults and of other developmental stages, life cycles including intermediate and final host specificity and preferred location within the final host. Also, new approaches to characterize the species should be included; isolates with similar morphology may be distinguished by molecular characterization of the species (sequencing of rDNA or other genes).

Currently available descriptions lack much of this vital information. Thus, it is impossible to construct a clear key for species determination. An attempt to organize the species of *Trichobilharzia* into a key has been realized in Blair and Islam's (1983) valuable review; the difficulties encountered in constructing the key are clearly presented by the authors. Rather than trying to construct another key of *Trichobilharzia* species, we have provided a list of species described from all over the world (Tables 1 and 2).

*Table 2* Overview of species with partly known developmental stages and life cycles (intermediate hosts unknown)

| *Trichobilharzia* species | Distribution | Natural and/or experimental bird hosts | Footnote |
|---|---|---|---|
| **Nasal species:** | | | |
| *T. aureliani* Fain, 1956 | Africa | Podicipediformes | |
| *T. duboisi* Fain, 1959 | Africa | Anseriformes | |
| *T. nasicola* Fain, 1955 | Africa | Anseriformes | |
| *T. rodhaini* Fain, 1955 | Africa | Ciconiiformes, Anseriformes | |
| *T. spinulata* Fain, 1955 | Africa | Anseriformes | |
| **Visceral species:** | | | |
| *T. anatina* Fain, 1955 | Africa | Anseriformes | |
| *T. berghei* Fain, 1955 | Africa | Anseriformes | |
| *T. burnetti* (Brackett, 1942) McMullen and Beaver, 1945 | N. America | Anseriformes | |
| *T. cerylei* Fain, 1956 | Africa | Coraciiformes | |
| *T. filiformis* (Szidat, 1938) McMullen and Beaver, 1945 | Europe | Anseriformes | |
| *T. guandongensis* Tsai *et al.*, 1979 | Asia | Not mentioned | 2 |
| *T. horiconensis* (Brackett, 1942) McMullen and Beaver, 1945 | N. America | Anseriformes | |
| *T. indica* Baugh, 1963 | Asia | Anseriformes | |
| *T. kegonsensis* (Brackett, 1942) McMullen and Beaver, 1945 | N. America | Anseriformes | |
| *T. kossarewi* Skrjabin and Zakharov, 1920 | Europe | Anseriformes | 1,2,3 |
| *T. kowalewskii* (Ejsmont, 1929) McMullen and Beaver, 1945 | Europe, Asia | Anseriformes | |
| *T. littlebi* (Byrd, 1956) Farley, 1971 | N. America | Passeriformes | 2,3 |
| *T. lonchurae* (Fischthal and Kuntz, 1973) Tsai *et al.*, 1979 | Asia | Passeriformes | 2 |
| *T. schoutedeni* Fain, 1955 | Africa | Anseriformes | |
| *T. waubesensis* (Brackett, 1942) 5 McMullen and Beaver, 194 | N. America | Anseriformes | |
| *T. zongshani* Tsai *et al.*, 1979 | Asia | not mentioned | 2 |

Synonymy of the following species has been questioned:
*T. kegonsensis – T. horiconensis – T. burnetti – T. waubesensis.*

[1] Because it is impossible to be certain that *T. kossarewi* is a synonym of *T. ocellata* (Blair and Islam, 1983 and section 3.2.), the former species is treated in this table separately as an insufficiently described *Trichobilharzia.*

[2] The original description was not available to the authors; the information is based on Blair and Islam (1983).

[3] The original description was not available to the authors; the information is based on Farley (1971).

## 4. DEVELOPMENT IN INTERMEDIATE HOSTS

### 4.1. Host-Finding and Penetration by Miracidia

Adult avian schistosomes produce eggs that, after maturation in the host, contain fully developed miracidia. The eggs of visceral schistosomes leave the avian host with the faeces. Within a short time (5–10 min, *T. szidati*, Neuhaus, 1952a; 2–3 h in *T. cameroni*, Wu, 1953) of being deposited in surface water, physiological cues such as increased light and change in osmotic pressure cause miracidia to hatch from the eggs (Meuleman *et al.*, 1984b; Xu and Dresden, 1990; Kalbe *et al.*, 1997). A nasal schistosome, such as *T. regenti*, deposits eggs in the nasal cavity of birds and miracidia hatch in the nasal mucosa, which may then be released into the water when the bird feeds or drinks (Horák *et al.*, 1998a). The body surface of a miracidium is covered with plates that bear cilia. Miracidia are fast swimmers, propelled by the beating action of these cilia. Miracidia lack a digestive system and cannot feed. The energy for swimming activity is derived from the breakdown of endogenously stored glycogen, mainly via the Krebs cycle (Tielens *et al.*, 1991). Consequently, miracidia can only swim for up to 20 h at 20 °C (*T. szidati*; see Neuhaus, 1952a) before they have depleted their energy reserves and die. In order to survive, miracidia must infect a snail within this time interval. Snails are obligatory intermediate hosts; the internal physiology of a suitable snail provides nutrients and other support for the differentiation and asexual reproduction of schistosome larvae.

Miracidia display specialized behaviours that enable them to find a host snail (reviewed by Haas and Haberl, 1997; Haas, 2000). After hatching from eggs, miracidia disperse by swimming in a relatively fast and linear fashion. This increases the chance for individual miracidia to find a host and it minimizes high infection rates in single snails. A preference for certain environmental conditions (light, temperature and gravity) causes miracidia to locate to microenvironments that are preferred by snail hosts. Snails release various secreted/excreted products (SEP), which results in an 'active space' of (gradients of) snail-generated chemicals around potential hosts. Miracidia are attracted by the macromolecular components of snail-SEP, which consist of glycoproteins larger than 30 kDa, termed 'miracidia-attracting glycoproteins' (MAGs) (Kalbe *et al.*, 2000). Upon entry into an active space of a snail, miracidia of *T. ocellata* change their linear swimming behaviour by increasing the rate of change of direction (RCD). Additionally, *T. ocellata* miracidia display a turn-back response (consisting of a sharp 180° turn) if the chemical cues fall below a threshold value (Kalbe *et al.*, 1997; Kock, 2001). In an aquatic environment, where currents may prevent the formation of linear chemical gradients, these markedly non-directional behaviours may be optimal for miracidia to locate and ultimately contact a moving snail.

If the appropriate cues are absent at the initial contact with a snail, miracidia resume their swimming behaviour and move away (contact without return response). The presence of pertinent macromolecular glycoconjugates in the mucus on the snail's surface causes the miracidia to remain close to the skin of the snail and to display 'repeated investigation' and 'attachment' behaviours (MacInnis 1965; Haas *et al.*, 1991; Haberl and Haas, 1992). Once attachment has occurred, clues of undetermined nature trigger penetration, effected by pulsing movements and release of the contents of apical penetration glands. These secretions remain to be characterized but they probably contain proteolytic enzymes that help miracidia to penetrate through the skin of the snail.

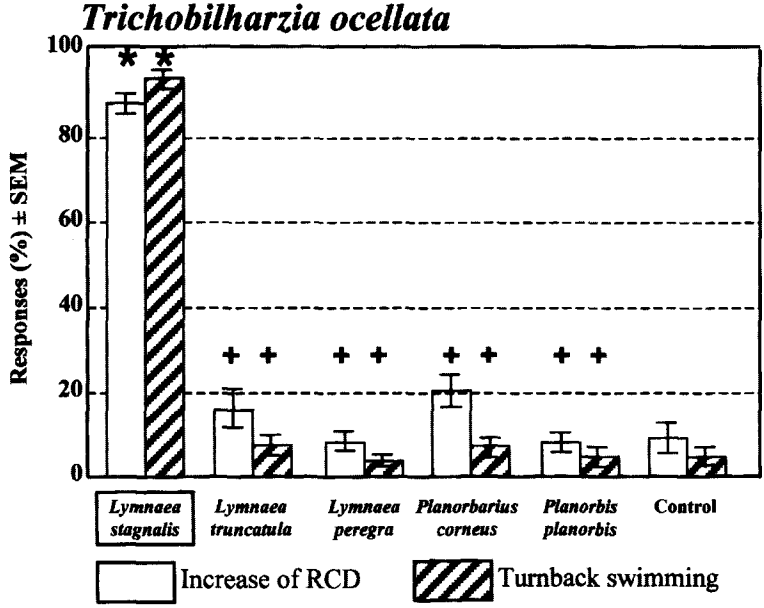


*Figure 6* Preference in host-finding by miracidia of *Trichobilharzia ocellata*. The miracidia employ snail-released chemicals for short-range orientation towards a host. Upon detection of appropriate cues, the miracidia change their linear swimming behaviour by increasing the rate of change of direction (RCD). The miracidia also display a turnback swimming response (a sharp 180° turn) if the chemical cues fall below a threshold concentration. *Trichobilharzia ocellata* initiated host-finding behaviour in response to snail-conditioned water (SCW) containing chemicals released by the host snail, *Lymnaea stagnalis* (boxed). SCW derived from non-host snails such as *Lymnaea truncatula*, *Lymnaea peregra* (both Lymnaeidae), *Planorbarius corneus*, and *Planorbis planorbis* (both Planorbidae) failed to evoke significant host-finding behaviour. This greatly increases the likelihood that *Trichobilharzia ocellata* penetrates a suitable host in which it can develop successfully. Control = water, $n$ = 189–685, * $P < 0.05$ versus control, + $P < 0.05$ versus response to SCW derived from *Lymnaea stagnalis*. Reproduced with permission from Kalbe *et al.* (1997).

Interestingly, not all schistosome species penetrate a snail through this route. Some schistosome species preferentially enter through natural openings such as the mouth or rectum of snails (Loker, 1978; Xia and Jourdane, 1991).

The reliance on snail-released chemicals to locate a host provides miracidia with a degree of specificity regarding the snail that they infect (Kalbe *et al.*, 1996). The MAGs produced by different snail species may have similar peptide backbones, but differ in saccharide composition. Miracidia are thought to differentiate between these glycosylation patterns in order to preferentially locate and infect particular snail species (Figure 6; Kalbe *et al.*, 2000; Kock, 2001). Such selective abilities become relevant as a particular schistosome species may successfully infect some snails, but fails to establish in other snail species that prove to be unsuitable as a host. For instance, *T. franki* establishes infections

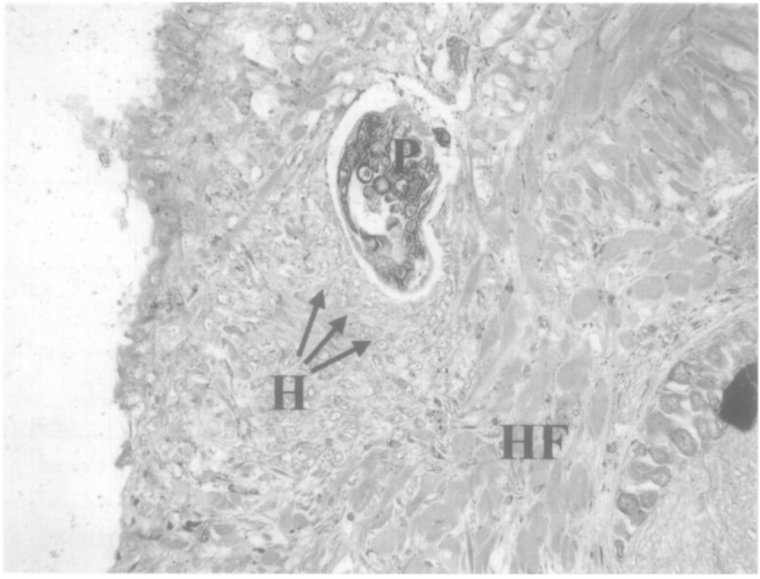


*Figure 7* Cross-section showing a critical phase in the interaction between an avian schistosome *Trichobilharzia regenti* and a snail *Lymnaea stagnalis*. Only hours after penetration, the parasite (P) resides in the margin of the head–foot of the snail (HF), and is developing from a miracidium into a mother sporocyst. The combination of the biological properties of both the parasite and the host decides whether the parasite will succeed, or whether it will be defeated by the host. To survive and establish an infection, the parasite requires a suitable intramolluscan environment. Also, the parasite must prevent a defence response from the snail. These conditions are met within host snails such as *Radix peregra* or *Radix ovata*. In this case, however, *Trichobilharzia regenti* penetrated into *Lymnaea stagnalis*, which is unsuitable as host. The parasite will die either from inadequate physiological conditions (such as lack of appropriate nutrients) or due to a defence response from the snail. The aggregation of haemocytes (H; phagocytic snail defence blood cells) indicates that this parasite will soon be encapsulated and eliminated. Giemsa staining. Author: L. Trefil, Parasitology, Charles University, Prague, Czech Republic.

in the snails *Radix auricularia* and *R. ovata*, but not in other lymnaeid snails such as *Lymnaea stagnalis* or *Stagnicola palustris*, whereas the reverse pattern applies to *T. ocellata* (Kock, 2001). The range of suitable intermediate hosts can be restricted to species- or even strain-level of the snail species present in a given environment. The augmented host-finding behaviour in response to *L. stagnalis*-released chemicals increases the likelihood that *T. ocellata* encounters a suitable host. However, a preference for some snails does not always prevent miracidia from penetrating non-host snails (e.g. Grassi *et al.*, 2001; Kock, 2001; Figure 7). Additionally, miracidia do not seem to avoid unsuitable strains of a particular host species. Physiological and immunobiological factors that decide the fate of schistosome parasites within snails are discussed below.

### 4.2. Intramolluscan Development: Stages and Infection Course

The entry into a snail host marks a transition from free-living to a parasitic phase in the life cycle of an avian schistosome. Studies of the avian schistosome *T. ocellata* (Sluiters, 1981; Amen *et al.*, 1991; Amen and Meuleman, 1992), supplemented with some observations from other schistosomes, provide an overview of the parasite stages, the asexual multiplication and the infection course within the snail.

After penetrating into a snail host, a miracidium undergoes dramatic changes in morphology and metabolism as it transforms into a mother sporocyst, the next larval life stage. During and after penetration, the parasite sheds the cilia-bearing epithelial plates. A new body covering forms from cytoplasmic extensions of cells that lie below a superficial muscle layer. The extended, anuclear cell projections (termed 'intercellular ridges') fuse to form a syncytial tegument that has a continuous cytoplasm without intervening cell membranes, and that remains connected with the lower-lying nucleated cell bodies (Meuleman *et al.*, 1978). The syncytial tegument represents a living interface that facilitates intimate metabolic interactions between the parasite and the internal environment of the snail host. The aerobic metabolism present in miracidia changes to a more fermentative metabolism. This allows sporocysts to be facultative anaerobes that can adjust to the variable conditions (from aerobe to anaerobe) that may occur inside the snail host (Tielens *et al.*, 1991, 1992).

At 4 days post-infection (p.i.), mother sporocysts of *T. ocellata* were present in subepidermal blood vessels and sinusoids of *L. stagnalis*, close to the site of penetration (Sluiters, 1981). Once fully transformed, mother sporocysts migrated further into the head–foot tissues of the snail. Additionally, germinal cells inside the mother sporocysts started to develop into daughter sporocysts, the next larval stage. From day 7 to day 11, individual mother sporocysts contained many maturing daughter sporocysts. Most mother sporocysts remained in the head–foot, but some migrated to the ovotestis or digestive

gland of the host. From day 12 onwards, mature daughter sporocysts emerged from the mother sporocysts and resided in the head–foot and the regions of the ovotestis or digestive gland. Individual mother sporocysts degenerated after releasing many daughters, however, intact mother sporocysts containing developing daughter sporocysts were still present at 33 days p.i.

On day 19, cercarial development from germinal cells inside individual daughter sporocysts became obvious. Daughter sporocysts now also occurred in the kidney, but mechanical damage to the organs of the host was not observed. After about a week, mature cercaria emerged from daughter sporocysts, migrated through blood vessels to the pulmonary region and left the snail by penetration of the body wall. It is hypothesized that a set of specialized glands serves for the passing of cercariae through the snail tissues (for *T. szidati*, see Neuhaus, 1952a).

The intramolluscan development of *T. ocellata* is temperature dependent. If snails were maintained at 20 °C, the infections reached patency (shedding of cercaria) at about 7 weeks p.i. (Amen *et al.*, 1991; Amen and Meuleman, 1992). At 25 °C, the prepatent time interval decreased to 4–5 weeks (Sluiters *et al.*, 1980; Sluiters, 1981). Parasite development (daughter sporocysts and cercaria) and cercarial shedding was observed to continue up to 83 days p.i. (Sluiters, 1981); the shedding of cercaria from *L. stagnalis* continued for longer than 123 days p.i. with *T. ocellata*, after which the experiment was terminated (Sluiters *et al.*, 1980). A single miracidium infection yielded thousands of cercaria, due to the asexual multiplication associated with each transition between the intramolluscan life stages. Infection with multiple miracidia increased the output of cercariae even further (Sluiters *et al.*, 1980).

### 4.3. Effects on the Snail Host

Infection with an avian schistosome has severe consequences for a snail. Remarkably, *T. ocellata* causes little or no apparent physical injury to host snails; infected snails usually live equally as long as non-infected control snails (Sluiters *et al.*, 1980; Sluiters, 1981). However, avian schistosomes actively and dramatically modify the internal physiology of a snail to maximize the resources (such as nutrients and space) that they derive from the host (for reviews see de Jong-Brink, 1995; Thompson, 1997).

To optimally obtain nutrients, schistosomes induce changes in the metabolism and biochemistry of the host. Infected snails have heightened metabolism (increased heart rate and respiration) and display changes in internal levels of proteins, carbohydrates, nitrogen and lipids (e.g. Meyer *et al.*, 1986; Thompson, 1997).

*Trichobilharzia ocellata*-infected *L. stagnalis* have increased body and shell size (McClelland and Bourns 1969; Sluiters *et al.*, 1980). The giant growth or

'gigantism' results from an increase in the haemolymph volume; the dry weight of infected snails increases only slightly relative to control snails (Joosse and van Elke, 1986). The increase in growth coincides with the occurrence of mature daughter sporocysts. *Trichobilharzia ocellata* causes gigantism probably to ensure adequate space for larval development within the snail. This phenomenon is considered to result from parasite-mediated modulation of the neuroendocrinology of the snail but the underlying mechanisms remain unclear (de Jong-Brink, 1995). It should be noted that gigantism does not occur in all schistosome–snail associations (Thompson, 1997).

Generally, schistosome infection causes 'parasitic castration', the cessation of reproduction in a snail host. Thus, resources (nutrients and space) that otherwise are used for reproduction become available to the parasite. If *T. ocellata* infects juvenile snails, the reproductive organs of *L. stagnalis* do not mature and these snails never produce eggs (Sluiters, 1981). *Trichobilharzia ocellata* retards but does not prevent the development of the reproductive organs in older *L. stagnalis*. Initially, these snails even have an increased production of eggs, but egg-laying ceases when the infection becomes patent (Schallig *et al.*, 1991). Parasitic castration is effected in an indirect fashion. Developing cercariae of *T. ocellata* contain a factor that causes *L. stagnalis* to release 'schistosomin'. Schistosomin inhibits ovoposition through antagonizing several female gonadotropic hormones (Hordijk *et al.*, 1991; Schallig *et al.*, 1992; de Jong-Brink *et al.*, 1992, 1995).

Hoek *et al.* (1997) demonstrated that infection with *T. ocellata* caused changes in the mRNA expression profile of the host, *L. stagnalis*. Among the affected transcripts were several neuroendocrine factors. By affecting the transcription of these factors, *T. ocellata* may indirectly change the physiology of the host. The changes that occur in the host are generally considered to be beneficial to the parasites. The extent of changes in host metabolism is variable, correlating with consecutive transitions between the different stages of intramolluscan larvae. Apparently, schistosome-mediated manipulations of the host physiology and metabolism ensure optimal usage of host resources for larval development while allowing the host to recover from periods of metabolic exhaustion (Thompson, 1997). It has been proposed to view an infected snail as an extended phenotype of the parasite (Dawkins, 1990). Obviously, the ability to shift the resources from host reproduction towards parasite development, thus reducing the fitness of the host to zero, makes an avian schistosome a severe pathogen for snails.

## 4.4. Internal Defence Reactions of Snail Intermediate Hosts

### *4.4.1. General comments on snail (lymnaeid) immunobiology*

Not all snail species are suitable hosts for schistosomes. For instance, digenean infection has never been observed from *Crepidula fornicata*, a marine

gastropod (Pechenik *et al.*, 2001). Field studies show that the incidence of schistosome infection in snail populations is usually low (Loy and Haas, 2001). Potentially, local adaptation reduces the suitability of snails as hosts for sympatric strains of schistosomes (Morand *et al.*, 1996). However, snails can also be naturally resistant to schistosomes (e.g. Joubert *et al.*, 1990; Grassi *et al.*, 2001). The resistance has a genetic basis. Selective breeding can yield snail strains that do not support parasite development (e.g. Richards *et al.*, 1992; Langand and Morand, 1998).

Snails possess potent internal defences that are capable of eliminating invading pathogens, including schistosome larvae (for reviews see: van der Knaap and Loker, 1990; Adema and Loker, 1997; Lardans and Dissous, 1998). In combination, the genetic backgrounds of both the parasite and the host decide the fate of an invading parasite. A snail in which a parasite cannot develop due to lack of nutrients or inappropriate pH is an unsuitable host. Other snails may provide the correct internal environment for a particular schistosome, but mount an internal defence and eliminate the invader. This renders the snail immunobiologically incompatible or resistant. In a suitable snail host, the internal defences fail to eliminate an invading schistosome, resulting in an immunobiologically compatible parasite–host association.

Snails (and invertebrates in general) lack lymphocytic defences (no antibodies and T-cell receptors, no immunological memory) and employ internal defences of an innate type. Lectins mediate self-non-self differentiation by pattern recognition of repetitive carbohydrate moieties that characterize the surface of pathogens (Janeway, 1989). Snail lectins occur in soluble form, and associated with the cell membrane of defence cells. Recent molecular studies suggest that snails produce complex and highly diverse lectins (Kurachi *et al.*, 1998; Zhang *et al.*, 2001). The binding of a snail lectin to pathogen-associated carbohydrates is thought to activate a snail defence response, comprising humoral and cellular components (van der Knaap *et al.*, 1982, 1983; Richards and Renwrantz, 1991; Horák and van der Knaap, 1997; Horák and Deme, 1998; Horák *et al.*, 1998c).

The humoral defences of snails include several agglutinins and toxic activities (for review see Adema and Loker, 1997). Study of gene expression in *L. stagnalis* after infection with *T. ocellata* (Hoek *et al.*, 1996, 1997) identified a cDNA transcript that was named molluscan defence molecule (MDM), based on sequence similarities with the haemolin, a soluble immune protein with opsonic properties, present in insects (Schmidt *et al.*, 1993). Planorbid snails produced increased amounts of several parasite-reactive plasma polypeptides in response to echinostome parasites. Among these are lectins of a large and diverse gene family of fibrinogen-related proteins (FREPs). FREPs have been found in several planorbid species; no sequence data are available to suggest that lymnaeid snails also employ FREPs (Adema *et al.*, 1997; Zhang, 2001). The functional aspects of both MDM and FREPs remain to be clarified further.

Cell-free plasma of several lymnaeid and planorbid snails contained an activity that killed incompatible intramolluscan trematode larvae *in vitro* (Sapp and Loker, 2000).

Haemocytes, phagocytic cells that circulate in the blood, constitute the major cellular defences of snails. Activated haemocytes migrate towards and then phagocytose or encapsulate pathogens. The internalized pathogens are eliminated by cell-mediated cytotoxicity. *In vitro* killing of incompatible schistosome larvae by haemocytes of *L. stagnalis* and other snails involves lysosomal enzymes and toxic chemicals such as reactive oxygen intermediates (Dikkeboom *et al.*, 1988; Adema *et al.*, 1993, 1994; Hahn *et al.*, 2001a) and nitric oxide (Hahn *et al.*, 2001b).

Several factors such as age of snails, pollution and the presence of other parasites reduce the functioning of the internal defences of snails (Dikkeboom *et al.*, 1985; Russo and Lagadic, 2000). Generally, however, snails have potent defences that successfully eliminate invading pathogens (Richards *et al.*, 1992; Grassi *et al.*, 2001). It is remarkable then that in some combinations, snails fail to eliminate an invader and become infected by schistosome parasites.

### *4.4.2. Parasite-induced changes in cellular and humoral immunity*

Schistosomes employ both passive and active strategies to survive the internal defences of snails in order to establish a successful infection. However, circumventing the host defences is likely to be a highly complex process that is successful only in a limited range of snail strains or species. Upon entry into a snail, a schistosome will deploy its particular survival strategies to try and prevent a host defence response. Only the defence system of a suitable snail is rendered ineffective; the internal defences of resistant snails remain unaffected and eliminate the schistosome parasite. It is likely that this leads to the restricted immunobiological compatibility between schistosomes and snails.

A schistosome can passively display surface moieties that are not recognized as foreign to evade recognition as non-self. If an invading pathogen is not detected, the internal defence system of the snail remains inactive. Indeed, the composition of carbohydrate moieties on the surface of *T. ocellata* and *T. szidati* changes considerably when a miracidium transforms into a sporocyst (Gerhardus *et al.*, 1991; Horák,1995), but the functional significance remains to be determined. Schistosomes may also mask their surface with host-like epitopes. This can be achieved by absorbing host-derived factors onto the surface (molecular masking). *Trichobilharzia ocellata* may employ this strategy: immediately after emerging from daughter sporocysts, cercaria were observed to acquire host-like factors onto their surface that bind antisera raised against plasma of the host, *L. stagnalis* (Roder *et al.*, 1977; van der Knaap *et al.*, 1985).

Schistosomes also display molecular mimicry, the expression of host-like determinants from genes of the parasite itself. The question has been raised whether similar gene products protect the parasite or whether sequence similarities between parasite and host have resulted from convergent evolution (Yoshino and Boswell, 1986; Damian, 1987; Dissous and Capron, 1995).

Schistosomes also actively inhibit host defences. After the initial observation that echinostome parasites rendered host snails susceptible to schistosomes for which they were normally resistant (Lie *et al.*, 1976), it was determined that schistosome parasites also reduce the defensive capabilities of snails (Lie and Heyneman, 1977). The interference hypothesis (Lie, 1982) proposes that schistosomes and other trematode parasites interfere with the function of snail haemocytes in order to survive within the host. *Trichobilharzia ocellata* mediates interference indirectly by releasing secreted and/or excreted products (SEP) that interact with the central nervous system (CNS) of the *L. stagnalis* to affect the internal defence system of the snail (Amen and de Jong-Brink, 1992). Concordant with these findings is the demonstration of altered gene expression in the CNS of *L. stagnalis* after infection with *T. ocellata* (Hoek *et al.*, 1997). Among the suppressed messages was that of the putative defence factor MDM (Hoek *et al.*, 1996). It is assumed that this reduces the efficiency of host defences, benefiting the parasite. Changes in the expression levels of neuroendocrine factors (Hoek *et al.*, 1997) are also thought to affect the host defences (de Jong-Brink, 1995).

The SEP released by schistosomes mediate direct interference effects towards snail haemocytes. Co-incubation of snail haemocytes with SEP collected from larval schistosomes or with sporocysts inhibits several functional properties of these defence cells such as intracellular protein synthesis (Lodes *et al.*, 1991), haemocyte motility (Lodes and Yoshino, 1990) and production of oxygen radicals (Connors *et al.*, 1991). *Trichobilharzia ocellata* inhibits phagocytosis (Amen *et al.*, 1992), efficiency of encapsulation (Adema *et al.*, 1994) and bacterial killing (Nuñez *et al.*, 1994) by haemocytes from *L. stagnalis*. In the latter case, the killing activity of *L. stagnalis* haemocytes was inhibited by a 40-kDa protein component of SEP from *T. ocellata*. Intriguingly, the 40-kDa factor did not affect the bactericidal activity from haemocytes of a non-host snail *Planorbarius corneus* (Nuñez and de Jong-Brink, 1997; Nuñez *et al.*, 1997). This finding seems to underline the fact that the capabilities for immunomodulation of schistosomes are restricted to affect only host snails. At this time, sequence information is not available for any candidate interference factors.

Clearly, much remains to be learned about the close and specific association between schistosomes and snails to understand fully how the larval development in the intermediate snail host culminates in the release of cercariae.

## 5. DEVELOPMENT IN VERTEBRATE HOSTS

### 5.1. Host-finding

The mature cercariae are released from snails in high quantities, especially on sunny days preceded by an overcast period. Cercaria are thought to use secretions of specialized glands to pass through the snail tissues (*T. szidati*, see Neuhaus, 1952a). Free-living cercariae need to infect a final host quickly as the parasites have only a short life span after emerging from the snail into the water (1–1.5 day at 24 °C; Neuhaus, 1952a). Characterization of the host-finding behaviour of *T. ocellata* (Haas 1992, 2001 for reviews) revealed that the cercariae exhibit a positive phototactic and a geonegative orientation and can swim both backwards and forwards (Figure 8). A forward swimming response towards shadows, water turbulence and touch affords a high probability of contact with a final host (Feiler and Haas, 1988a).

Prior to an initial contact, cercariae are selectively attracted to, and attach to, substrates that display physical and chemical properties of warm-blooded vertebrate skin. Not all warm surfaces are equally attractive; cercariae of *T. ocellata* can differentiate their preferred temperature with a sensitivity of only 1 °C (Feiler and Haas, 1988a). Cercariae also react to chemical signals. *Trichobilharzia* sp. cercariae released from *Physa acuta* were attracted by linoleic acid (Graczyk and Shiff, 2000), a fatty acid that also attracts *S. mansoni* (Shiff *et al.*, 1993; Shiff and Graczyk, 1994). Skin surface lipids such as ceramides and cholesterol are important stimuli for attachment of *T. ocellata* (Feiler and Haas, 1988b). Feathers covered by the uropygial gland secretions lack these particular lipids allowing cercariae to preferentially attach to the skin of birds. Due to a similar lipid composition of the skin of birds and mammals, the cercariae are not able to avoid penetration into mammals and cause cercarial dermatitis (see section 6.2.1.).

### 5.2. Penetration of the Vertebrate Skin

After contact with the host the cercariae of *T. ocellata* show several behavioural patterns: attachment to the host, enduring contact, leech-like creeping to a suitable entry site (wrinkles in the skin or openings of hair follicles) and penetration (Figure 8). On average, the creeping behaviour lasts 8 s, ranging from 0 to 80 s (Haas and Haberl, 1997; Haas and van de Roemer, 1998). Not all cercariae initiate penetration efforts following attachment. Appleton and Brock (1986) estimated that 85.2% of *Trichobilharzia* sp. cercariae (released from *Lymnaea natalensis*) attempted to penetrate mouse skin. After 1 h, 59% of the cercariae had proceeded to enter the skin, whereas the remaining 41% were

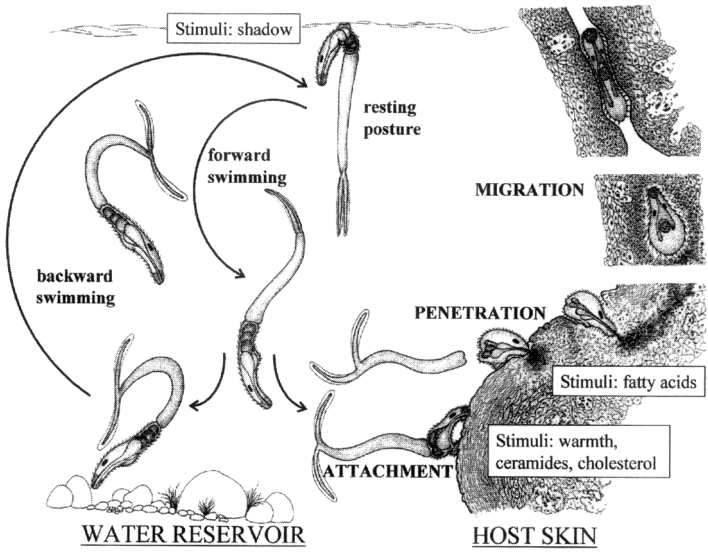


*Figure 8* Behaviour of *Trichobilharzia* before and after the penetration into a vertebrate host (behaviour of free-living cercariae modified after Haas, 1992).

The free-living cercarial stage of *T. ocellata* shows phototactic and geonegative orientation. Additionally, it displays four types of behaviour: (1) Resting posture is a motionless position in which parasites cling to the water surface with the acetabulum, the tail hangs downward and the furcae are relaxed; cercariae do not respond to water currents or touch. (2) Forward swimming (body first). This behaviour is stimulated mainly by passing shadows. The direction of swimming is directed away from the light source; the furcae of the tail are folded together; only in this phase do cercariae respond to thermal and chemical vertebrate cues that induce attachment. (3) Backward swimming (tail first) towards light (upward) is stimulated by a contact with inappropriate substrates. (4) During the passive phase cercariae sink down using the furcae as a drag anchor. The active phase (2 or 3) alternates with the passive phase (1 or 4).

After contact with the host, *T. ocellata* shows four patterns of behaviour: (1) attachment to the host (stimulated by warmth and surface lipids – ceramides and cholesterol); (2) enduring contact; (3) creeping to a suitable entry site; and (4) penetration (stimulated by fatty acids). At the start and during penetration, the contents of the penetration glands are released. The penetration process is accompanied by penetration movements (contractions and elongations of the body, mechanically supported by the spines that cover the body), shedding of the tail and transformation of the tegument. The gland secretions surround the schistosomulum and line the entry tunnel. After the initial phase of skin infection, visceral *Trichobilharzia* species usually enter the lymphatic or venous system whereas nasal *Trichobilharzia* migrate via peripheral nerves.

still attached by the suckers to the epidermis. Comparable observations were made from *T. regenti* (Hrádková and Horák, 2002). Perhaps cercariae do not always encounter appropriate signals to initiate penetration of the host. However, cercariae can also establish (experimental) infection perorally, penetrating mucosa of the oral cavity (Macy *et al.*, 1955). This suggests that cercariae are not highly restricted in recognizing and penetrating the different epithelia of a vertebrate.

Whereas warmth alone has no effect, penetration is stimulated by free fatty acids (saturated acids with 9–14 C atoms and unsaturated acids; the penetration-stimulating effect of the latter acids increases with the number of double bonds; Feiler and Haas, 1988a; Haas and van de Roemer, 1998). The high levels of such fatty acids in human skin stimulate higher rates of cercarial penetration in humans than in the natural duck host. The nature of stimulatory host signals may be species- or genus-specific; another bird schistosome, *Austrobilharzia terrigalensis*, initiates penetration in response to free sterols, whereas fatty acids have only a weak effect (Clegg, 1969).

The cercariae start the penetration process by attaching the head organ to the skin surface, in a similar fashion as described for human schistosomes (*S. mansoni*). Like *S. mansoni* (Howells *et al.*, 1975), *T. ocellata* sheds the tail at this time by the contraction of a sphincter muscle at the cercarial hindbody (Haas and van de Roemer, 1998). Sometimes the tail is shed earlier during the creeping phase. The contents of the penetration glands are released onto the skin. These contain proteolytic enzymes, including elastase, a 30-kDa serine proteinase (*T. ocellata*; see Bahgat *et al.*, 2001; Bahgat and Ruppel, 2002). This can be observed histologically by a distinct lysis of the stratum corneum (Bourns *et al.*, 1973; Haas and van de Romer, 1998). Moreover, detection with fluorescein-labelled lectins showed that gland deposits surround the penetrating parasite (*T. szidati*) and line the resulting entry tunnel in the host tissue (Horák *et al.*, 1998b). The gland secretions contain additional types of bioactive molecules. *Trichobilharzia szidati* releases a lectin specific for β-1,3–glucan and glycosaminoglycans that may be involved in recognition of host connective tissue and activation of parasite effector molecules (e.g. proteases). Other than carbohydrate-binding properties, no additional activities have been detected for this lectin (Horák *et al.*, 1997). The lytic attack on the skin and underlying tissues, combined with intermittent contractions of the body which is covered with tegumental spines, allows cercariae to disrupt and penetrate the skin of the host. Usually, cercariae of *T. ocellata* penetrate in a nearly surface-parallel direction to the stratum corneum before entering deeper tissue layers. On average, the parasites pass through the stratum corneum within 4–5 min and the stratum germinativum is penetrated at 3 h p.i. (Bourns *et al.*, 1973; Haas and van de Roemer, 1998).

### 5.3. Cercaria–Schistosomulum Transformation and Immune Evasion

With the transition from free-living to parasitic life inside the final host, the parasite undergoes a second transformation, from cercaria to schistosomulum. This transformation is accompanied by a substantial reconstruction of the surface. The thick cercarial glycocalyx is shed, which considerably changes the antigenic properties of the parasite. This is demonstrated by low recognition of the parasite's surface by lectin probes and anti-cercarial antibodies. This state remains for several hours or even days and may represent a way to escape recognition by the host immune system (Horák *et al.*, 1998b). The outer tegumental membrane is replaced by a double membrane (Horák *et al.*, 1998b) that is typical for all blood-dwelling adult trematodes (spirorchids, sanguinicolids, schistosomes; McLaren and Hockley, 1977). The outer membrane of human schistosomes is thought to also have immune-evasive properties.

Additional factors may protect the parasite from immune attack. The cercariae of *T. ocellata* possess enzymes that convert $C_{20}$ polyunsaturated fatty acids (most notably arachidonic acid) into eicosanoids (lipid mediators) such as prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids. Interestingly, eicosanoids are produced at levels similar to those of human schistosomes (Nevhutalu *et al.*, 1993). These bioactive compounds may cause vasodilatation in host tissues, and suppress immune functions such as superoxide production by human neutrophils (Nevhutalu *et al.*, 1993) and the migration of epidermal Langerhans cells, which play a key role in the immune defence system (Angeli *et al.*, 2001).

### 5.4. Migration

Shortly after completion of the transformation, schistosomula of the visceral species of *Trichobilharzia* leave the skin and enter the blood vessels of the host to mature and spend their adult life in the circulatory system (see section 5.4.5 for information on nasal species). Adult parasites usually reside in portal or intestinal veins of naturally infected hosts, sites that are typical also for species of the genus *Schistosoma* (for review see Basch, 1991). Based on this similarity, it is expected that *Trichobilharzia* follows the same migration route as *Schistosoma* (for review see Bayssade-Dufour *et al.*, 1994), entering lymphatic or venous vessels and continuing via the right heart to the lungs, the left heart and the systemic circulation to the final location. The receptors and mechanisms that guide the path of migration through the host are not understood (Basch, 1991). Interestingly, the parasites migrate along similar routes in both (immunologically and physiologically) compatible final (bird) hosts and incompatible hosts. However, parasites do not start reproduction in incompatible hosts and die at various intervals p.i.

### *5.4.1. Visceral schistosomes in the skin phase*

*(a) Compatible hosts.* A comparison of the number of cercariae that penetrated the skin and the number of worms recovered from internal organs indicates that many parasites fail to cross the skin. Ellis *et al.* (1975) suggested that schistosomula that are unable to break the cornified layers are permanently trapped in the skin. The passage of schistosomes through the skin seems to depend mainly on the immunity status of the host (see section 6.1.4.). Relative to primary infections, the number of *T. ocellata* schistosomula trapped in the skin of ducks increased with repeated challenge infections (Bourns *et al.*, 1973; Ellis *et al.*, 1975; Rau *et al.*, 1975). Successful schistosomula of *T. ocellata* leave the duck skin within 3 days p.i. (Bourns *et al.*, 1973). *Trichobilharzia* migrates rapidly after entering the circulation, especially in comparison with *S. mansoni* (Haas and Pietsch, 1991). By days 3 and 4 p.i., 36% of the parasites originally present in the skin were detected in various internal organs (Bourns *et al.*, 1973; Haas and Pietsch, 1991).

*(b) Incompatible hosts.* Avian schistosomes, including *Trichobilharzia* spp., may or may not be able to cross the skin of an inappropriate vertebrate host. For instance, Cercaria parocellata did not penetrate beyond the epidermis of humans (Macfarlane, 1952). As observed from the bird schistosome *A. terrigalensis*, failure to penetrate results in increased mortality of parasites in the skin (Rai and Clegg, 1968). In contrast, some schistosomula of *T. ocellata* leave the mouse skin soon after penetration, although others can still be extracted from the skin by day 4 p.i. (Haas and Pietsch, 1991). As in the bird hosts, survival of the parasites depends heavily on the immune status of the host (see section 6.2.1.). In previously unsensitized rabbits *T. ocellata* and *T. stagnicolae* survived up to 48 h p.i. However, many of the worms had died in the skin of sensitized rabbits by 6 h p.i. (Olivier and Weinstein, 1953).

### *5.4.2. Visceral schistosomes in the lung phase*

*(a) Compatible host.* On the way towards the systemic circulation, the lungs represent the first target organ that is invaded by migrating schistosomula, as reported for several species: *T. cameroni*, *T. oregonensis*, *T. parocellata* and *T. ocellata* (McMullen and Beaver, 1945; Wu, 1953; Macy *et al.*, 1955; Islam and Copeman, 1986). Within 1–2 days after penetrating the skin *T. ocellata* invades the lungs and remains there between 4 and 16 days p.i. During this time the parasites grow and feed on the host blood (Bourns *et al.*, 1973; Ellis *et al.*, 1975; Haas and Pietsch, 1991; Horák and Kolářová, 2000). Once in the lungs the parasites leave the venous system and probably remain extravascularly in the air spaces for a certain amount of time. They migrate from air capillaries and parabronchi to secondary bronchi, then invade the bronchial epithelium

and gain entrance into vessels (Bourns *et al.*, 1973). The lungs represent a difficult barrier and only some schistosomula are able to pass through. Even during primoinfections of compatible hosts, schistosomula are frequently trapped during the lung passage. In contrast to the lungs, a lower recovery rate for *T. ocellata* worms was reported for duck livers and intestine (e.g. Haas and Pietsch, 1991).

*(b) Incompatible host.* In several cases, avian schistosomes invade the lungs of incompatible hosts. This was first described for schistosomula of *T. stagnicolae* in Swiss mice, *T. ocellata* in Swiss mice, hamsters and monkeys, and *T. physellae* in mice at 4–7 day intervals p.i. (Olivier, 1953). A pulmonary phase was also recorded from mice infected with *T. brevis* (Basch, 1966), *Trichobilharzia* sp. (Appleton and Brock, 1986) and *T. szidati* (Horák and Kolářová, 2000). Similar observations were reported for other schistosome genera, i.e. *Austrobilharzia*, *Bilharziella* and *Ornithobilharzia* (Bacha *et al.*, 1982; Horák and Kolářová, 2000; Bayssade-Dufour *et al.*, 2001). This suggests that lung migrations are common to all visceral species of schistosomes.

The migrating schistosomula can reach the lungs within 10 h p.i. (*T. ocellata* in mouse; Haas and Pietsch, 1991). Usually, the survival time in an incompatible host is short. However, *T. szidati* worms may persist in the mouse lungs for up to 10 days p.i. (Horák and Kolářová, 2000). Haas and Pietsch (1991) reported that, despite a significant decrease in parasite number within the first 5 days, schistosomula of *T. ocellata* survived in the lungs of mice up to 6 days p.i.

Subsequently, worms disappear from the incompatible hosts. The factors influencing survival of lung schistosomula are poorly understood. Schistosomula of *T. szidati* seem metabolically active in the lungs of incompatible hosts; their gut contains a dark pigment evidently derived from digestion of erythrocytes of the host (Horák and Kolářová, 2000). Perhaps the parasites are elimated by a host immune response. However, electron microscopy of *T. szidati* in the lungs of mice showed no damage to the tegument and an absence of host immune cells (Horák and Kolářová, 2000). The authors hypothesized that failure of the parasite during a primary infection of an incompatible host may result from some immunologically unrelated factors (absence of essential nutrients, etc.). A comparison of the pulmonary phase of infection showed that *T. szidati* schistosomula grow and feed rapidly in a duck, whereas in a mouse they seem to be inhibited in development (Horák and Kolářová, 2000). As suggested by Kolářová (2001), survival of parasites in the lungs can also be influenced by their size; schistosomula of certain species can be too large to enter lung capillaries and to migrate in a non-specific host.

### *5.4.3. Visceral schistosomes in the liver phase, final location and egg production*

*(a) Compatible host.* After re-entering the systemic circulation, the parasites migrate to a final location. In most cases, adult schistosomes concentrate in the portal or intestinal veins of the venous system. This suggests that the schistosomula of visceral avian schistosomes develop into adults in the portal veins, similar to *Schistosoma* spp. The few available data indicate that the prepatent period for *Trichobilharzia* infections is about 12–14 days (Neuhaus, 1952a; Basch, 1966; Bourns *et al.*, 1973; Meuleman *et al.*, 1984b). The stimuli that guide the migration of adult worms and the exact location for egg-laying remain to be fully characterized. However, adult worms of different species appear to favour particular sites for oviposition. Depending on the species of *Trichobilharzia*, parasite eggs have been detected in the intestinal wall of various parts of the small and large intestines, including the cloaca of birds (McMullen and Beaver, 1945; Wu, 1953; Yamaguti, 1971). The migration of adults to sites where egg-laying occurs may be similar to that proposed for *A. variglandis* (Wood and Bacha, 1983). The female worms travel from vessels of the intestinal serosa through the muscularis externa and crawl by means of body contractions into small veins of the mucosa. After the release of eggs, the worms return to the serosa. This allows the small mucosal veins to contract around the newly deposited eggs, keeping them in that place.

The eggs pass from the mucosal vessels through the connective tissue of the lamina propria into the intestinal lumen. As suggested for human schistosomes, the progression of eggs may be due to a combination of host inflammatory reactions and egg-derived proteases, resulting in lysis of the tissues. The finding of frequent accumulations of leucocytes around eggs located within blood vessels supports this view (Wood and Bacha, 1983). Muscular contractions of the host may further help the eggs to pass through the connective tissue into the intestinal lumen (Bloch, 1980). Finally, the eggs are released from the intestines with the faeces when the bird defecates.

It is not clear whether adult avian schistosomes spend their entire life in the portal and/or intestinal veins. On at least two occasions, adult worms have been encountered in the intestinal tissues. Adult *T. ocellata* migrate to veins of the small intestine and subsequently penetrate the mucosa, sometimes even approaching the tops of villi (Bourns *et al.*, 1973). Similarly, Neuhaus (1952a) recorded mature adults of *T. szidati* in muscularis submucosa and the mucosa of the intestinal wall. Both *T. ocellata* and *T. szidati* disappeared from the host intestines about day 21 p.i. This time also coincides with the end of the patency of infection; the bird host ceases to release parasite eggs in the faeces (Bourns *et al.*, 1973; P. Horák, unpublished data). Because all schistosomes normally occur in the blood system, their location in the intestinal tissues may represent a terminal phase of the infection. Hypothetically, the flukes escape from the

blood vessels during this phase and are destroyed in the tissues or enter the intestinal lumen, die and leave the host. The death of the adult worms could explain the observed brevity of *Trichobilharzia* infections.

The fate of the majority of adult worms in the 'postpatent' period remains to be determined, although it is generally assumed that adult worms die soon after depositing their eggs. However, a limited number of worms may leave the intestinal area to return to the liver. Mature *T. parocellata* were found in portal veins of the liver in a duck at 86 days p.i. (Islam and Copeman, 1986), and *T. ocellata* was observed in the latter organ even at 248 and 370 days p.i. (Bourns *et al.*, 1973). Interestingly, dead and degenerating females of *T. physellae* were found within the interlobular bile ducts (Pence and Rhodes, 1982). Remarkably, adult parasites survive for some time in dead hosts; Szidat (1938) recovered living worms from decomposing birds that had died several days earlier.

The factors that determine the life span of these parasites remain unclear. Nevertheless, the patency of *Trichobilharzia* infections and potentially also the life span of adult avian schistosomes are considerably shorter than those of human schistosomes (for review see Basch, 1991).

*(b) Incompatible host.* Immature worms are rarely found in the liver of non-specific hosts. However, radiolabelled *T. ocellata* were sporadically detected in the mouse liver and intestine on days 2–5 p.i. (Haas and Pietsch, 1991). The longevity of these parasites in the liver remains unknown, but appears to be shorter than that in the lungs.

### *5.4.4. Egg dissemination and atypical migration of visceral schistosomes*

As occurs in human schistosomiasis (for review see Bacha *et al.*, 1982), part of the eggs of *Trichobilharzia* species fails to leave the bird host and is disseminated via the circulatory system to the liver, lungs and other organs (McMullen and Beaver, 1945; Wu, 1953). The number of eggs in various organs, and associated pathology (see section 6), increase with higher worm burdens. In heavy infections, adult flukes may reside in atypical locations within the host. The infection by 300–400 specimens of *T. physellae* resulted in equal distribution of worms over the lungs, liver and intestine of a canary (McMullen and Beaver, 1945). It is likely that additional observations of adult parasites in atypical sites, e.g. *T. ocellata* in the lungs (Bourns *et al.*, 1973) and *Trichobilharzia* sp. in gonadal, gastric, oesophageal, jugular, femoral and thyroidal veins (Wojcinski *et al.*, 1987) and in interlobular bile ducts (Pence and Rhodes, 1982), were due to high infection doses also.

### *5.4.5. Nasal schistosomes*

Some schistosome species mature in the nasal area of a final host. Of only nine species that were described to date, *S. nasale* belongs to the genus *Schistosoma*. The others are representatives of the genus *Trichobilharzia* (Horák *et al.*, 1998a). Except for *T. regenti*, there are no details available regarding the migration routes to the nasal tissues.

*(a) Compatible host.* The migration route of a nasal schistosome such as *T. regenti* differs dramatically from those of visceral schistosomes (Horák *et al.*, 1999; Hrádková and Horák, 2002). After penetrating the skin of a duck, *T. regenti* invades peripheral nerves. The schistosomula then migrate further through the central nervous system (CNS), progressing through the spinal cord and the brain. Ultimately, the schistosomula locate to blood vessels in the nasal area where they complete their maturation to adult worms, mate and produce eggs. The progression through the host CNS is fast: schistosomula can be detected in peripheral nerves by day 1.5 p.i., in the spinal cord by day 2 p.i and in the brain by day 12 p.i. (Hrádková and Horák, 2002). The nasal area is reached by day 13 p.i. (Horák *et al.*, 1999). Eggs are produced by females as early as 14 days p.i. (Horák *et al.*, 1999). Contrary to visceral schistosomes, miracidia hatch from the eggs directly in the nasal soft tissues (Horák *et al.*, 1998a). The life span of *T. regenti* in a duck is estimated to be 23–25 days. The end of the patent period appears to be characterized by migration of adults from vessels into the surrounding extravasal tissues of the nasal area (Horák *et al.*, 1999; Kolářová *et al.*, 2001).

*(b) Incompatible host.* Infection of *T. regenti* in mice is also accompanied by invasion by schistosomula of the CNS. The parasites enter the peripheral nerves, migrate to the spinal cord (by day 2 p.i.), and reach the brain by day 3 p.i. (Hrádková and Horák, 2002). Contrary to the establishment and reproduction of worms in ducks, the infection of mice fails and results in the death of parasites in various parts of the CNS (Horák and Kolářová, 2001). The parasites triggered a host immune response (see section 6.2.2.). This can partly explain the incomplete development of the worms (Kolářová *et al.*, 2001).

A comparative study of the migration of *T. regenti* in suitable versus unsuitable (including immunodeficient and immunocompetent) vertebrate hosts disclosed differences in the rate of migration during the first 3 days p.i. (Hrádková and Horák, 2002). During this time, worms were recovered from the synsacral and thoracic spinal cord of ducks. In several strains of mice tested, the *T. regenti* had progressed further, having reached the cervical spinal cord and medulla oblongata, and in SCID mice, even the cerebellum. Differences in body size of the host may also affect the rate of migration, as suggested for visceral schistosomes (McMullen and Beaver, 1945). The migration rate of worms was faster in the CNS of SCID mice than in that of immunocompetent (BALB/c and hr/hr) mice (Hrádková and Horák, 2002).

Interestingly, the highest numbers of worms were found in the synsacral spinal cord of ducks and the thoracic spinal cord of mice. Many parasites remained in these locations (also during the later phases of infection) and only a small percentage of worms migrated further.

On rare occasions, juveniles of *T. regenti* and *T. arcuata* were observed in the lungs of both duck and murine hosts (Islam, 1986b; Horák *et al.*, 1999; Hrádková and Horák, 2002). If these parasites retain the ability to reach their final location, it can be hypothesized that these parasites use the arterial system of the host to migrate from the lungs to the nasal area of a duck.

## 6. VERTEBRATE IMMUNE RESPONSE AND PATHOLOGY

Schistosomes are considered to be highly pathogenic for migratory waterfowl (Graczyk *et al.*, 1993). The general paucity of reports dealing with clinical aspects, pathology and immunity of trichobilharziasis can be explained in part by the ease with which the parasites can be overlooked during gross necropsy due to their small size and occurrence within blood vessels (Wojcinski *et al.*, 1987). The available data focus mostly on symptoms of patent infections in birds. However, recent investigations have elucidated that immature flukes also may cause severe tissue injuries. Therefore, the spectrum of syndromes is probably wider than previously considered.

Cercarial dermatitis in mammals is but one familiar aspect of the pathology that bird schistosomes cause in incompatible hosts. Despite the failure to develop fully, the parasites penetrate and migrate in mammals during early phases of infection in a similar fashion to that observed in avian hosts. These novel findings warrant that the role of these parasites in other pathological changes of tissues and organs should be reassessed (Horák and Kolářová, 2001).

### 6.1. Compatible Hosts

#### *6.1.1. Factors influencing severity of infections*

Particular schistosome infections in waterfowl differ in severity. In general, pathogenicity of each species is determined by parasite biology (e.g. metabolism, feeding, mobility) and morphology (e.g. size), preferred site of the adult worms, the worm load and the duration of infection. Severe damage to tissue and organs results from accumulation of numerous parasites in vessels and dissemination of many eggs of the parasite to various organs of the host (Wojcinski *et al.*, 1987). Whereas mild infections with visceral schistosomes often are subclinical or even asymptomatic (Macy *et al.*, 1955; Basch, 1966),

even a small number of nasal schistosomes in close proximity to the brain may cause severe syndromes. The severity of injuries may progress with time as the parasites persist in the host.

Also immature schistosomes may severely impair the host. In some instances, invasion of the lungs by high numbers of visceral schistosomes induces sufficient lung damage to cause death (McMullen and Beaver, 1945; Wu, 1953). During the prepatent period of infection, migrating schistosomula of *T. regenti* can mechanically damage and destroy nervous tissue. Potentially, the parasite also releases metabolites and other secretory or excretory products that may be neurotoxic. The number of parasites additively increases the pathology (Kolářová *et al.*, 2001).

The resulting clinical picture is influenced by innate susceptibility and suitability as well as by acquired immunity of the hosts. In the field, the prevalence of *Trichobilharzia* infections differs among various avian species (e.g. Appleton, 1986; Loken *et al.*, 1995) and usually the prevalence in adults is lower than in young birds (Guth *et al.*, 1979). The study of sera from birds that were repeatedly infected showed that acquired immunity increases with age and previous exposure to *Trichobilharzia* parasites. *Trichobilharzia*-released antigens evoke immune reactions around the parasites. Dead worms induce the strongest responses, probably due to the release of large amounts of antigens (Kolářová *et al.*, 2001). Specifically, *T. regenti*-infected birds produce antibodies against gut-associated antigens (GAA) of adult worms (Kouřilová and Kolářová, 2002). It is of interest that repeated infections by cercariae result in a reduction of severity of *T. ocellata* infection in birds (Ellis *et al.*, 1975; Rau *et al.*, 1975). Nevertheless, transfer of lymphoid cells and/or immune serum did not convey significant levels of passive immunity to *T. ocellata* in ducks (Bourns and Ellis, 1975).

### *6.1.2. Clinical features*

The majority of syndromes that are usually associated with trichobilharziasis occur during the patent phase of infection, due to injuries to tissues and organs that develop in close proximity to the sites where egg-laying worms of *Trichobilharzia* reside. However, cercarial invasion into the skin and the migration of immature parasites also cause various disorders. For instance, pulmonary symptoms may appear shortly after the penetration by visceral schistosomes. Infections of ducks by *T. regenti* showed that the prepatent phase can be manifested by neuromotor symptoms (balance or orientation disorders and pareses) due to the migration of this nasal schistosome through the CNS of the host (Horák *et al.*, 1999).

Whereas birds appear to tolerate mild infections with visceral schistosomes relatively well (Macy *et al.*, 1955; Basch, 1966), heavy infections may cause

swelling of feet and legs, emaciation, weight loss and stunted growth (Macy *et al.*, 1955; Wojcinski *et al.*, 1987). Moreover, death was ascribed to *Trichobilharzia* infections: in birds infected experimentally with *T. ocellata*, *T. oregonensis* and *T. elvae* (Brumpt, 1931; Macy *et al.*, 1955), and in wildfowl harbouring *T. physellae* (Pence and Rhodes, 1982), *Trichobilharzia* sp. (Graczyk *et al.*, 1993) and *Trichobilharzia* sp. + *Dendritobilharzia* sp. (Wojcinski *et al.*, 1987). The patent period of *T. regenti* infections can be associated with bleeding of the nasal mucosa (Horák *et al.*, 1999).

The dissemination of eggs to various organs and tissues may cause non-typical symptoms during the chronic phase. These include proliferative conjunctivitis in Hawaiian goose due to *Trichobilharzia* sp. eggs (Schmäschke *et al.*, 1992) and neurological problems displayed by birds that have eggs of *Trichobilharzia* sp. in the brain (Graczyk *et al.*, 1993).

### *6.1.3. Gross pathology*

*Trichobilharzia* infections are initially characterized by skin symptoms. Penetration of *T. szidati* and *T. regenti* cercariae in skin of duck frequently leads to rapidly developing macular eruptions (Horák *et al.*, unpublished data), followed by crust formation and scaling of the affected tissues at 2–3 days p.i. (Ellis *et al.*, 1975). Subsequently, migrating schistosomula may cause haemorrhage, inflammation and subsequent consolidation of the tissue in the lungs (McMullen and Beaver, 1945) and, in cases of heavy infections, enlargement and abscessation of the liver (McLeod and Little, 1942).

Patent infections with low numbers of parasites may result in two different types of lesions (Pence and Rhodes, 1982). (1) Minor lesions associated with the eggs occur mostly in the liver and intestines. The surface of livers of ducks infected with *T. brevis* (Basch, 1966) and *T. oregonensis* (Macy *et al.*, 1955) displayed white cysts 150 and 175 μm in diameter, respectively. Potentially, severe infections cause livers to enlarge and have a pale aspect, and cross-sections disclose a friability of the liver tissue (Pence and Rhodes, 1982). The intestinal mucosa contains granulomas (evident as reddish and soot-like spots), although weak infections are limited to some sloughing of the internal intestinal wall (McLeod and Little, 1942). The egg dissemination by venous circulation may also result in lesions in other organs. In Hawaiian geese, whitish proliferative alterations occurred in the sclera, nictitating membrane and conjunctiva, accompanied occasionally by microabscesses (Schmäschke *et al.*, 1992). (2) The accumulation of numerous adults in mesenteric veins leads to major lesions. These encompass thrombosis, thickening and congestion of the intestine with a granular fibrino-haemorrhagic exudate adherent to the mucosal surface (due to host immune reactions), pulmonary congestion and hepatomegaly (Wojcinski *et al.*, 1987).

On occasion, the presence of egg-laying nasal schistosomes causes haemorrhages and petechiae in the nasal cavity of birds (Fain, 1956a; Horák *et al.*, 1999); an undetermined *Trichobilharzia* species caused rhinitis in a swan (Palmer and Ossent, 1984).

### *6.1.4. Histopathology and immunology*

The main pathological alterations caused by bird schistosomes in a host are probably due to eggs rather than to adult parasites (Szidat and Wigand, 1934; Szidat, 1938). Nevertheless, in some cases, tissue lesions can be attributed also to immature and mature schistosomes (Pence and Rhodes, 1982).

*(a) Skin lesions.* Soon after invasion (5 min p.i.), the parasites cause a distinct lysis of the *stratum corneum* in the duck skin (Bourns *et al.*, 1973; Haas and van de Roemer, 1998). Later on, the parasites are surrounded by an inflammatory reaction, the intensity of which depends on the timing of infection and immune status of the afflicted host. In chickens and pigeons initially infected by *Ornithobilharzia canaliculata*, the inflammation around the parasites was observed as early as 12 h p.i. Later, cell infiltration (histiocytes and heterophils) became stronger, accompanied by perivascular infiltration in the dermis (Morales *et al.*, 1971). However, the reaction in the skin of initially infected animals is probably not strong enough to prevent the subsequent migration of schistosomula. The data of Ellis *et al.* (1975) on worm load reduction in ducks challenged by *T. ocellata* suggest that repeated infections result in stimulation of host immunity to the extent that it increases the trapping of parasites in the host skin. A similar effect was described for *Trichobilharzia* infections of an incompatible host (Figure 9a; Kouřilová and Kolářová, 2001) or for *S. mansoni* in a compatible host (von Lichtenberg and Ritchie, 1961).

*(b) Lung lesions.* Lung lesions are caused by migrating schistosomula (usually visceral schistosomes); the parasites occupy air spaces (Bourns *et al.*, 1973; Ellis *et al.*, 1975) and feed on red blood cells. During early initial infections, the parasites evoke only weak inflammations. Challenge infections of birds led to stronger cellular infiltration (predominantly lymphoid cells) around *T. ocellata*. The observation of dead and dying worms in the centre of these inflammations in the lungs confirmed the effectiveness of the immune response in immune hosts (Ellis *et al.*, 1975). During the chronic phase, the eggs of *Trichobilharzia* incited minor lesions, varying from none to marked granulomatous reactions (Wojcinski *et al.*, 1987). In heavy infections, adults of *O. canaliculata* invaded the lungs of pigeons. The vascular system displayed medial hyperplasia, lymphotic endarteritis and segmental proliferation of vascular endothelium. The tissue of the lungs contained granulomatous reactions (consisting mostly of macrophages, histiocytes, heterophils and lymphocytes) that surrounded remnants of dead parasites (Morales *et al.*, 1971).

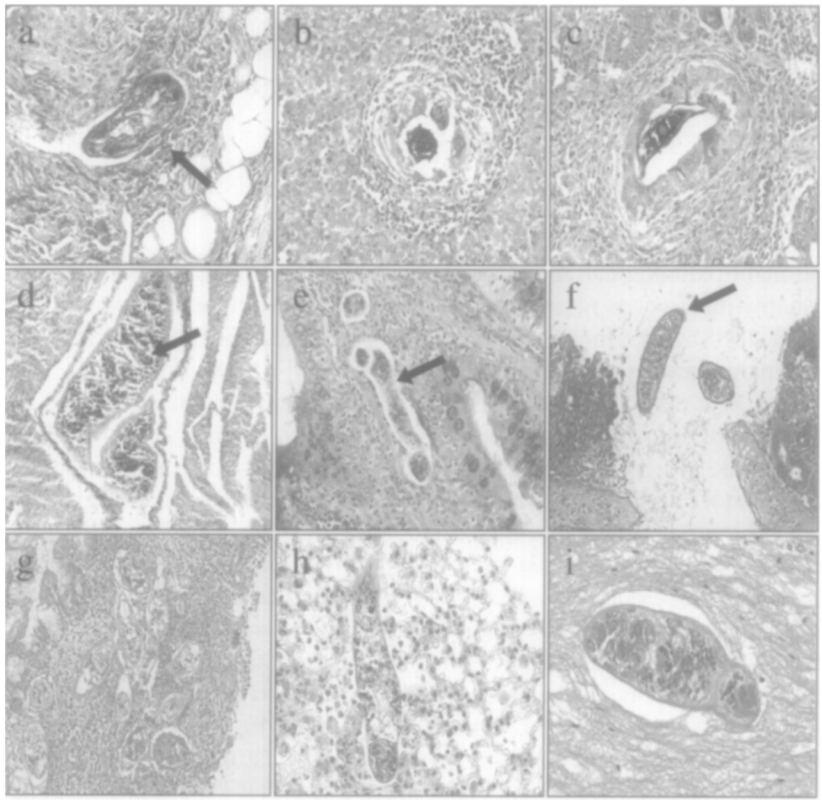


*Figure 9* Histopathological findings in compatible and incompatible hosts infected either by nasal (*Trichobilharzia regenti)* or visceral (*T. szidati*) bird schistosomes. (a) *T. regenti* schistosomula in the skin of a mouse, reinfected for the fifth time; 4 h p.i. (PAS). The schistosomula approaches the epidermis (stratum reticulare). An inflammatory reaction, consisting of eosinophils, neutrophils and macrophages (arrow) surrounds the parasite. (b) An egg of *T. szidati* in the liver of a duck, 21 days after primary infection (PAS). Usually, eggs are surrounded by inflammatory cells, an inner layer of giant cells and histiocytes, lymphocytes, plasma cells and macrophages towards the periphery. (c) An egg of *T. szidati* in the muscularis mucosae of the intestine of a duck (PAS). The cellular reaction is similar to that in (b). (d) *Trichobilharzia* sp. mature flukes (arrow), located intravascularly in the intestinal vessels of a naturally infected whooper swan (HE). (e) *T. szidati* in the duck intestinal wall – primary infection of lamina propria; 21 days p.i. (PAS). In certain cases the worms (arrow) reside in an extravascular location. (f) Adults of *T. regenti* in the duck nasal mucosa, 16 days after primary infection (HE). Worms are located intra- or extravascularly (arrow). (g) The eggs of *T. regenti* in the duck nasal mucosa, 16 days after primary infection (HE). Several miracidia occur free in the mucosa. Some miracidia are surrounded by a dense accumulation of eosinophils, heterophils, histiocytes and mononuclear cells, including voluminous giant cells. (h) *T. szidati* schistosomula in the lungs during primary infection of a mouse; 3 days p.i. (PAS). The immune reaction consisted of lymphocytes, neutrophils and macrophages. (i) *T. regenti* immature worms in the white matter of the thoracic spinal cord during primary infection of mice; 3 days p.i. (HE). Note the slight cellular infiltration around the parasites. Author of Figure 9a,i: P. Kouřilová, Department of Tropical Medicine, Charles University, Prague, Czech Republic. Staining: periodic acid-Schiff reaction (PAS); hematoxylin-eosin (HE).

*(c) Liver lesions.* Schistosome eggs (Figure 9b) are carried to the liver via the portal veins during the chronic phase of infection (e.g. Wojcinski *et al.*, 1987). The eggs usually cause granulomatous reactions in the liver (Graczyk *et al.*, 1993). Adult *Trichobilharzia* also cause liver lesions. Obstructive fibrosis of the portal triads may develop in case of heavy infections, during which adults may invade the bile ducts also (Pence and Rhodes, 1982). Dead worms, if transported to the liver (by the circulation), elicit mixed granulocytic inflammation. These large lesions are accompanied by tissue necrosis around the worms (Pence and Rhodes, 1982).

*(d) Intestinal lesions.* Intestinal lesions are mostly due to eggs. The eggs are usually located in close proximity to adult worms. Whereas slight immune reactions occur around viable eggs, granulomas are often associated with eggs that undergo degeneration or mineralization (Figure 9c; McLeod and Little, 1942; Wojcinski *et al.*, 1987). Additional pathology may be caused by loosely aggregated groups of adult *Trichobilharzia* worms in serosal and mesenteric blood vessels (Figure 9d; McLeod and Little, 1942), and by adult worms that are located extravascularly in the intestinal tissue (Figure 9e). Accumulation of numerous worms may be accompanied by thrombi in the veins and subsequent extensive haemorrhage and perivascular mononuclear cell infiltration around vessels (Wojcinski *et al.*, 1987). Heavy and chronic infections may result in thickening of the intestinal wall (Wojcinski *et al.*, 1987) similar to that caused by *Austrobilharzia variglandis* (Wood and Bacha, 1983).

*(e) CNS lesions.* The nervous tissue incurs damage from eggs of visceral and nasal schistosomes that are distributed by the circulatory system. In a similar way to that described for *Dendritobilharzia* sp. (Levine *et al.*, 1956; Wilson *et al.*, 1982), eggs of *Trichobilharzia* caused granulomatous encephalitis in waterfowl. Disseminated granulomas containing centrally located eggs surrounded by giant cells, macrophages, lymphocytes, heterophils and fibroblasts were noted in close proximity to the blood vessels of the *cerebrum* and *cerebellum* (Graczyk *et al.*, 1993).

The affinity of immature worms of nasal schistosomes (*T. regenti*) for nervous tissue can cause severe injury due to migration and establishment of parasites in the CNS (Kolářová *et al.*, 2001). The presence of the worms induces inflammatory reactions and pathological changes in the nervous tissue (spongiosis, eosinophilic perivasculitis and dystrophic and necrotic changes of neurons). Usually, more pronounced inflammatory reactions develop at sites with numerous parasites or around older, dead and disintegrating worms.

*(f) Nasal lesions.* The lesions are produced by eggs and adults of nasal schistosomes (Fain 1956a; Kolářová *et al.*, 2001). Whereas the eggs were detected only in the nasal mucosa, the adults (Figure 9f) were located intra- and extravascularly (Kolářová *et al.*, 2001). Pathological changes of the tissues surrounding adult worms have not been observed to date, but it is likely that these will be similar to those described for visceral schistosomes in other parts

of the body. Ageing of the eggs resulted in various host reactions (Figure 9g), ranging from focal accumulation of cells to the formation of granulomas (Kolářová *et al.*, 2001), similar to those described for visceral schistosomes (e.g. Wood and Bacha, 1983). In some cases, the observation of free miracidia surrounded by an inflammatory reaction (Kolářová *et al.*, 2001) supported the notion that these miracidia hatch directly in the host tissues (Horák *et al.*, 1998a).

*(g) Other findings.* Severe infections with visceral species of *Trichobilharzia* may result in occurrence of eggs, and both immature and adult parasites in unusual sites (Wojcinski *et al.*, 1987). In addition to the portal and mesenteric veins (normal locations), parasites were also discovered in caudal renal, pulmonary, gonadal, gastric, oesophageal, jugular, femoral and thyroidal veins of birds. Eggs were detected in liver, colon, small intestine, lungs, kidney, spleen as well as in tissues of adrenal/gonads, brain, gizzard, proventriculus, oesophagus, skeletal muscles and foot web. Schmäschke *et al.* (1992) reported lesions containing *Trichobilharzia* sp. eggs with viable miracidia in the sclera, nictitating membrane and conjunctiva of geese. Parasites and eggs can induce immune reactions and pathology in all these unusual locations.

### *6.1.5. Diagnosis and therapy*

The diagnosis of *Trichobilharzia* infections is focused primarily on microscopical detection of eggs in bird excrements. Sedimentation techniques (Kassai, 1999) facilitate the recovery of eggs with developed miracidia from faecal samples of birds infected with visceral schistosomes. Egg concentrations are usually low, and this necessitates examination of whole droppings (Appleton, 1983). This is achieved by filtering and concentration (by means of a formol–ether technique) of the samples prior to microscopical examination (Appleton, 1986). In the case of nasal schistosomes, fresh exudates from nasal cavities are examined for eggs or free miracidia. Samples can be obtained also by rinsing the nasal cavity with saline or water (Horák, unpublished data). In some cases, tissue biopsies can be examined histologically (Schmäschke *et al.*, 1992).

Necropsy can reveal eggs in intestinal and nasal scrapings; adults and eggs can be detected in squashed tissue samples or in stained cross sections. Immunodiagnostic tests are not used routinely in veterinary practice, but they can be helpful in some cases. Antibodies against gut-associated antigens (GAA) of adult flukes can be detected from ducks harbouring patent *T. regenti* infections by means of indirect immunofluorescence and cross-sections of adult schistosomes (IFAT) (Kouřilová and Kolářová, 2002). Although sensitive, this assay cross-reacted with GAA of different schistosome species (including *Schistosoma*).

Praziquantel seems to be a highly effective antihelminthic for the treatment of bird schistosome infections (Blankespoor and Reimink, 1991; Schmäschke *et al.*, 1992; Müller *et al.*, 1993; Reimink *et al.*, 1995). A threefold application of 200 mg to ducks with patent *Trichobilharzia* infections at 24-h intervals led to a permanent reduction in the release of eggs with viable miracidia (Müller *et al.*, 1993). A single dose of 200 mg/kg significantly reduced parasite load in common mergansers (Blankespoor *et al.*, 2001). Doses of 22 mg/duck/day, applied for 1 week during prepatency, prevented the development of schistosomes completely (Müller *et al.*, 1993).

## 6.2. Incompatible Hosts

### *6.2.1. Cercarial dermatitis*

Cercarial dermatitis is an allergic response to the penetration of schistosome larvae into mammalian skin (Cort, 1928). It develops after repeated contacts (host sensitization) with the cercariae (Olivier, 1949). Cercariae of the genus *Trichobilharzia* are the most common causative agent (Kolářová *et al.*, 1997).

*(a) Clinical features and gross pathology.* The skin syndromes develop in various mammals, including, for example, rabbits and dogs (Herber, 1938; Augustine and Weller, 1949; Olivier, 1953) but have mostly been described from afflicted humans (e.g. Cort, 1928; Vogel, 1930a; Brackett, 1940; Olivier, 1949; Haemmerli, 1953; Gianotti and Luvoni, 1958; Bearup and Langsford, 1966; Margono, 1968; Hoeffler, 1977; Blair and Copeman, 1977; Appleton and Lethbridge, 1979; Eklu-Natey *et al.*, 1985; Baird and Wear, 1987; Wiley *et al.*, 1992; Chamot *et al.*, 1998). The associated symptoms show only minor differences between humans and animals.

Grossly, cercarial dermatitis represents a maculopapular skin eruption associated with intense itching. Cercarial penetration into the skin results in a prickling sensation (primary itching) within 4–20 min, persisting for up to 1 h. Transitory maculae (up to 10 mm in diameter) appear at the sites of penetration of each cercaria. Local urtical reactions have also been reported as discrete indurated papulae that reach 1–5 mm in diameter. Although the maculae usually disappear in a few hours, they may persist to be replaced by distinct and indurated papulae of 3–5 mm in diameter (10–15 h p.i.) The development of papulae is accompanied by intense itching (secondary itching). An erythematous zone may develop around the papulae with a central punctum. When the papulae are confluent, the whole exposed area may be elevated and oedematous. On the second or third day p.i., vesicles (1–2 mm in diameter) may form on the papulae and these often rupture when rubbed or scratched. Papulae regress progressively after about 4 days and usually disappear by day 10 p.i. to leave a pigmented spot (1–4 mm in diameter) that persists for a month or

longer. Sporadic and intermittent pruritus may accompany the skin eruption; it tends to disappear within several days. The time course of skin symptoms depends on the sensitivity of the afflicted person (Olivier, 1949).

Only mild reactions occur during initial infections, and the disease manifests itself only by development of maculae. If papulae develop, they are usually small (1–2 mm), innocuous and inconspicuous. Usually, no secondary itching, oedema or diffuse erythema are detected. Sometimes a delayed formation (as late as 8 days p.i.) of small papulae was noted. In sensibilized hosts, papulae may occur within one hour of penetration by cercariae. In this case, the papulae have vesicles and increase to 3–8 mm in diameter. They are associated with erythema (10–100 mm in diameter), oedema and intensive pruritus. The symptoms may persist for a relatively long period, at least 20 days in one case (Bechtold *et al.*, 1997). The erythema starts to regress by day 3 p.i.; however, the size depends among other things on the extent of rubbing or scratching induced by the pruritus. Excoriation may occur with severe itching. Pustules do not form unless the lesions become secondarily infected. Generalized skin reactions are rare but may start by day 2 p.i. and persist for 2 days. The rash consists of small (about 2 mm), hard and pink papulae, which are very mildly and intermittently itchy (Olivier, 1949).

Japanese rice planters frequently suffer considerably from repeated infections by cercariae. In severe cases, the patients develop a chronic type of dermatitis called 'koganbyo' in which papulo-vesicular eruptions appear mainly on the dorsal side of hands and fingers; sloughing and injury of the skin is extensive (Oda, 1973).

Massive infections in humans may also cause fever, limb swelling, nausea and diarrhea. Skin lesions occur only on parts of the body that were in contact with cercariae, including parts under swimming suits. The itching can range from negligible to unbearable, potentially creating a noted insomnia (Chamot *et al.*, 1998).

Sensibilization may persist for years in humans (Olivier, 1949). Although few data are available on the development of resistance against cercarial dermatitis, Macfarlane (1949) reported immune individuals among adults, but not in children. Repeated contacts with various species of cercariae might lead to desensibilization (Berg and Reiter, 1960).

All representatives of the genus *Trichobilharzia*, and other non-human schistosomes, seem to produce similar symptoms when infecting humans (Brackett, 1940; Cort, 1950; Batten, 1956; Malek and Armstrong, 1967; Appleton and Lethbridge, 1979; Wiley *et al.*, 1992). There are no significant differences between dermatitis occurring in fresh and salt water bodies (Kolářová *et al.*, 1989). By contrast, human schistosomes usually induce milder skin reactions (Basch, 1991). This difference may stem from an inability of *T. ocellata* to produce a molecule analogous to the 16.8-kDa anti-inflammatory factor from *S. mansoni* (Ramaswamy *et al.*, 1996). In the

case of the latter parasite, the activation of the receptor for parasite-derived prostaglandin D2 dramatically reduced the skin hypersensitivity response after challenge (Angeli *et al.*, 2001).

Generally, it is difficult to distinguish whether skin reactions were caused by bird or human schistosomes. In some areas, bird and human schistosomes may co-occur, sometimes even in the same snail species (Nassi, 1987). The similarity in symptoms underlines the necessity to consider all travellers to be positive for schistosomiasis if they acquired 'cercarial dermatitis' in areas that are endemic for human schistosomes.

*(b) Histopathology and immunology.* Pathological effects associated with penetration of *Trichobilharzia* cercariae into mammals primarily depend on the sensitivity of the afflicted hosts. No marked differences were noted for cellular responses either of various mammalian species against one *Trichobilharzia* species or one mammal against different species (and genera) of bird schistosomes. Initial infections of rabbit and mouse skin by *Trichobilharzia* (Figure 9a) exhibited a mixed inflammatory exudate with few eosinophils and neutrophils, starting by 6 h p.i. (Augustine and Weller, 1949; Olivier, 1953; Kouřilová and Kolářová, 2001). Repeated infections of mice simultaneously resulted in subacute oedematous dermatitis and increased numbers of schistosomula trapped in the skin (Kouřilová, 2001), similar to that recorded from mice exposed to *Gigantobilharzia* (Batten, 1956) and *Ornithobilharzia* (Bayssade-Dufour *et al.*, 2001). The infiltration by melanophages and sinusal hyperplasia, with accumulation of melanin-laden macrophages in subcutaneous lymph nodes, was detected only 30 min after the last challenge of mice infected with *Ornithobilharzia* sp.; the mobilization of melanotic pigment is probably secondary due to pruritus and scratching of the tissue (Bayssade-Dufour *et al.*, 2001).

Strong inflammation around the worms in humans probably arises in hosts that have been infected repeatedly. The observations on this topic were made from sections of biopsies taken from sites with papulae (e.g. Vogel, 1930b; Brackett, 1940; Macfarlane, 1949; Haemmerli, 1953; Gianotti and Luvoni, 1958; Margono, 1968; Appleton, 1984). The occurrence of papulae suggests that the skin biopsies were obtained from sensibilized subjects. The most comprehensive study of Haemmerli (1953) described a three-phase cellular response (leucocytic, lymphocytic and histiocytic), which led to the elimination of *Trichobilharzia* schistosomula. During the leucocytic phase (3–9 h p.i.), the parasites were intact. The tegument of the parasite started to degrade at 24 h p.i. (lymphocytic phase) and schistosomula were completely destroyed during the histiocytic phase (36–52 h p.i.). The process was accompanied by perivasculitis and spongiosis of the area surrounding the parasites. Tissue repair (72 h p.i.) was accompanied by parakeratosis with reduced perivascular infiltration in the surrounding tissue; afterwards, cell melanotic pigmentation occurred (100 h p.i.).

The progression of the skin reactions described above may be determined by the degree of host sensibilization (Gay *et al.*, 1999). Whereas a mild human reaction to *T. ocellata* and *T. szidati* comprised only leucocytic vascularitis with perivascular dermal infiltrates of lymphomonocytic and polynuclear type, a strong reaction in another patient was characterized by lymphocytic vascularitis with lymphocytic dermatitis that occurred as early as 10 h p.i.

The use of IFAT- and ELISA-based methods disclosed that *T. szidati* infection in mice led to production of antibodies specific for cercariae of *T. szidati* and *S. mansoni* (Kolářová *et al.*, 1994). However, the mouse antisera did not react with (skin and lung phase) schistosomula (Horák *et al.*, 1998b). It was observed that the parasite loses the main antigenic structure when the cercariae lose the carbohydrate-rich glycocalyx during penetration of the host skin.

### *6.2.2. Additional syndromes*

*Trichobilharzia* infections may cause other types of symptoms in various species of mammals. Under certain circumstances (e.g. first contact with cercariae), the transformed schistosomula can escape and migrate in the mammalian body (for review see Horák and Kolářová, 2001). Schistosomula of various bird schistosomes (*Trichobilharzia* sp., *T. ocellata*, *T. szidati*, *T. stagnicolae*, *T. physellae*, *T. brevis* as well as *Bilharziella polonica* and *Ornithobilharzia* sp.) were detected in the lungs of mice, hamsters, rabbits, rhesus monkeys and gerbils (Olivier, 1953; Appleton and Brock, 1986; Haas and Pietsch, 1991; Horák and Kolářová, 2000; Bayssade-Dufour *et al.*, 2001), and additionally in organs such as liver, kidney, heart and intestines (Haas and Pietsch, 1991). In some of these animals, initial infection resulted in minute petechiae in the lungs. During the early phases of such infections in mice the majority of these lesions were bright red, distinct, discrete and apparently distributed at random over the pleural surface of the lungs. In some cases, deeper, larger and dark red haemorrhages with diffuse and indistinct margins developed with progression of the infection (Olivier, 1953). The lungs of *Ornithobilharzia*-infected gerbils contained numerous whitish nodules alongside pulmonary and collateral arteries, and the peribronchial trees. Some nodules were scattered in the parenchyma (Bayssade-Dufour *et al.*, 2001).

In the lungs of *T. szidati*-initially infected mice, many schistosomula (Figure 9h) resided in the air spaces on day 4 p.i. (L. Kolářová, unpublished data). Immune reactions developed around many intact parasites; the inflammations were most intense around older schistosomula (Moravcová and Kolářová, 2001).

Previously exposed mammals generally develop stronger immune responses against the parasites. Examination of the lungs of gerbils that were re-infected with *Ornithobilharzia* disclosed that most parasites suffered damage to the tegument. Moreover, lymphocytic vasculitis occurred around live worms and worms that had incurred some tegumental damage. The resulting pathology was accompanied by macrophage alveolitis in the peripheral lung parenchyma (Bayssade-Dufour *et al.*, 2001).

Immature parasites of *T. regenti*, a nasal species of *Trichobilharzia*, are neurotropic and also migrate through the CNS of mammals. Histological examination disclosed both intact (Figure 9i) and destroyed worms in the nervous tissue of mice (Horák *et al.*, 1999). Compared with birds, mice displayed more vigorous cellular responses to the parasites. The strong inflammatory reactions around disintegrating parasites were associated with dystrophic and necrotic changes of neurons in the surrounding tissues (Kolářová *et al.*, 2001). The intense inflammatory response may in part be the reason why *T. regenti* fails to develop completely in a non-specific host. The establishment of *T. regenti* in the CNS, and the associated pathology, may lead to changes in neurobehavioural reactions of affected mammals, similar to those described from human neuroinfections by the genus *Schistosoma* (Scrimgeour and Gajdusek 1985; Lambertucci, 1993). However, in the latter case, the pathology develops due to the dissemination of parasite eggs to the CNS.

### *6.2.3. Diagnosis and therapy*

Differential diagnosis of cercarial dermatitis is difficult. The resulting lesions resemble those that result from dermatitis of bacterial etiology, contact dermatitis or insect bites from chiggers, fleas and mosquitoes (Hoeffler, 1977). However, clinical and epidemiological data, together with parasitological and immunological diagnostic methods, may combine to identify cercariae as the correct etiological agent. Three criteria are the most important among other clinical and epidemiological data: (1) recent history of a contact with natural water bodies (i.e. within the previous 96 h); (2) development of a papular dermatitis with severe itching between 12 and 24 h after exposure; and (3) distribution of these lesions only on parts of the body that were immersed in the water (Appleton, 1984).

Infrequently, schistosomula can be detected in skin biopsies taken from sites where papulae have developed. However, this method can only be successful shortly after exposure. The parasites are usually destroyed in (or migrate out of) the human skin within 24–72 h p.i. (e.g. Haemmerli, 1953).

Additionally, laboratory tests that monitor the immune reactions in patients can aid in the diagnosis of cercarial dermatitis. Cercarial dermatitis may be

associated with increased eosinophil counts, as observed in human volunteers exposed to *Heterobilharzia americana* (Malek and Armstrong, 1967). Skin tests have been developed, based on the evaluation of erythema that develops around injected antigens, prepared by homogenization of either cercariae of bird schistosomes (Macfarlane, 1949) or adults of *S. mansoni* (Cort, 1936; Augustine and Weller, 1949; Moore *et al.*, 1968; Blackburn and Ma, 1971; Wiedermann *et al.*, 1973; Krampitz *et al.*, 1974) or *S. japonicum* (Hsü and Ameel, 1956). Unfortunately, these tests lack specificity and are frequently insensitive. Therefore, they cannot be used for definitive diagnosis of cercarial dermatitis (Wiederman *et al.*, 1973).

Other methods rely on detection of antibody responses for diagnosis. The 'Cercarienhüllenreaktion', a complement fixation test, IFAT and ELISA have been used to assess the titres of specific antibodies against cercariae (of either *Trichobilharzia* or *Schistosoma*) or against homogenates of *S. mansoni* adults (Vogel and Minning, 1949a,b; Hendricks and Cort, 1956; Moore *et al.*, 1968; Knight and Worms, 1972; Krampitz *et al.*, 1974; Kimmig and Meier, 1985; Kolářová *et al.*, 1994; Pilz *et al.*, 1995). Although these techniques are more sensitive than skin tests, they are not specific. Thus, they cannot be performed for a differential diagnosis between cercarial dermatitis and human schistosomiasis. Recently, an IFAT approach to test the antibody reactivity with schistosomular and adult GAA of both *Trichobilharzia* and *Schistosoma* species allowed the correct distinction of antisera. Thus, this particular method seems promising for differential diagnosis of cercarial dermatitis caused by bird schistosomes (Kouřilová and Kolářová, 2002).

At present, the therapy of cercarial dermatitis is only symptomatic. Abirritant powders may be applied to the skin and, in serious cases, the use of systemic antihistaminics (tablets or better in gels) or mild corticosteroids should be considered (Hoeffler, 1977).

## 7. EPIDEMIOLOGY OF CERCARIAL DERMATITIS

Cercarial dermatitis has been recognized since historical times. In 1847, Fujii (cited by Oda, 1973) reported skin eruptions in Japanese rice planters. Naegeli (1923) described clinical symptoms of this 'water-borne' disease in Germany, hypothesizing that it was caused by 'animal plancton'. Finally, in 1928, Cort in North America identified the causative agent and the disease was named schistosome dermatitis. Many reports have accumulated since and the infection is presently known under different names in various areas of the world, e.g. swimmer's itch (Christensen and Green, 1928), Zerkarien-Dermatitis (Vogel, 1930b), dermatite des nageurs (Brumpt, 1931), bouton de canicule des baigneurs (Desportes, 1944/45), kabure (Fujii, 1847, cited by

Oda, 1973), koganbyo (Tanabe, 1948), sawah itch (Buckley, 1938), etc. In most areas, the parasite species that cause human cercarial dermatitis have not been precisely identified due to a variety of practical problems and technical difficulties (see section 3). This is unfortunate as unequivocal identification of the causative agent (including data on life cycles) is a prerequisite for estimation of health risks and for designing control measures (see sections 3 and 6).

### 7.1. Distribution

With the exception of Antarctica, cercarial dermatitis occurs globally (Hoeffler, 1982). It has been reported from North and Central America (USA, Canada, Haiti, Salvador, Mexico, Cuba), Australia, New Zealand, Asia (Formosa, Malaysia, Philippines) (for review, see Hoeffler, 1982), Japan (Oda, 1973; Suzuki and Kawanaka, 1980), India (Narain *et al.*, 1994; Agrawal *et al.*, 2000), Thailand (Kullavanijaya and Wongwaisayawan, 1993), Vietnam (Landmann *et al.*, 1961), China (Liu *et al.*, 1977), Iran (Sabha and Malek, 1979), Africa (South Africa; Appleton, 1984) and Europe (Denmark, Finland, Great Britain, the Netherlands, Slovak Republic, Czech Republic) (for review see Kolářová *et al.*, 1989), Austria (Graefe *et al.*, 1973; Dvořák *et al.*, 1999), European countries of the former Soviet Union (e.g. Raisyte, 1974; Tsyrkunov, 1987; Beer and German, 1994), France (Léger and Martin-Lœhr, 1999), Germany (for review see Allgöwer and Matuschka, 1993), Iceland (Kolářová *et al.*, 1999), Italy (Nobile *et al.*, 1996; Golo *et al.*, 1998), Norway (Thune, 1994), Spain (Simon-Vicente, 1983), Sweden (Thors and Linder, 2001) and Switzerland (Eklu-Natey *et al.*, 1985; Chamot, 1998).

Probably, the disease is most prominently distributed along the major flyways of the migratory birds: for example, in North America, the dermatitis seems to concentrate along the Mississippi flyway (Jarcho and van Burkalow, 1952) and the Pacific distribution may follow the Neoarctic–Hawaiian, Asiatic–Palauan and Japanese–Marianan flyways (Chu, 1958). According to this view, the Palaearctic–African bird migration (Moreau, 1972) may influence the distribution of cercarial dermatitis in Europe and Africa.

The transient presence of waterfowl is essential for establishment of the parasite life cycle in a particular water reservoir. Most of the birds probably become infected in nesting areas; however, in some cases, birds may also transport mature (within the host) or larval (within snails transported on bird legs) schistosomes to and from the wintering locations (Woodruff and Mulvey, 1997; Wesselingh *et al.*, 1999). Thus, bird migrations may be expected to introduce certain parasite species into new geographical areas. However, no data are available to suggest that avian schistosomes have expanded their geographical range in this fashion.

## 7.2. Epidemiology

Cercarial dermatitis is a common, non-communicable cutaneous disease that is widely neglected. The occurrence of the disease depends upon complex interactions among the parasites, molluscan and definitive hosts, and humans as they share the same environment under conditions that favour parasite transmission (Hoeffler, 1982). Depending on the causative species, cercarial dermatitis can develop in fresh- or salt-water bodies (for review see Horák and Kolářová, 2001). At present, the most frequently reported cercariae belong to the genus *Trichobilharzia* (Horák and Kolářová, 2001), larval development of which takes place only in freshwater bodies.

Cercarial dermatitis occurs seasonally. It is most prevalent during warmer months when both the release of cercariae from snail intermediate hosts and the number of people that have contact with water reach peak levels (Cort *et al.*, 1940; Appleton and Lethbridge, 1979). In addition to factors that relate to the biology of the parasites, the frequency of human cercarial dermatitis also depends on the behaviour of bathers (duration of swimming), previous contact with the parasite (history of cercarial dermatitis) and, possibly, individual susceptibility to the cercariae. A multivariate analysis (Chamot *et al.*, 1998) showed that the time of day, barometric pressure, maximum air temperature and duration of swimming activity strongly predict not only the incidence of cercarial dermatitis but also the number of skin lesions.

A history of cercarial dermatitis should be assumed if strong skin reactions occur early after infection (Chamot *et al.*, 1998). Both sexes and all age groups can be affected equally under similar conditions (Chamot *et al.*, 1998). However, the time interval of exposure may differ for various age groups. Children between 5 and 9 years are at highest risk because they spend more time in the water during recreational activities (Appleton and Lethbridge, 1979). In rice planters or pickers, for instance, adults were the most affected group (Appleton, 1984).

At present, cercarial dermatitis is regarded as an emerging disease (de Gentile *et al.*, 1996). High eutrophication of water reservoirs (Allgöwer and Effelsberg, 1991), colonization of ponds by susceptible snails and by nesting ducks, combined with long periods of sunshine in the summer, are important factors that have led to recent increases in the number of outbreaks of cercarial dermatitis (de Gentile *et al.*, 1996). Also, the disease may be acquired when cleaning an aquarium containing field-collected snails that harbor avian schistosomes (Bastert *et al.*, 1998; Fölster-Holst *et al.*, 2001).

## 7.3. Identification of Potential Transmission Sites for Cercarial Dermatitis

The occurrence of cercarial dermatitis is delineated by the distribution of intermediate hosts infected by schistosomes. With respect to species-specific physical, chemical and biological requirements, freshwater snails live in various habitats, differing in size and origin (natural or man-made). Usually, the distribution of snails within larger habitats is discontinuous, often due to a preference for aquatic vegetation that offers protection and sites for egg-laying (Sturrock, 1993). Identification of reservoirs that pose a risk for humans relies on detection of bird schistosomes.

The examination of water samples may disclose the presence of cercariae in a particular water body. However, this is usually difficult due to low parasite concentrations, and various methods of cercariometry – filtration, the use of phototaxic response equipment and continuous flow centrifugation – have been developed to detect (human) schistosomes (for review see Théron, 1986); perhaps these may also be applied in the search for bird schistosomes.

Moreover, cercariae can be 'collected' by a trap containing a matrix with unsaturated fatty acids (linoleic acid). This substrate stimulates the attachment and penetration by cercariae. Any immobilized larvae are subsequently visualized for counting. This method to monitor schistosome-infested waters was primarily developed for *S. mansoni* (Shiff *et al.*, 1993; Shiff and Graczyk, 1994), but could be applied also for *Trichobilharzia* (Graczyk and Shiff, 2000). Molecular methods can further increase the sensitivity of detection, e.g. a PCR assay based on a highly repeated sequence of *S. haematobium* allows detection of single cercariae and as little as 10 fg of schistosomal DNA (Hamburger *et al.*, 2001).

Determination of field-collected cercarial species is a complicated matter, as described in section 3. Surprisingly, the findings of bird schistosome cercariae are frequently confirmed by experimental infections of volunteers. Several novel insights into the potential pathogenic effects of these parasites (see section 5) warrant caution against performing experimental infections in humans.

The source of infection is identified mainly by examination of water snails. Two methods are commonly used: cercarial emergence and dissection of snails. Exclusive use of cercarial emergence underestimates the value of parasite prevalence and overlooks parasites that are present but not shed by snails at the time of examination. This method yielded false results in 59.1% of cases when compared with the dissection of snails (Curtis and Hubbard, 1990). The occurrence of the parasites fluctuates during the year; these differences are due to cyclic reproduction and changes in the age distribution within snail populations. It is recommended to examine snails of various sizes to determine parasite prevalence more accurately (Hurley *et al.*, 1994).

Higher numbers of infected snails increase the infection risk for humans.

However, *Trichobilharzia* infection rates in lymnaeids are usually low, ranging between 0.3 and 5.2% (Meyer, 1964; van den Broek, 1965; Dönges, 1965; Bearup and Langsford, 1966; Graefe *et al.*, 1973; Wills *et al.*, 1976; Kolářová *et al.*, 1992; Müller and Kimmig, 1994; Hurley *et al.*, 1994; Loken *et al.*, 1995; Niewiadomska *et al.*, 1997; Väyrynen *et al.*, 2000; Loy and Haas, 2001). Except for *L. auricularia*, prevalences that exceed 5% are exceptional and may falsely result from examination of only a low number of snails (Loy and Haas, 2001). The high prevalences of parasites (22 and 26%), recorded from *L. auricularia* in Bohemia and Germany, respectively (Kolářová *et al.*, 1989; Müller and Kimmig, 1994), may result from disturbances in the ecological balance of the lakes that were sampled, leading to increased sizes of snail populations (Kolářová *et al.*, 1989).

The proportion of infected snails at any time depends upon complex interactions of biotic and abiotic factors, including susceptibility of particular snail hosts, and number, distribution and behaviour of definitive hosts. Environmental factors such as temperature are also important (Webbe, 1982). It appears that most snails become infected during the first summer of their life (Hoeffler, 1982). Snail infections in the late summer probably secure survival of schistosomes in the water body. It is obvious that snails containing parasites overwinter and cercarial shedding starts during warm days of the next spring. In this way, the parasite infects new birds when they return to their breeding areas (McMullen and Beaver, 1945; Jarcho and van Burkalow, 1952). On the other hand, summer infections of the snails might be responsible for distribution of schistosomes to the bird wintering areas.

Most commonly, infections of birds by avian schistosomes are diagnosed through faecal examination. This approach is appropriate to detect visceral schistosomes, but it will fail to show the presence of nasal schistosomes. The latter parasites are also common in waterfowl (Rudolfová *et al.*, in press). Therefore, different methods to examine birds should be applied to obtain correct values for prevalence of infection (see also section 6.1.5).

The prevalence of trichobilharziasis in birds can be quite high in comparison with snails. Faecal surveys indicated the following prevalences of *Trichobilharzia* spp.: 60.5% in spurwing goose (Appleton, 1986), 69.6% in domestic duck (Liu *et al.*, 1977), 83.9% in common merganser, 2.6% in Canada goose and 4.9% in gulls (Loken *et al.*, 1995). Guth *et al.* (1979) demonstrated prevalences of 12.0 and 46.3% of visceral schistosomiasis in adult and young Anseriformes, respectively. The investigation of wildfowl (genus *Aythya* and *Anas*) during two consecutive years showed that prevalence of nasal schistosomes was constant at 24.0% (Rudolfová *et al.*, in press).

Migratory birds probably do not acquire heavy infections because they leave the infective habitat, whereas non-migratory birds may suffer severely from trichobilharziasis and die (Wilson *et al.*, 1982). However, the properties

of resident snail populations that determine susceptibility or resistance can lead to different infection rates at particular localities.

## 7.4. Control

Effective control of cercarial dermatitis must be based on analysis of an entire biological system. Only then can the interruption of the schistosome life cycle be achieved. Intervention efforts can be directed at the level of either intermediate or definitive hosts. The control of intermediate hosts is usually aimed at elimination of snails and/or prevention of snail reproduction. At the definitive host level, the focus is to eliminate schistosome infection in birds.

The control of snail populations may employ chemical, mechanical and biological approaches (reviewed by Allgöwer and Matuschka, 1993). Since the first use of copper sulphate (Taylor and Baylis, 1930) to kill snails in an effort to control cercarial dermatitis, various chemical compounds that are toxic for snails (molluscicides) have been used for this purpose. Many of these preparations are derived from various copper compounds (e.g. Brackett, 1939; McMullen and Brackett, 1948). The use of these compounds may yield inconsistent results, depending on abiotic properties of the water to be treated and the experience of personnel involved in the control (for review see Blankespoor and Reimink, 1991). For example, treatment of a water body with copper sulphate can cause copper to precipitate as copper carbonates. Accumulation of copper in the reservoir sediments can cause death of various aquatic organisms and reduce the recreational value of the lake. Surprisingly, after treatment with a copper-based molluscicide, snails continued to reproduce and spread at a rapid rate (Blankespoor and Reimink, 1991). There is even evidence that, over an extended time, snail populations develop (partial) resistance to copper sulphate (Blankespoor *et al.*, 1985).

Among other synthetic compounds, such as B-2 (sodium 2,5-dichloro-4-bromophenol) or NaPCP (sodium pentachlorpentate niclosamide), niclosamide (Bayluscide®) is still the molluscicide of choice. Although it is expensive, niclosamide is highly effective against all life stages of the snail and also against schistosome larvae. Whereas niclosamide is non-toxic for humans, domestic animals and crops, it is, unfortunately, toxic for fish (for review see Perrett and Whitfield, 1996). Moreover, the application of niclosamide did not prevent the re-colonization of sites by remaining snails (Lardans and Dissous, 1998). A promising alternative is provided by the ongoing development of plant molluscicides (Singh and Singh, 2000). However, the availability of these preparations and their effectiveness depend on the local situation and native flora (Perrett and Whitfield, 1996). Finally, the application of various bacterial products could be considered for control of snails and parasites. The eluate of *Bacillus thuringiensis israelensis* contains a

water-soluble M-exotoxin which is toxic for *S. mansoni* and *T. szidati* cercariae and corresponding snail hosts (Horák *et al.*, 1996).

The removal of aquatic vegetation and snails from swimming areas can be an effective control measure (e.g. Graefe *et al.*, 1973). Virtually all snails can also be eliminated from large bodies of water by mechanical disturbance of the snail habitat (using boats mounted with rototillers or tractors equipped with a rake), provided that the intervention is performed in shallow (littoral) areas with high concentration of snails at the time of reproduction and early development (Leighton *et al.*, 2000). Alternatively, small lakes can be emptied temporarily to dry out the bottom, remove mud and cover places reserved for bathing with a new layer of river sand (Kolářová *et al.*, 1989).

Generally, biological control of bird schistosomes aims to regulate or reduce snail and/or parasite populations. The fitness of schistosome intermediate hosts can be affected significantly by removal of food sources and introduction of natural predators (e.g. fish) or competing snail species (resistant to schistosomes) (Haas, 1985; Allgöwer and Matuschka, 1993). The introduction of parasites that attack or compete with larval schistosomes within the snail host, or that induce high mortality or castration of snails has been explored (for review see Lim and Heyneman, 1972). For example, intramolluscan larvae of echinostome parasites are predators of schistosome larvae that may co-occur in the same snail. Introduction of (eggs of) *Echinostoma audyi* led to some level of control of intramolluscan larvae of *T. brevis* in the snail *Lymnaea rubiginosa* (Ow-Yang *et al.*, 1970; Lie and Ow-Yang, 1973). Many factors affect the feasibility of biological control, and these approaches should be taken only under semi-controlled conditions, such as in small lakes. Also it may take considerable time before these approaches yield demonstrable effects. Because of this, these methods have little practical value.

At the level of definitive hosts, control efforts have explored prevention of bird contact with infested waters and chemotherapy of birds. Whereas the first approach (e.g. relocation of breeding bird host species to other areas; Blankespoor and Reimink, 1991) is difficult to implement on a large scale in the field, the treatment of birds with antihelminthics is rather promising. Based on a study in which birds were captured and treated with praziquantel to kill adult worms, Blankespoor and Reimink (1991) concluded that treatment of birds is more effective than the use of copper sulphate; also it is less expensive and has no detrimental effects on the environment.

### 7.5. Prophylaxis

Theoretically, human infections are prevented by prohibiting access to water that harbours cercariae of avian schistosomes. However, various restrictions in water activities are usually not respected by bathers (Allgöwer and Matuschka,

1993; Chamot *et al.*, 1998). Some protection against cercarial dermatitis can be achieved by rubbing the skin to full dryness immediately after water contact, before cercarial penetration occurs (Brackett, 1939; Haas and van de Roemer, 1998). A thorough coating of the body with vaseline provides complete protection against infection (McLeod and Little, 1942). The value of protective clothing (long trousers, hip boots and waders) is unpredictable. Recently, *N,N*-diethyl-*m*-toluamide (DEET) was found to have a repellent effect against various cercariae of the genus *Schistosoma* (Salafsky *et al.*, 1999, 2001); when incorporated in liposomes ('lipodeet'), the application on skin was 100% effective in preventing penetration by cercariae. A similar prophylactic effect can be expected against penetration by schistosome larvae from genera that cause cercarial dermatitis.

## 8. CONCLUSION

The association between avian schistosomes of the genus *Trichobilharzia* and cercarial dermatitis in mammals has been recognized for many decades. However, difficulties in obtaining adult parasites from birds, combined with extensive morphological similarities, continue to complicate both the unequivocal identification at species level and the characterization of specific biological properties of distinct species within the genus *Trichobilharzia*. The systematics of the genus requires a major revision, including clarification of the position and validity of the type species *T. ocellata*. New, more extensive criteria to identify a species of *Trichobilharzia*, combining morphology, characteristics of the life cycle and molecular sequence data, may begin to resolve this issue.

Species of *Trichobilharzia* display a remarkable specificity towards (species of) snail intermediate hosts. The parasite modifies the physiology and internal defences of a snail to complete intramolluscan development successfully. The transmission of a *Trichobilharzia* species by only certain snails is thought to reflect the highly specialized nature of these complex interactions between the parasite and the intermediate host.

As parasites, *Trichobilharzia* species are serious pathogens for birds. Comparable to pathology caused by parasites of the genus *Schistosoma* in mammals, trichobilharziasis is accompanied by injuries due to parasites that migrate through the viscera or the CNS (in the case of nasal species of *Trichobilharzia*) of birds. Moreover, adult worms and parasite eggs that have become trapped in the tissues evoke the formation of granuloma.

The ability of cercariae to penetrate into mammals is linked to more than just cercarial dermatitis alone. Study of primary infections in rodents revealed that some immature *Trichobilharzia* worms also migrate through the body of

mammals. Strikingly, they are also neurotropic in mammals. The invasion of nasal species of *Trichobilharzia* into the nervous tissues of experimental rodents potentially led to neuromotor disorders. This suggests that cercarial dermatitis, the elimination of penetrating cercariae in the skin, represents a protective reaction of a sensitized organism against the parasite. At present, there are no data on the pathology of primary infections in large mammals and humans. However, the cosmopolitan occurrence of *Trichobilharzia* species and the frequent exposure of animals and humans to these parasites (cercarial dermatitis is an emerging disease) provide ample justification to intensify study of clinical and pathological aspects related to trichobilharziasis in mammals, including humans.

In summary, species of the genus *Trichobilharzia* display striking transitions in morphology, a complex life-cycle and fascinating properties that enable these parasites to survive in intermediate and final hosts. The inconspicuous nature of their endoparasitic life style may have led to a considerable underestimation of the pathogenicity and medical/veterinary importance of *Trichobilharzia*.

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# The Consequences of Reducing Transmission of *Plasmodium falciparum* in Africa

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## ABSTRACT

Malaria transmission intensity in Africa varies over several log orders, from less than one infected bite per year to more than one thousand. In this review

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we examine the consequences in terms of age pattern, clinical spectrum and overall burden of disease and discuss the possible implications for interventions that reduce exposure to infected bites. With very low transmission intensity, all age groups are susceptible to severe malaria. With increasing transmission intensities, older children and adults suffer less severe disease and with high transmission rates the majority of severe cases occur in infants under one year of age. This pattern reflects the increasingly rapid acquisition of immune responses that limit the life-threatening effects of malaria with increasing exposure to the parasite. The clinical spectrum of severe malaria varies with transmission: with high transmission, severe malarial anaemia dominates and cerebral malaria is rare. As one moves towards lower transmission rates, cerebral malaria accounts for an increasingly large proportion of cases. Although the population risk of severe disease falls with age, the risk of death at an individual level may rise with age after an initial fall from very high case fatality rates in children aged under 6 months. Of central interest to malaria control is how the overall amount of disease in childhood varies with transmission. Data from a number of sources suggest that, with low transmission, the amount of malarial disease rises with increasing exposure but that this saturates relatively early. A key issue is whether the same pattern obtains for deaths, both those directly due to malaria and those from all causes. The methodological limitations of ecological comparisons between different areas are discussed before presenting a review of attempts to use this approach in Africa. This suggests that children living in areas of low malarial endemicity have all-cause mortality rates about half of those of children living in areas of moderate to high transmission. Deaths in the first year of life rise linearly with increasing exposure to malaria over a wide range of transmission intensities; by contrast all-cause mortality in children aged 0–4 years appears to saturate at relatively low transmission intensities. These data suggest that interventions that reduce exposure to malaria parasites, such as insecticide-treated bed nets (ITNs), will have the greatest chance of a sustained effect when used in areas where disease burdens are high but the frequency of parasite exposure is low-to-moderate. In conditions of high transmission, initial reductions in mortality may prove difficult to sustain as the reduced level of transmission may still lie on the part of the curve where mortality has saturated. However, at all levels of transmission the overall balance of benefits, including reduced load on families and health services from non-life-threatening malaria, favours the widespread introduction of ITNs in endemic areas of Africa.

## 1. INTRODUCTION

Over the last decade there has been a broadening of the epidemiological approaches to malaria, from an emphasis on vector–parasite dynamics to an

approach that is beginning to capture the impact on morbidity and mortality. Coincidentally, several contentious issues have re-emerged from a debate that began over 50 years ago. These debates have swung between emotive claims to informed opinion based upon limited evidence. The debate centres on whether reducing the risk of infection with *Plasmodium falciparum* will always lead to reduction in the life-time risks of death from malaria.

A characteristic of malaria in most parts of sub-Saharan Africa is that infection with *P. falciparum* is common and that death from malaria, whilst numerically considerable, is relatively rare compared to the life-time risk and frequency of infection. Most severe outcomes and death following infection occur in children. It has therefore been widely held that the acquisition of immunity during childhood is an important survival mechanism for populations living under conditions of stable, endemic malaria transmission. The cost, in terms of child mortality, is high, sufficiently high to have selected for several innate survival mechanisms such as the genetic polymorphisms associated with red blood cell structure and function (Hill, 1992).

Interfering with 'nature's balance' under stable, endemic conditions of transmission led some to question the virtue of indoor residual house-spraying as a means of malaria control in Africa during the optimistic eradication era of the late 1940s and early 1950s (Garnham, 1949; Wilson, 1949; WHO, 1950; Wilson *et al.*, 1950; Dobson *et al.*, 2000). Over 5 years ago this debate was reopened in the light of ecological comparisons of measures of mortality from malaria and measures of parasite challenge (Snow and Marsh, 1995). There followed a series of analyses of the same and different data (Molineaux, 1996; Trape and Rogier, 1996; Anonymous, 1997; Brown, 1997; D'Alessandro and Coosemans, 1997; Greenwood, 1997; Lengeler *et al.*, 1997; Trape, 1997; Smith, T.A. *et al.*, 2001). Interpretations of these comparative studies can be broadly divided into those which concluded that malaria mortality amongst children aged less than 5 years rises linearly with increasing transmission intensity and those which showed that initial linear rises in mortality risks saturate under conditions of moderate-to-high transmission.

There are several issues nested within the primary debate that demand careful interpretation. First, under conditions of stable, endemic transmission, just how quickly do the risks of a poor clinical outcome following infection decline with age? Second, is the host's ability to develop functional immunity independent of age? Third, and a logical extension, do the cumulative risks of a poor clinical outcome towards the end of childhood vary between communities with different forces of transmission? Fourth, and perhaps of greater importance to the public health debate, does *P. falciparum* infection *per se* influence the risks of disease or death not necessarily directly due to malaria? Finally, is there a point at which a linear association between mortality and transmission intensity saturates, and, if so, what is this endemicity range, what is its

geographical extent in Africa, and how successful can interventions be in reducing infection risks to place populations below this range?

## 2. THE AGE PATTERNS OF SEVERE MALARIA MORBIDITY: DEVELOPING FUNCTIONAL IMMUNITY

There have been several descriptions of the relative, age-specific risks of severe paediatric morbidity attributed to *P. falciparum* infection across Africa. Figure 1 provides a summary of the data available from hospital settings located within two crudely defined transmission settings. The hospital data have been extracted from eight areas where less than 50% of the childhood communities using the hospital were likely to be infected with *P. falciparum* when surveyed at cross section (low-to-moderate intensity transmission). The remaining four hospitals served populations where the prevalence of infection among children was greater than 75% (high-intensity transmission). Characteristic of severe disease under conditions of high-intensity transmission is the overwhelming burden among infants (55–66% of all childhood malaria admissions) with far fewer cases amongst children aged 4 years (less than 4%). Conversely, under condi-

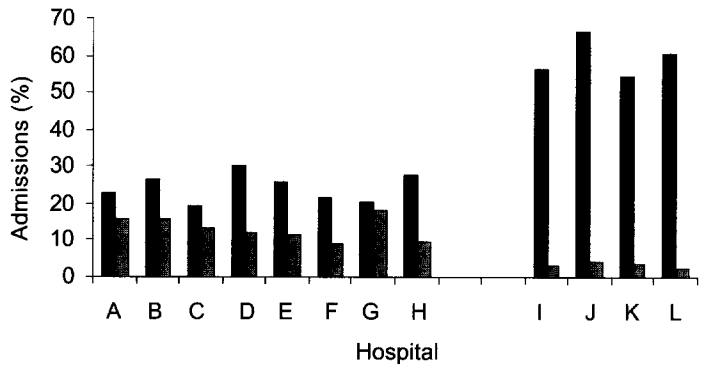


*Figure 1* Percentage of paediatric (0–4 years) malaria admissions aged less than 1 year (black) or aged 4 years (grey) from eight areas of low-to-moderate intensity transmission (A: Bakau, The Gambia (Snow *et al.*, 1997); B: Kericho, Kenya (Shanks and Snow, unpublished data), C: Humera, Ethiopia (Seboxa and Snow, 1997), D: Kilifi Township, Kenya (Snow and Marsh, unpublished data); E: Kinshasa, DRC (Greenberg *et al.*, 1989); F: Brazzaville, Congo (Trape *et al.*, 1987); G: Sukuta, The Gambia (Snow *et al.*, 1997); F: Kilifi rural, north, Kenya (Snow *et al.*, 1997)) and four areas of intense transmission (I: Kilifi rural, south, Kenya (Snow *et al.*, 1997); J: Nchelenge, Zambia (Gernaat *et al.*, 1998); K: Siaya, Kenya (Snow *et al.*, 1997); L: Idete and Lumeno,Tanzania (T. Teuscher, unpublished data)).

tions of low-to-moderate intensity transmission the age-structured, proportional burden appears to be spread more evenly across childhood. A consistent finding in all such studies is that the clinical presentations of complicated falciparum malaria also vary according to the intensity of transmission. The frequency of cerebral malaria declines with increasing intensity of transmission whilst severe malaria anaemia increasingly dominates the clinical burden (Slutsker *et al.*, 1994; Snow *et al.*, 1994, 1997; Modiano *et al.*, 1998). One further important observation illustrated in Figure 1 is that, at all intensities of transmission, the risks of severe disease are always higher in infancy compared to the fifth year of life, implying that a degree of immunity occurs very early in life.

To examine the speed with which this functional immune response is acquired we studied the risks of developing severe malaria in infants presenting to hospital in four distinct transmission settings (Snow *et al.*, 1997). During the first 3 months of life the incidence of severe malaria was low in all settings, presumably as a result of the protection afforded by passively acquired maternal immunoglobulin G (McGregor, 1965), the physiological protection offered through the continued presence of fetal haemoglobin (Pasvol *et al.*, 1977) and possibly riboflavin deficiency (Bates *et al.*, 1982). Thereafter, under conditions of high transmission, peak incidence in severe disease was reached by the fifth to seventh months of life, after which it significantly declined before the first birthday. In the two areas of low-to-moderate transmission, the incidence of severe disease continued to rise past the first birthday. The incidence data in infancy were modelled against the force of infection, as measured through the infant parasitological conversion rate amongst asymptomatic infants living in the same communities (Gupta *et al.*, 1999). The best-fit models suggested that a significant degree of functional immunity against severe disease occurred following exposure to relatively few new infections. It is important to recognize that the same may not be true for a child's ability to mount an effective immune response to infection *per se*, nor one that prevents mild clinical episodes. The prevalence of both these states continues to remain high throughout childhood in almost every transmission setting. Preventing these states may require exposure to a wide range of parasite polymorphisms whilst the epidemiological data on severe malaria outcomes lend support to a degree of 'strain'-transcending immunity (Gupta *et al.*, 1999).

There appears to be a general consensus that the intensity of *P. falciparum* transmission provides a useful indication of the likely age-structured risk of severe clinical disease in a given population. It also seems reasonable to assume that the declining incidence of severe outcomes following infection is in some way a function of the degree of parasite exposure from birth. Whether the outcomes of a clinical episode of malaria are better or worse as children progress through infancy and childhood is therefore important to define.

## 3. THE EFFECTS OF AGE ON THE RISK OF DISEASE OUTCOMES

With several infectious diseases, notably measles and rubella, primary exposure to the pathogen later in life results in a poorer clinical outcome compared to an exposure in early life (Anderson and May, 1991). With malaria, several authors have argued that the opposite may be true when an older child is better able to recover from primary infections than younger children or to respond to treatment (D'Alessandro and Coosemans, 1997; Greenwood, 1997). There are probably good behavioural reasons to imagine this would be the case when an older child can draw attention to symptoms more quickly than young children. However empirical descriptions of this age-effect or any biological basis for its existence have until recently not been well addressed.

Baird and colleagues have described the relationship between age, infection and severity of outcome through a series of studies amongst both immune and naïve populations in Irian Jaya, Indonesia (Baird *et al.*, 1993, 1998; Baird, 1998). Whilst it appears that, among non-immune migrants to falciparum endemic areas, adults develop a stronger and faster immune response to infection than children, the clinical outcomes of infection are worse in adulthood than those in childhood. This suggests some increasing constitutional ability of the immune system to mount a response to infection as patients get older. This response however may not provide any additional advantage in dealing with the primary infection. Two further analyses of risks and outcomes of falciparum malaria among non-immune migrants support the findings from Irian Jaya. The risk of clinical malaria among non-immune Israeli travellers was highest amongst the group below 40 years of age but the risks of developing severe, complicated malaria were four times higher among those aged over 40 years (Schwartz *et al.*, 2001). Similarly, in the United States of America *P. falciparum* malaria case-fatality rates among non-immune travellers aged over 20 years were 10 times higher than those among travellers aged less than 20 years (Greenberg and Lobel, 1990).

When studying the effects of age upon disease risks in highly endemic areas such as Africa, there is a problem differentiating the coincidental effects of cumulative parasite exposure with time from those of age *per se*. One useful and available source of information is the survival chances of those who have developed disease. We have previously compiled case-fatalities amongst severe and/or complicated malaria admissions from several clinical settings across Africa where hospitals are located in a wide range of endemicities (Figure 2; Marsh and Snow, 2000). One striking feature is that very young children (1–5 months of age) have a particularly poor prognosis following admission with complicated malaria. Between 6 and 23 months, case-fatalities decline but thereafter show, despite wide confidence intervals, a consistent rise with increasing age of the child. This is consistent with the findings from non-immune travellers and

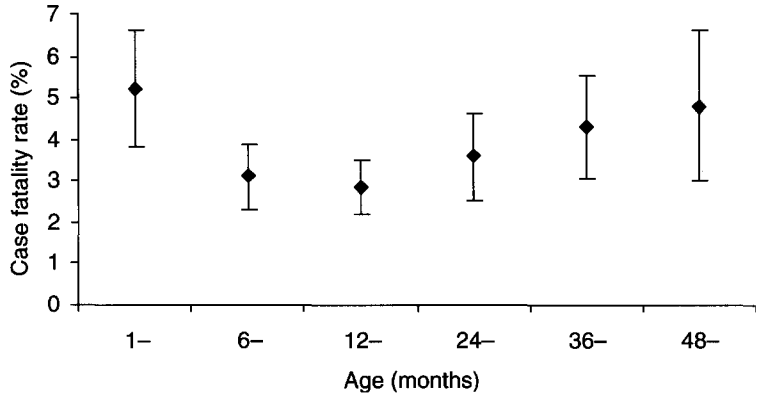


*Figure 2* Age-specific case-fatalities (percentage; 95% confidence interval) among paediatric admissions from 10 locations representing the transmission intensity range common to Africa (Marsh and Snow, 2000).

supports a general principle that, whilst incidence of severe disease may decline throughout childhood, the risks of a fatal outcome increase.

## 4. RATES OF PAEDIATRIC MALARIA MORBIDITY ACROSS THE VARIED TRANSMISSION SETTINGS OF AFRICA

There have been several attempts to examine differences in morbidity between communities living under different intensities of transmission. Ellman *et al.* (1998) recorded the monthly period prevalence of fever associated with a peripheral *P. falciparum* parasitaemia > 4000 parasites per microlitre ('malaria cases') among children aged 6–71 months in five clusters of villages located at different altitudes in Muheza district, Tanzania. As altitude increased the frequency of 'malaria cases' decreased, although the only significant reduction in risk was in the two clusters where the prevalence of 'asymptomatic' infection was lowest (46% and 33%). Conversely, in two similar rural populations of Senegal subjected to challenges of around 20 and 200 infected bites per person per year, respectively, the total lifetime experience of clinical episodes of malaria was higher in the population receiving the lower challenge (Trape and Rogier, 1996). However, there is circularity in the definition of morbid cases of malaria during fever surveys under varying intensities of transmission. Etiological fraction estimates of the numbers of fevers attributed to a given parasitic infection will differ between areas where infection is rare and those where infection is frequent (Smith, T.A. *et al.*, 1994).

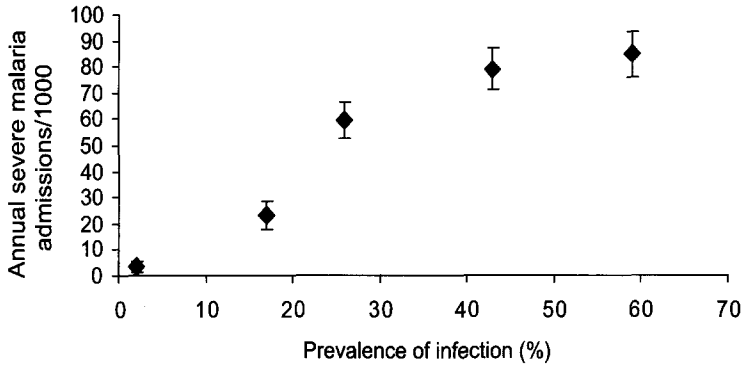


*Figure 3* The annual incidence of hospitalization with severe malaria per 1000 infants aged 1–11 months (with 95% confidence interval) from five communities located within easy access of the hospital site against the prevalence of *Plasmodium falciparum* infection among asymptomatic infants aged 3–11 months from these communities (Snow *et al.*, 1998a).

A clearer distinction can be made with the definition of severe, complicated malaria. Trape and colleagues (1987) reviewed the hospital records in Brazzaville, where populations living in close proximity and using the same health services were exposed to a wide range of challenges. The incidence of cases of severe, life-threatening, malaria was similar at all levels of transmission but maximum values were observed in areas of moderate transmission. More recently, we used a standardized protocol to examine prospectively the incidence of severe malaria in five different populations across Africa having similar access to, and utilization of, hospital services but living with a wide range of malaria transmission levels (Snow *et al.*, 1997, 1998a). For infants, the risks of hospitalization with a severe malaria event rose with increasing prevalence of 'asymptomatic' infection defined amongst infants in these communities (Figure 3). The combined risks throughout childhood (1–59 months) were lowest among populations exposed to very low intensity parasite transmission (childhood infection prevalence in the community less than 5%). However, as with the previous studies amongst older children, the highest risk occurred in communities exposed to moderate intensity transmission (infection prevalence 40–50%) and risk declined in populations exposed to high transmission (infection prevalence > 75%) (Figure 4).

Thus, with severe clinical outcomes examined using a range of methodologies, a consistent pattern seemed to emerge. Nevertheless, reliance upon hospital data in the comparison of community risk has been much criticized. It has been argued that communities vary enormously in their utilization of hospitals, one determinant being a community's perception of disease severity. The latter is particularly important owing to reported differences in the clinical presentations of life-threatening disease with age and endemicity (Greenwood,

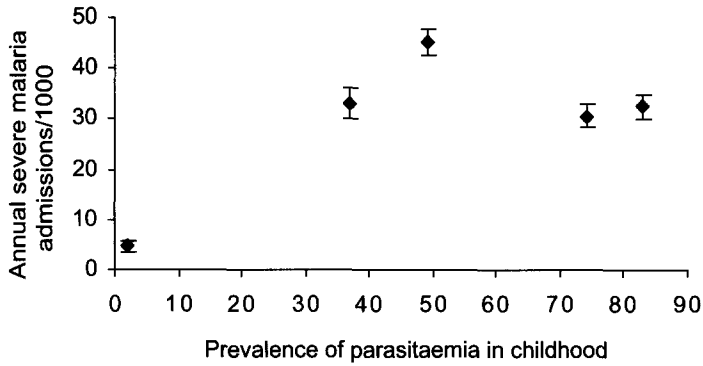


*Figure 4* The annual incidence of severe malaria admissions per 1000 children aged 1–59 months (with 95% confidence interval) from five communities with different malaria transmission intensities (Snow *et al.*, 1997).

1997; Smith, T. A. *et al.*, 2001). Furthermore, one feature of hospitalized cases of severe disease, whilst serving as a clinical proxy for mortality, is characterized by survival at discharge. Hospital case-fatalities can be high but invariably 90% of admissions do survive and are returned to the community.

## 5. THE RISKS OF MORTALITY DURING CHILDHOOD WITH INCREASING INTENSITY OF MALARIA TRANSMISSION

The critical issue to arise is whether one can conclude that the patterns described from studies of hospitalized malaria morbidity would remain true for deaths from malaria in the community. During an early examination of this we reviewed available data on malaria-specific mortality at different levels of transmission (Snow and Marsh, 1995). The limited data covered a range of endemicities, mortality measurement techniques and time periods. The data were consistent with any number of interpretations: malaria mortality rising, reaching a plateau, or declining as transmission intensity increased.

We concluded that the data were not sufficiently robust to draw definitive conclusions. Important reservations were raised about the ecological comparisons made between different communities separated sometimes by over 50 years, when changing access to treatment and other child survival initiatives would have confounded any comparison. Furthermore, the measurements of mortality risks ranged from civil registration systems to more contemporary systems of active demographic surveillance. Finally, the clinical presentation of malaria often shares symptoms with other infectious diseases, notably fever, diarrhoea and respiratory abnormalities and the attribution of malaria as the

primary cause of a paediatric death through interviews with bereaved relatives lacks both sensitivity and specificity (Anker *et al.*, 1999).

Two more recent analyses of mortality data amongst infants and children in Africa against a range of transmission conditions have tried to redress some of the deficiencies in our earlier comparative studies. First, Chandromohan *et al.* (2001) analysed contemporary data from a collaborative programme of demographic surveillance in two of the three sentinel demographic surveillance system (DSS) districts of a single country, Tanzania. The authors described one 'mesohyperendemic' district and three transects of a 'low malaria risk' district, according to whether communities are located at 1001–1250 m, 1251–1500 m or above 1500 m. The analysis considered the age-structured acute febrile mortality risks, rather than all-cause mortality, and showed that these risks were low in areas above 1251 m, at intermediate levels between 1001 and 1250 m, and highest where stable endemic transmission had been recorded, in Morogoro district. Interpretation of these data at the lower altitude transects was hampered by the lack of any empirical knowledge of either infection prevalence or sporozoite inoculation rates. The most persuasive conclusion to be drawn was that, where transmission is restricted by low ambient temperature, mortality from malaria is rare amongst all age groups from infancy through to the tenth birthday.

T.A. Smith and colleagues (2001) have provided the second more recent extension of the debate. They revisited the ecological comparisons of the relationship between entomological inoculation rates (EIR) and childhood mortality outcomes, and argued in favour of a comparison of all-cause mortality rather than malaria-specific mortality. This is an important extension for several reasons beyond the technical difficulties in defining malaria-specific mortality. First, cause-specific mortality implies that a single cause is responsible for each death, when in reality malaria may be a secondary, coincidental or co-primary cause of death. Second, the effects of a single malaria infection may go beyond the direct consequences of surviving that infection, when the parasitized host may not experience any direct clinical effect of the infection but susceptibility to the pathological consequences of other infectious agents may be amplified, leading to 'indirect mortality'.

Smith, T.A. *et al.* (2001) therefore first considered the rates of all-cause infant mortality from 20 DSS studies undertaken since 1980 plotted against an estimate of the intensity of transmission as judged by the EIR (Figure 5). The spread of infant mortality risk was considerable but there was a significant positive association between all-cause infant mortality and increasing intensity of transmission. To examine whether this association was maintained throughout childhood, the authors extended their analysis to consider mortality risks between the first and fifth birthdays. Two striking observations were: (i) all-cause mortality among older children varied less than infant mortality across the transmission intensity range; and (ii) it did not support the notion that

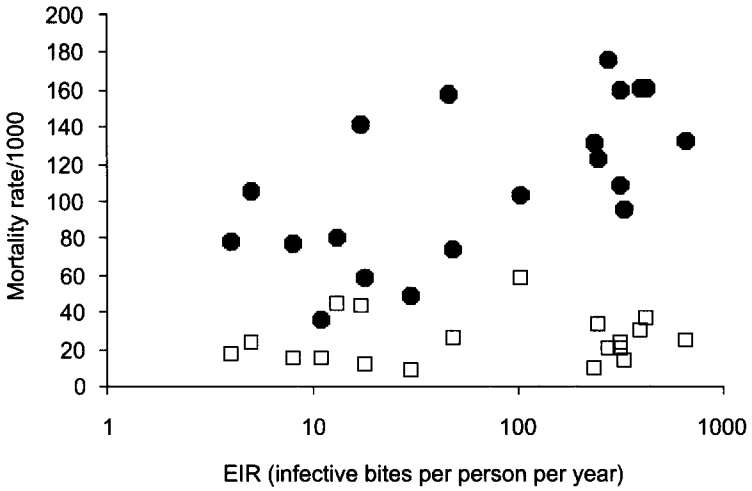


*Figure 5* Distribution of all-cause mortality 0–11 months (●) and 12-59 months (□) from 20 sites in Africa by the annual *P. falciparum* entomological inoculation rate (EIR) (Smith, T.A. *et al.*, 2001).

mortality in this age range rose with increasing EIR (Figure 5). The combined mortality risks from birth through to the fifth birthday did suggest a tendency towards higher mortality with increasing EIR, driven principally by the effects during infancy, but this association was not significant. This observation suggests that a delay in the risk of death early in life may increase the numbers of years of life saved but may not alter the number of deaths if death were simply delayed until later in childhood. Given the epidemiological concerns raised through earlier studies on the possible 'delayed' risks of severe malaria morbidity, this observation deserves further review.

## 6. LIMITATIONS OF THE *x*-AXIS

Ecological comparisons are open to much criticism. Every demographic site has its own idiosyncrasies in terms of socioeconomic variables, access to and effectiveness of health services, prevalence of human immunodeficiency virus or the genetic make-up of the population. All things are not equal and epidemiologists comparing risks between geographical areas can at best support or cast doubt on a general principle. Nevertheless, data on mortality outcomes plotted against estimates of the frequency of parasite challenge remain our only primary source of evidence to examine the relationship between exposure

intensity and disease outcome. The one area where it is possible to insist upon a degree of comparability is in the methodologies used to define the parameters of interest.

The EIR is the product of the human biting rate (the number of vectors biting an individual over a fixed period of time, usually expressed per year) and the sporozoite rate (the proportion of vectors with sporozoites in the salivary glands). Estimates of sporozoite challenge across broad geographical areas are fraught with methodological problems. First, the frequency of parasite exposure varies considerably within small geographical areas and even between households (Cattanni *et al.*, 1985; Mbogo *et al.*, 1995). Previous comparisons of the risks of parasite challenge from birth and disease outcomes have often selected one point estimate of EIR from a single household or a collection of households to represent the wide areas from which mortality has been documented. Second, whilst the EIR defines the frequency of contact between hosts and infected vectors, methods used to measure it in the field vary (Githeko *et al.*, 1996). Whether sampling of vectors is undertaken indoors or outdoors with light-traps placed close to the bed, with pyrethrum spray catches, or by human bait catches, affects the calculation of the EIR. The overall consequence is that EIR data are hard to reconcile as standard measures between sites and may convey a degree of precision in the estimation of parasite challenge that simply does not exist. A final comment for the purposes of ecological comparisons is that a focus on estimates of sporozoite inoculation excludes the use of considerable additional mortality data for which other proxies of transmission intensity exist.

A more readily available, and widely used, index of transmission intensity is the parasite rate (Metselaar and Van Theil, 1959). This is often presented as the prevalence of *P. falciparum* infection among children and has the advantage over entomological measures of endemicity in that surveys often cover more comprehensively the geographical areas used to define mortality. Prevalence of infection crudely corresponds to the frequency and duration of parasite exposure but does not provide a precise estimate of the number of new infections received by a child each year. The non-linear relationship between challenge and infection prevalence has long been recognized (MacDondald, 1957). Given the long persistence of parasitaemia after a single infection (Eyles and Young, 1951), it might be expected that parasite prevalence would become an increasingly blunt instrument at higher levels of transmission.

Beier and colleagues (1999) have recently reviewed coincidental measures of the parasite prevalence in childhood and standardized estimates of EIR. Their analysis showed that over 70% of the variance in parasite prevalence could be explained by logarithmic classification of the EIR, overall and when analysed by region – East and West Africa – or 'ecological zone'. Broadly, no

study area with a prevalence of infection in childhood below 50% had an EIR greater than 15. Conversely all areas with a prevalence of infection greater than 80% had an EIR in excess of 200. Analysis of the logarithmic classifications of the EIR showed that an EIR of less than one infective bite per adult per year encompassed an interquartile range of infection prevalence between 1 and 25%. EIRs between 1 and 10 had an infection prevalence range of 25–50%, and EIRs between 11 and 100 covered the prevalence range between 51% and 74%. The highest EIRs (in excess of 100) were associated with infection prevalences above 74%.

The analysis by Beier and colleagues (1999) strongly suggested that approaching measures of exposure as categorical rather than continuous variables may be important to overcome the inadequacies in the measures available to estimate parasite challenge. This is important for reasons not only related to the methodological difficulties. The future mapping of the spatial distribution of transmission intensity, populations exposed to risk and the likely impact of intervention strategies will most probably be based upon categorical classes of risk and benefit.

## 7. RE-EXAMINING THE MORTALITY AND TRANSMISSION INTENSITY EVIDENCE

To revisit the analysis provided by Smith, T.A. *et al.* (2001) we have used mortality data from a database constructed as a resource to document the public health burden associated with *P. falciparum* malaria in Africa*. For purposes of comparison with previous analyses, data have been restricted to survey reports since 1980 but only where all-cause mortality experiences from birth through to the fifth birthday were recorded prospectively. For each mortality study, we have identified a geographically specific cross-sectional estimate of

* The Burden of Malaria in Africa (BOMA) project was established in 1998 to identify all published and unpublished sources of mortality, morbidity and disability risks associated with *P. falciparum* infection among communities in Africa. Data have been identified through searches of electronic databases (Medline, Embase and Popline) and manual searches of pre-electronic English and French tropical and regional biomedical journals. In addition, unpublished Ministry of Health and regional conference material has been accessed from libraries in Kenya, Uganda, Sudan and South Africa. All material has been cross-referenced to locate additional sources of information. Correspondence with authors has been used to provide age- and time-specific data for each published survey report when this information was not available. Data have been abstracted, geopositioned and matched to markers of malaria endemicity on a pre-coded pro forma and entered into a relational database (Access 97, Microsoft®, 1996). To date, this database contains information on 153 temporally and/or geographically independent reports of the age-specific incident risks of malaria mortality since 1912 defined through a variety of techniques among communities located in 24 countries in Africa.

the prevalence of infection among children aged between 0 and 15 years of age (Table 1, pp. 252–3). The 26 mortality estimates have been grouped according to whether the prevalence of infection was less than 25% (low transmission intensity), 25–50% (low-to-moderate transmission intensity), 51–74% (moderate-to-high transmission intensity) or greater than 74% (high transmission intensity) (Figure 6).

One important observation is that an African child stands a significantly greater chance of surviving childhood if he or she lives in an area of low malaria risk. Children living in low transmission areas of Africa experience almost half the mortality risks compared to those who live under conditions of moderate-to-high transmission intensity. This is entirely consistent with the observations derived from the study of severe disease (Figures 3 and 4) and acute febrile mortality risk (Chandramohan *et al.*, 2001). The risk of a poor malaria outcome under such conditions of transmission is likely to be directly related to the chance of encounters with infection. For all-cause mortality this observation has greater significance as it demonstrates clearly the contribution malaria makes to the overall chances of survival. Nevertheless, it could be argued that areas of low malaria risk are those of greater economic growth and stability. Indeed, the areas we have classified as low malaria risk are high intensity agricultural regions of east and southern Africa (Transvaal, South

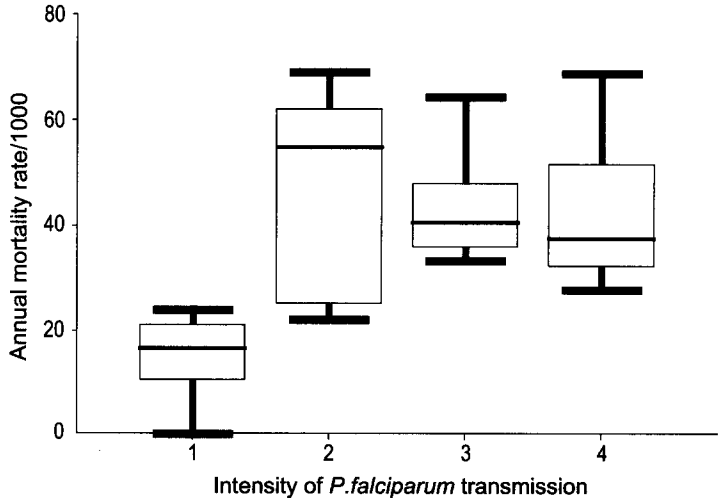


*Figure 6* Box plot showing median (central lines), 25%, 75% quartile ranges around the median (box depth) and upper and lower limits (T-bars) of all-cause childhood mortality per 1000 children aged 0–4 years per annum recorded during the surveillance of communities located in (1) areas of low, (2) low-to-moderate, (3) moderate-to-high or (4) high intensity transmission as defined in Table 1.

Africa, Muranga and Kericho in Kenya and Hai in Tanzania). By extension our analysis may simply be an ecological fallacy, reflecting more a poverty divide with malaria maintaining some communities in the vicious trap of poor health. On a global scale, this has been well articulated by Sachs and Malaney (2002); at a continental level, it has been less well defined. It would be hard to distinguish the true cause or effect of the role played by malaria but whatever explanation one favours it remains true that within the series of data presented in Figure 6, living under conditions of low malaria risk improves the chance of surviving childhood.

The second striking observation, consistent with the analysis provided by Smith, T.A. *et al.* (2001), is that across a broad range of malaria transmission intensities all-cause mortality under 5 years of age appears to vary very little. This pattern of all-cause childhood mortality is strikingly similar to the pattern described for severe morbidity presenting to hospital (Figure 4). The notion of a mortality 'plateau' is most evident from the comparison of median estimates of mortality under 5 years within the moderate-to-high and high transmission categories. These transmission conditions cover a wide range of settings encompassing childhood infection prevalence rates from 50 to 90%. The mortality ranges within areas of low-to-moderate intensity transmission are harder to interpret. What is clear is that mortality risk probably rises sharply under relatively small changes in the frequency of parasite challenge. At the lower end of this transmission category, mortality rates were comparable to those in endemic sites in the low intensity transmission range. Within this range, a small increase in the prevalence of infection was associated with a much larger rise in mortality risk (Table 1). At some ill-defined point within this range of transmission conditions mortality, as with severe disease risk, probably reaches its point of saturation. This nadir is probably higher than previously proposed for malaria-specific mortality by Trape and Rogier (1996), who argued that saturation occurs at conditions supporting one infective bite every 8 years.

As one would expect with any ecological comparison, the ranges of mortality within any given transmission category vary considerably and are probably the result of differences between communities in factors other than the frequency of infection. Nevertheless, for the same reasons we feel comfortable with the proposition that all-cause mortality below 5 years of age is considerably lower under conditions of low intensity transmission because we feel that there is little evidence that mortality in this age group rises over a broad range of low-to-moderate, moderate-to-high and high transmission intensities.

## 8. RECONCILING THE ROLE OF DIRECT AND INDIRECT EFFECTS OF *P. FALCIPARUM* ON CHILD SURVIVAL WITH THE SATURATION OF ALL-CAUSE MORTALITY WITH INCREASING TRANSMISSION

Several authors have argued that indirect mortality is a significant feature of malaria infection and that intense transmission will lead to a state of chronic parasitization that provides a generalized enhancement of risk of early mortality (Greenwood, 1997; Molineaux, 1997). Data used in support of indirect malaria mortality were derived from results obtained during trials of chemoprophylaxis (Greenwood *et al.*, 1988) and insecticide-treated bed nets (ITNs) (Alonso *et al.*, 1993b) performed in The Gambia. These trials showed that the effects upon all-cause mortality were larger than would have been expected given the baseline estimates of malaria-specific mortality defined through verbal autopsy, although it must also be true that this could be interpreted as further evidence of the inadequacy of the verbal autopsy technique. The second and widely cited sources of evidence in support of indirect malaria mortality are the historical and precipitous declines in all-cause mortality associated with aggressive vector control programmes in Sri Lanka, Guatemala and the Pare region of Tanzania (Molineaux, 1985; 1996; Bradley, 1991, 1992). However, as most authors commenting on these data highlight, vector control was implemented coincidentally with generalized improvements in the provision of curative services (actually instigated in the Pare region because of fears of 'rebound' mortality'; Smith, A. and Pringle, 1967) or because of economic development (Langford, 1996). This general principle we will return to later.

There is persuasive evidence of the indirect role played by malaria in increasing the risks of low birth weight babies for women living under increasing intensities of malaria transmission. Low birth weight is a major determinant of neonatal and post-neonatal mortality to the extent that it has been suggested that 25% of all neonatal deaths can be attributed to malaria during pregnancy (Goodman *et al.*, 1999). Infancy also marks a period of particular risk for invasive bacterial infections. Paediatric bacteraemias are most common among children aged 0–6 months and recent more detailed clinical descriptions of their pathology and prognosis suggest that malarial infections may increase the risk of a fatal outcome in hospital settings. Finally, chronic and repeated infections during early childhood lead to a state of anaemia and other states of undernutrition so that the combined effects may enhance the susceptibility to generalized morbidity and mortality. Anaemia, micronutrient deficiencies and wasting are particularly marked during the first year of life in most malaria endemic communities. These 'indirect' mortality risks may well exert their greatest influence during a period of an infant's life when he or she is at lowest risk of a direct fatal outcome following infection. As the intensity of transmission increases, the risks of indirect, early infant mortality may

increase but this would be balanced against survivors more rapidly becoming immune to the direct effects of severe malaria. Furthermore, it would appear from the analysis of all-cause mortality throughout childhood (Figures 5 and 6) that under conditions of frequent parasite challenge in Africa the indirect effects of infection on child health are outweighed by the direct consequences of infection.

## 9. INSECTICIDE-TREATED BED NETS IN AFRICA

One of the most significant advances in malaria control in Africa over the last 15 years has been the results of carefully designed clinical trials of ITNs (insecticide-treated bed nets) and curtains. The combined results of these trials suggest that the use of ITNs can reduce all-cause childhood mortality by 17% (Lengeler, 1998). The trials were undertaken across a range of baseline endemicities in Africa and one early observation was that protective efficacy appeared to decline with increasing intensity of transmission (Table 2, p. 254). Nevertheless, it has been suggested that comparisons of results obtained from randomized controlled trials (RCTs) should focus on attributable risks (numbers of lives saved) rather than rate ratios (protective efficacy) (Lengeler *et al.*, 1998). When reanalysed, the trial data showed that there was little difference in the number of lives saved (1 or 6–59 months of age) whether the trials were undertaken in high or low intensity transmission locations (Lengeler *et al.*, 1998). This may suggest that ITNs are likely to be of sustained public health value across the entire transmission spectrum, but such an assertion requires at least two basic assumptions. First, the results of the RCTs can be sustained for more than 1 or 2 years and a realignment of mortality risk following changes in infection risk from birth must not affect long-term childhood survival. Second, if the trend in protective efficacies in favour of areas of lower intensity transmission indeed exists, then childhood mortality (0–4 years) would need to be consistently higher at increasing intensities of transmission to balance any declining protective efficacy. As discussed above, there is little evidence in support of this.

Only two published RCTs have been undertaken in areas of high intensity transmission (Table 2). Both studies, in northern Ghana (Binka *et al.*, 1996) and Burkina Faso (Habluetzel *et al.*, 1997), showed reduced protective efficacy during the second year of the trials compared to the first year. The unadjusted rate ratios of the intervention to control mortality amongst children aged 6–59 months was 0.72 during year 1 compared with 1.01 during year 2 in Ghana and 0.84 during year 1 and 1.04 during year 2 in Burkina Faso. One further RCT, in an area of intense, perennial transmission at Asembo Bay, western Kenya, where strict randomization was maintained for 2 years, has recently been

*Table 1* All-cause childhood mortality (0–4 years) at 26 sites in Africa according to categorical estimates of the intensity of *Plasmodium falciparum* transmission[a]

| Site[b] | Childhood infection prevalence[c,d] | Dates of mortality surveillance | Person-years exposure to risk (0–4 years) | All-cause mortality events (0–4 years) | Mortality rate (under 5 years) |
|---|---|---|---|---|---|
| **Low endemicity areas[e]** | | | | | |
| 1. Agincourt, South Africa | 6 [0–9] | 1992–95 | 2,880,000 | 216 | 0.075 |
| 2. Hai district, Tanzania | 4 [0–9] | 1992–95 | 74,494 | 1,233 | 16.55 |
| 3. Kericho, Kenya | 12 [0–9] | 1997–98 | 30,623 | 325 | 10.61 |
| 4. Mbarara, Uganda | 18 [0–3] | 1988–89 | 4,320 | 104 | 24.07 |
| 5. Muranga, Kenya | 24 [0–15] | 1985–88 | 1,952 | 251 | 21.0 |
| **Low to moderate endemicity areas[f]** | | | | | |
| 6. Dar es Salaam, Tanzania | 28 [0–9] | 1992–95 | 34,023 | 883 | 25.95 |
| 7. Bandim II, Guinea Bissau | 35 [1–5] | 1987–90 | 2,753 | 153 | 55.58 |
| 8. Katana, Democratic Republic of Congo (DHC) | 35 [1–9] | 1986–87 | 5,187 | 358 | 69.02 |
| 9. Farafenni, North Bank, The Gambia | 39 [0.5–6] | 1982–83 | 2,505 | 171 | 68.3 |
| 10. Mlomp, Senegal | 46 [2–9] | 1985–95 | 7,886 | 194 | 24.6 |
| 11. Kilifi, Kenya | 49 [0–9] | 1991–93 | 20,679 | 455 | 22.0 |
| 12. Niakar, Senegal | 50 [2–9] | 1984–95 | 58,982 | 3,242 | 54.97 |
| **Moderate to high endemicity areas[g]** | | | | | |
| 13. Morogoro district, Tanzania | 52 [0–2] | 1992–95 | 50,071 | 1,795 | 35.85 |
| 14. Kangondjan, Burkina Faso | 54 [0.5–9] | 1982–86 | 1,271 | 43 | 36.20 |
| 15. Farafenni South Bank hamlets, The Gambia | 55 [1–5] | 1988–90 | 3,130 | 150 | 47.92 |
| 16. Bo, Sierra Leone | 59 [0–7] | 1990 | 776 | 35 | 45.1 |
| 17. Farafenni South Bank PHC villages, The Gambia | 66 [1–5] | 1988–89 | 2,263 | 146 | 64.52 |
| 18. Upper River Division, The Gambia | 71 [1–4] | 1989–93 | 113,319 | 3,776 | 33.32 |
| **High endemicity areas[h]** | | | | | |
| 19. Imbo Sud, Lac Nyanza, Burundi | 76 [2–9] | 1990–91 | 3,815 | 160 | 41.94 |
| 20. Bagamoyo, Tanzania | 82 [0.5–3.5] | 1992–94 | 5,850 | 192 | 32.82 |
| 21. Pahou and environs, Benin | 83 [0–15] | 1989 | 1,037 | 29 | 27.97 |
| 22. Asembo Bay, Kenya | 83 [1–4] | 1997 | NA | NA | 59.9 |
| 23. Navrongo, Ghana | 87 [0–7] | 1990 | 1,065 | 35 | 32.86 |
| 24. Tanga, Tanzania | 88 [1–4] | 1992–93 | 2,072 | 90 | 43.44 |
| 25. Kilombero, Tanzania | 90 [1–9] | 1996–97 | 8,761 | 279 | 31.85 |
| 26. Bandafassi, Senegal | 95 [2–9] | 1984–95 | 16,975 | 1,167 | 68.75 |

*Table 1 continued*

[a] An important methodological difference from that used in the review provided by Smith, T.A. *et al.* (2001) is our exclusion of data based upon modelled assumptions about mortality risks during the first 6 months of life in surveys where these events were not empirically reported. Where multiple mortality surveys were undertaken over several years, these have been collapsed to provide a single mortality estimate. For surveys undertaken in the same area but separated by several years, we have selected only the most recent mortality estimates (for example Saradidi in the Asembo Bay area of Kenya (Spencer *et al.*, 1987; Smith, T.A. *et al.*, 2001), Imbo Sud, Burundi (Coosemans, 1987; Dellacollette and Barutwanayo, 1993) and Bagamoyo in Tanzania (Mtango and Neuvians, 1986; Premji *et al.*, 1997)). Mortality surveys undertaken at Linzolo (DRC) have not been included because the entry point for survival analysis was birth at the district hospital (Carme *et al.*, 1992). Four further mortality estimates have not been included as it was not possible to identify any estimates of infection prevalence or EIR from the locations of the demographic surveillance (Bwamanda, DRC (Van den Broeck *et al.*, 1993); Chingwale, Malawi (Lindskog *et al.*, 1988); Lama-Doonka, Somalia (Ibrahim *et al.*, 1996) and Alamata, Ethiopia (Kidane and Morrow, 2000)).

[b] References: (1) Khan *et al.* (1999); (2) Tanzania (1997); (3) Shanks *et al.* (1999, 2000); (4) Vella *et al.* (1992); (5) Mirza *et al.* (1990); (6) Tanzania (1997); (7) Molbak *et al.* (1992); (8) Delacollette *et al.* (1989); (9) Greenwood *et al.* (1988); (10) Trape *et al.* (1998); (11) Snow *et al.* (1994b); (12) Trape *et al.* (1998); (13) Tanzania (1997); (14) Gazin (1990); (15) Alonso *et al.* (1993a, b); (16)Barnish *et al.* (1993); (17) Alonso *et al.* (1993a, b); (18) Jaffar *et al.* (1997); (19) Delacollette and Barutwanayo (1993); (20) Premji *et al.* (1997); (21) Velema *et al.* (1991a, b); (22) Smith, T.A. *et al.* (2001); (23) VAST (1993); (24) Salum *et al.* (1994); (25) Armstrong-Schellenberg *et al.* (1999); (26) Trape *et al.* (1998).

[c] Age range (years) in brackets.

[d] References: (1) Brink (1958); (2) MARA/ARMA collaboration: modelled maps of malaria transmission in West Africa (http://www.mara.org.za/internal); (3) Shanks *et al.* (2000); (4) Jelliffe and Jelliffe (1963); (5)Njoroge (1987); (6) Yamagata (1996); (7) Smedman *et al.* (1983); (8) Delacollette *et al.* (1990); (9) Greenwood *et al.* (1987); (10) Trape *et al.* (1998); (11) Snow *et al.* (1997); (12) Trape *et al.* (1998); (13) Van den Homberg (1994); (14) Gazin (1990); (15) Alonso *et al.* (1993a, b); (16) Barnish *et al.* (1993); (17) Alonso *et al.* (1993a, b); (18) Thomson *et al.* (1994); (19) Delacollette and Barutwanayo (1993); (20) Shiff *et al.* (1995); (21) Akogbeto *et al.* (1992); (22) Bloland *et al.* (1999); (23) Binka *et al.* (1994); (24) Lyimo *et al.* (1991); (25) Smith, T.A. *et al.* (1993); (26) Trape *et al.* (1998).

[e] Prevalence of infection 1–24% (EIR < 1).

[f] Prevalence of infection 25–50% (EIR 1–10).

[g] Prevalence of infection 51–74% (EIR 11–100).

[h] Prevalence of infection > 74% (EIR > 100).

NA, not applicable.

*Table 2* Mortality reductions recorded during randomized controlled trials of insecticide-treated bed nets in Africa and prevalence of *Plasmodium falciparum* infection in children not using treated nets[a]

| | Unadjusted rate ratio (%)[b] | Duration of trial (years) | Baseline or control estimates of infection prevalence amongst children aged 0–9 years (%) |
|---|---|---|---|
| The Gambia[c] | 23 | 1 | 39[c] |
| Kilifi, Kenya[d] | 29 | 2 | 49[e] |
| Oubritenga, Burkina Faso[f] | 14 | 2 | 85[g] |
| Navrongo, Ghana[h] | 17 | 2 | 87[i] |

[a] See also Lengeler *et al.* (1998).
[b] 1 – (intervention mortality rate)/(control mortality rate) × 100.
[c] D'Alessandro *et al.* (1995).
[d] Nevill *et al.* (1996).
[e] Snow *et al.* (1997).
[f] Habluetzel *et al.* (1997).
[g] Habluetzel *et al.* (1999).
[h] Binka *et al.* (1996).
[i] Binka *et al.* (1994).

completed. Preliminary examination of these results indicated an important reduction in mortality although the data also confirmed reduced protective efficacy against all-cause mortality among children aged less than 5 years during the second, compared to the first, year of the trial (P. Philips-Howard, personal communication). Conversely, the only RCT maintained over 2 years in an area of low-to-moderate transmission intensity (Kilifi, Kenya; Nevill *et al.*, 1996) showed little variation between trial years in the unadjusted rate ratios for all-cause mortality among children aged 6–59 months (0.70 in year 1 and 0.74 in year 2; unpublished data).

It is important to recognize that, overall, most of the trials carried out in a broad spectrum of transmission conditions, with the exception of that in Burkina Faso, achieved a significant effect on child survival over the combined surveillance period and they were not designed to undertake any sub-group analysis. Nevertheless, there remains a strong suggestion that the initial gains afforded by ITNs may in some settings decline with duration of use by the childhood population. There could be a variety of reasons why this may occur. Amongst these must be included the possibility that, as children age with changing conditions of parasite challenge, mortality risk readjusts. This would be consistent with the ecological and clinical evidence currently available.

Such a realignment of mortality risk under conditions of high transmission would not be important if the risk were balanced against the same level of

protective efficacy against childhood mortality at 0–4 years as has been reported in areas of lower intensity transmission or if childhood mortality were consistently higher under conditions of high transmission. Neither position appears to be supported by the available evidence. The results from the RCTs suggest that the protection is higher under conditions that favour low-to-moderate intensity transmission (Table 2). Neither Smith, T.A. *et al.* (2001) nor the data in Figure 6 favours the notion that all-cause mortality throughout childhood is any higher under conditions of moderate-to-high transmission.

## 10. WHO WILL BENEFIT MOST FROM REDUCTIONS IN PARASITE EXPOSURE?

The positive linear association between severe, life-threatening malaria morbidity, death from malaria or death from other causes during the first year of life and increasing intensity of transmission is supported by a variety of empirical data sources reviewed in Sections 5 and 7 above. However, the idea that this association is maintained throughout childhood is not supported by similar analyses of all-cause mortality or severe malaria morbidity. This is important for long-term public health questions surrounding the implementation of interventions aimed at reducing parasite challenge.

One clear and unambiguous position is that all-cause mortality among children less than 5 years of age is lowest as one approaches the $x$–$y$ intercept with transmission intensity. An area on the extreme left of the $x$ transmission axis probably encompasses a sharp inflection in the risks of severe or fatal disease outcomes. Of interest, therefore, is how likely ITNs, or any other control method, would be to reduce the prevalence of infection below this point (see Figure 6) and how many people live within a range of endemicities likely to benefit most from this proportional reduction in infection risk.

During the RCTs of ITNs the prevalence of *P. falciparum* infection in childhood was reduced by as little as 7% in Burkina Faso (Habluetzel *et al.*, 1999), and as much as 30% in Kilifi, Kenya (unpublished data). In The Gambia, a range of reductions in infection prevalence was observed between the five matched communities from nil to over 50% (D'Alessandro *et al.*, 1995). In many communities located in areas of low-to-moderate transmission intensity, a 30–50% reduction in infection prevalence would be sufficient to place childhood communities in the category of low mortality risk and arguably the sustained impacts of ITNs are likely to be largest under such conditions. However, in areas of high (or higher) transmission such a reduction would result in parasite prevalences still above this threshold. In such circumstances, potential long-term realignment of disease risk could limit the number of severe disease episodes avoided or lives saved.

Africa includes an enormous range of transmission conditions from one infectious bite every 5–10 years to several infectious bites every night (Hay *et al.*, 2000). Preliminary maps of infection risks suggest that relatively few people live under high transmission conditions. Only 9% of the population of west Africa and 14% of that of Kenya live in areas with a prevalence of infection in excess of 70% (Snow *et al.*, 1998b; Kleinschmidt *et al.*, 2001). New developments in mapping malaria infection risks in Africa should allow better definition of the location and numbers of people living in areas likely to achieve maximum gains from different control activities (Rogers *et al.*, 2002).

## 11. CONCLUSIONS

The African continent exhibits enormous heterogeneity in both the risks of infection with *P. falciparum* and death within the first 5 years of life. Variation in the risks of exposure to infectious agents and mortality forms the rationale for mathematical approaches to the epidemiology of many disease systems. However, in contrast to diseases like measles, rubella or several veterinary diseases, where it has been possible to estimate age-structured risks against the force of transmission, the malaria community's efforts have been hampered by the lack of reliable quantification of either exposure or disease outcome risks. Yet, we must begin to draw together the most plausible epidemiological evidence in order to define a public health framework for the likely successes of existing and new interventions.

This review has provided a digest of some of the issues that have re-emerged over the last 5 years in relation to the question of how malaria transmission intensity relates to the pattern, spectrum and magnitude of disease outcomes in childhood. There are several areas where a level of general agreement can be defined. First, under all conditions of stable transmission the incidence of a severe clinical outcome following malaria infection declines rapidly in childhood. However, clinical outcome for the individual may worsen with increasing age. These two features of clinical malaria are consistent with the basic principle of endemic stability proposed for other infectious diseases (Coleman *et al.*, 2001). Second, at a community level the decline in the risk of a severe disease outcome occurs earlier in life when the frequency of parasite exposure from birth is greatest (highest transmission intensity). Third, severe life-threatening malaria morbidity and all-cause mortality during infancy rise consistently with increasing intensity of transmission and it seems reasonable to assume that immunologically naïve infants would benefit from any reduction in the risk of parasite challenge from any starting point.

What will continue to be contentious is whether or not malaria-specific and all-cause mortality throughout childhood reach a point of saturation with

increasing intensity of transmission. We believe that the data presented from a series of independent ecological comparisons strongly suggest that they do. Furthermore, examination of the data from trials which have reduced the frequency of parasite exposure among cohorts of children using ITNs suggests that under conditions of high transmission early successes of the intervention may be diluted with duration of use. Realignment of the speed with which children develop functional immunity in the face of new conditions of parasite challenge from birth provides a plausible explanation.

Early successes achieved by ITNs under all transmission conditions are likely to be concentrated amongst young, immunologically naïve infants, a group at the highest risk of death from both malaria and a wide range of other causes, and these effects should be sustained. The long-term success of ITNs, in terms of overall reduction in childhood mortality, will be greatest where disease burdens are high but the frequency of parasite exposure is low-to-moderate. Fortunately, notwithstanding the need for more accurate mapping of transmission intensities, it seems likely that large parts of Africa fall into this category. In areas of high transmission, interventions may have less chance of reducing exposure to the point where one would expect sustained reduction in overall mortality through childhood. However, this should not limit the widescale use of ITNs under all transmission conditions for a number of reasons. First, under all transmission conditions, the major early reduction in childhood mortality will be enormously important and will give impetus to all other parts of the malaria control strategy. Second, although this review has concentrated on relationships between transmission and life-threatening malaria, reduction in the massive load of less severe disease will have a major impact on health services in terms of both the costs of treating malaria and the development of drug resistance, and it is likely that this effect will be sustained under all transmission conditions. Finally, all predictions of what may happen when transmission is reduced have to be qualified by caution concerning the limitations of ecological comparisons on which such predictions are based. In the end, it will be the widespread introduction of successful control measures that will throw most light on the relationship between malaria transmission and outcome.

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# Cytokine-Mediated Host Responses during Schistosome Infections; Walking the Fine Line between Immunological Control and Immunopathology

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## ABSTRACT

Most helminth infections of humans and animals induce similar immune responses, which are characterised by the production of Th2-associated cytokines (interleukin (IL)-4, IL-5, IL-9, IL-10, IL-13) and antibodies (IgG1 – mouse, IgG4 – man, IgE – both). This type-2-biased immune phenotype generally persists for the duration of the infection. Although similar types of immune responses are also triggered during allergy, atopy and anaphylaxis, chronic helminth-induced type-2-associated responses are usually held in check by appropriately regulated control mechanisms that limit the destructive potential of prolonged cytokine bias. Among numerous reported activities, helminth-induced type-2-associated immune responses have been linked to the expulsion of gastrointestinal nematodes and the formation of circumoval granulomas during schistosomiasis. However, what happens when this highly regulated, and often beneficial, type-2 immune response becomes chronic, improperly controlled, or exaggerated during helminth infections? Using schistosomiasis as a model disease, we describe the lethal consequences of inappropriate immune response induction by reviewing the literature generated from experimental animal studies and human epidemiological investigations. Development of severe and non-overlapping immunopathological phenotypes will be discussed in the context of immune deviation and in the setting of chronic and/or hyper-polarised cytokine environments.

## 1. INTRODUCTION

Parasitic worm infections caused mainly by organisms contained within the Phyla Platyhelminthes and Nematoda remain one of the major causes of human morbidity and mortality in the developing world today. These infections are so common that recent summaries of the worldwide prevalence of all major human helminth diseases have shown that there are more than enough existing infections for every living person to harbour at least one parasite if worm infections were spread evenly across the population of the world (Le Riche, 1967)! However, the vast majority of all severely debilitating human infections occur in the tropics and sub-tropics. Here, they account for severe malnutrition, blindness, haemorrhaging, fever, diarrhoea, and a variety of other clinical maladies. Despite the existence of anti-helminth chemotherapies, the accumulation of crippling and chronic disorders such as those mentioned above not only can lead to an increase in individual morbidity and mortality rates but also can induce a spiralling collapse of the socioeconomic status of an affected village, community, or even a whole country. Why? One reason is that

helminth infections induce common symptoms of other illnesses. Because of this they are often misdiagnosed and subsequently treated incorrectly by medical practitioners. Even if these infections are properly diagnosed and identified in a timely fashion, many people fail to get the appropriate treatment due to political, economic, or social reasons. In addition, the currently available and most widely used chemotherapies today (albendazole, mebendazole, diethylcarbamazine, ivermectin, and praziquantel) can fail to induce life-long protection and, therefore alleviate infection and morbidity only temporarily. Taken together, these factors can potentially lead to a population of chronically ill individuals not contributing to the productive development of their community. Ultimately, this state of 'medical emergency' leads to countries suffering (in some instances staggering) financial losses that may be unrecoverable. Concerns over the medical and economic health of developing countries have fuelled research related to (1) the search for new chemotherapies through novel drug design, (2) the accurate identification of 'at risk' populations through epidemiological approaches, (3) the delivery and administration of drugs through humanitarian efforts, (4) the characterisation of chemotherapuetic and immunoprophylactic targets, and (5) the understanding of helminth/host interactions through immunobiological investigations.

This review will focus on one particular aspect of helminth/host interactions – the host's inappropriate development of highly skewed, imbalanced and deleterious immunopathological inflammatory reactions that evolve during schistosome infection. These reactions, unlike those properly regulated and balanced ones commonly associated with non-lethal chronic persistence of schistosome parasites, lead to elevated morbidity and mortality rates. Because distinct types of atypical immunopathologies are increasingly being reported in schistosome infections of experimental animal models and human populations, investigators have questioned whether these pathologies are, in fact, uncommon. The consensus amongst most schistosome investigators, therefore, is that it is important to identify the immune correlates, pathways and molecules that associate with severe pathological reactions during schistosomiasis. Once identified, they need to be properly characterised and understood in order for strategies of intervention or prevention to be implemented successfully.

The purpose of this review is to summarise how a balanced immune response develops and is maintained in the host during schistosomiasis and to discuss the deleterious consequences of skewed, atypical and exaggerated immune response induction. Specifically, a focus will be presented that defines the different pathological states induced by inappropriate immune responses, describes how these distinct states may develop during schistosomiasis, and discusses whether similar pathological reactions occur in other helminth infections using examples from both human and animal studies.

## 2. SCHISTOSOMIASIS

### 2.1. Background

Human schistosomiasis, caused by three major species of trematodes (*Schistosoma mansoni*, *S. japonicum* and *S. haematobium*), is transmitted by cercariae released from water-dwelling snails. *Schistosoma mansoni* and *S. japonicum* cause intestinal and hepatic schistosomiasis whereas *S. haematobium* causes urinary schistosomiasis. Intestinal and hepatic schistosomiasis is found mainly in sub-Saharan Africa (*S. mansoni*), South America (*S. mansoni*), the Middle East (*S. mansoni*) and Asia (*S. japonicum*) whereas urinary schistosomiasis (*S. haematobium*) is generally endemic to North and sub-Saharan Africa. Currently, schistosomes are estimated to infect more than 200 million people worldwide with as many as 600 million more individuals in danger of acquiring schistosomiasis due to mass immigrations, emigrations and newly created irrigation projects (Bergquist and Colley, 1998). These particular metazoan organisms are large, extracellular, macro-endoparasites that undergo larval to adult metamorphosis within various tissues of an infected host.

Helminths, as a group, are supreme survival strategists, having evolved structures, behaviours and mechanisms that allow for the successful continuation of life in harsh host environments such as blood, the gastrointestinal tract and the lymphatics (among many others). Schistosomes are no exception; *S. mansoni*, *S. japonicum*, and *S. haematobium* all live the majority of their life spans in the blood of their definitive hosts (vertebrate mammals) and have evolved various survival strategies (Pearce and Sher, 1987). Reaching sexual maturity in the blood stream, male and female schistosomes (unlike other trematodes, which are hermaphroditic, schistosomes are dioecious) mate and each pair produces hundreds (*S. mansoni* and *S. haematobium*) to thousands (*S. japonicum*) of eggs every day. Many of these eggs will ultimately be transmitted to the outside environment either by passing with the faeces (*S. japonicum* and *S. mansoni*) or in the urine (*S. haematobium*). In a suitable water environment, the eggs will hatch viable larval parasite stages (miracidia) and the search for an intermediate snail host ensues. Four to five weeks following miracidial penetration and asexual reproduction of intramolluscan schistosome parasite life-stages, snails will begin to shed a second larval stage (cercariae), which is infectious to humans and other mammalian definitive hosts. Although some pathological consequences develop when a host is infected by cercariae (Mulvihill and Burnett, 1990), these are usually minor and ill-defined in comparison to those changes that evolve in response to the eggs that become trapped in host tissues during a patent infection. In fact, extrapolations taken from murine studies suggest it is the schistosome egg that induces the majority of all pathological reactions in an infected individual.

Although in most cases the host develops control mechanisms that prevent severe egg-associated pathology from occurring, some individuals will suffer from the hepatosplenic form of the disease. While severe periportal (Symmers pipestem)/hepatic fibrosis, portal hypertension, the development of portosystemic collaterals and oesophageal varices have all been documented during schistosome-induced hepatosplenomegaly (Cheever, 1968), other severely damaging infection-related disease states (mildly related to fibrosis) also may be associated with the parasite (Mwatha *et al.*, 1998). These different disease states need to be further characterised. Therefore, understanding how schistosomes induce distinct pathological reactions in the host and how the host protects itself from succumbing to severe damage is an important priority in modern schistosome-related immunological investigations.

## 2.2. The Egg and the Granuloma

Schistosome eggs, and the products that they release, are potent stimulators of cell-mediated delayed-type host inflammatory reactions (Boros and Warren, 1970). In the case of *S. mansoni*, these eggs are deposited in the hepatic and mesenteric vasculature where they are intended for transmission across the small intestine into the lumen and from there to the outside environment of the definitive host. In this obligatory process (for continuation of the life cycle), some of these eggs become lodged and trapped in the intestinal wall. Additionally, as blood flow in the hepatic and mesenteric vasculature travels towards the liver (away from the intestines), many eggs are swept here and become lodged in the capillary sinusoids. The host, recognising the trapped eggs in the intestine and liver as dangerous foreign entities, responds vigorously by inducing a circumoval granulomatous inflammatory reaction composed of eosinophils, T cells, mast cells, B cells, macrophages, giant cells and some neutrophils. The granuloma has been well investigated in the murine model of infection, where research has shown that this structure is influenced by antibodies (Jankovic *et al.*, 1998), chemokines (Park *et al.*, 2001), cytokines (Cheever *et al.*, 1998), adhesion molecules (Ritter and McKerrow, 1996) and apoptosis (Lundy *et al.*, 2001; Rumbley *et al.*, 2001). Thus, because of the constant turnover of cell populations, granulomas change in size during infection. They reach a peak size around 8 weeks post-infection and begin to 'down-modulate' their volume around 12–16 weeks post-infection. B cells and antibodies appear to be integral to the process of down-modulation (Jankovic *et al.*, 1998). Because of their circumoval localisation and dense cellularity, many investigators have shown that granulomas are host-protective. The evidence for this concept comes from infection studies in T cell-depleted mice (Mathew and Boros, 1986), thymectomised mice (Domingo and Warren, 1967; Cheever *et al.*, 1985), SCID mice (Amiri *et al.*, 1992; Cheever *et al.*, 1999) and

nude mice (Cheever *et al.*, 1989). In each of these examples, the host fails to form a well-organised, circumoval granuloma and ultimately dies. Although the exact cause of death is not entirely known, Dunne and Doenhoff have provided evidence which demonstrates that certain *S. mansoni* (not observed in *S. japonicum* or *S. haematobium* infections) egg molecules are highly toxic to liver parenchyma cells (Doenhoff *et al.*, 1981; Dunne *et al.*, 1981; Dunne and Doenhoff, 1983). Therefore, if the host fails to form a mature circumoval granuloma during *S. mansoni* infections, these 'hepatotoxic' egg antigens can leach out into the surrounding parenchyma and induce extensive liver damage. Although some of these egg molecules have been biochemically characterised (Dunne *et al.*, 1991), their molecular identities are currently unknown.

From these early and elegant studies, it was apparent that T cells were important for granuloma formation and host survival. However, some of these studies also demonstrated that host T cell populations were paramount to the schistosome's survival as well (e.g. Doenhoff *et al.*, 1986; Cheever *et al.*, 1999). Specifically, it was illustrated that the absence of host T cells led to a state in which schistosome eggs could not efficiently traverse the intestinal lumen, which ultimately interrupted the continuation of the parasite's life cycle. Therefore, the survival of both host and parasite are intimately related to the generation of a stable and activated T cell population during infection. At the time of these initial experiments, it was unclear which population of T cell was responsible for the observed effects on granuloma inflammation, host survival and parasite egg transmission, although $CD4^+$ and $CD8^+$ T cell populations (either expressing $\alpha/\beta$ T cell receptors or $\gamma/\delta$ T cell receptors) were the most likely candidates. Therefore, a series of seminal studies were undertaken to demonstrate firmly which cell population positively influenced the development of circumoval granulomas as well as host and parasite survival.

Infection of mice deficient for MHC II (Abeta (o)) (Hernandez *et al.*, 1997; Angyalosi *et al.*, 1998), $\alpha/\beta$ T cell receptors (Iacomini *et al.*, 1995) or $CD4^+$ T cells (Fallon *et al.*, 2000a) led to a similar pathological outcome as infected thymectomised, SCID, nude or T cell-depleted mice. This common pathology was characterised by abrogated granuloma inflammation, liver damage and increased mortality rates. A study of $CD4^+$ T cell-deficient humans (HIV co-infected) also revealed the importance of this cell population in mediating *S. mansoni* egg excretion (Karanja *et al.*, 1997). In this study, HIV and schistosome co-infected individuals (in comparison to HIV–, schistosome+ individuals) had a significant defect in egg excretion. This requirement of $CD4^+$ T cells to mediate egg passage across the gut lumen is quite similar to the phenomenon observed in infected SCID mice (Cheever *et al.*, 1999).

The contribution of other T cell populations to granuloma formation was further examined in subsequent studies. Infection of mice deficient for MHC I (TAP knockout mice or $\beta$-2 microglobulin knockout mice) (Hernandez *et al.*,

1997; Yap *et al.*, 1997), γ/δ T cell receptors (Iacomini *et al.*, 1995) or $CD8^+$ T cells (Yap *et al.*, 1997) resulted in the formation of circumoval granulomas similar to infected gene-sufficient or wild-type mice. The results of these studies and others (artificial *in vitro* granuloma formation; Doughty *et al.*, 1987) conclusively demonstrated that α/β $CD4^+$ T cells interacting with MHC class II molecules on antigen-presenting cells are absolutely required for the development of host-protective granulomas and successful, long-term host/parasite survival. Furthermore, these studies additionally demonstrated that γ/δ $CD4^+$ or $CD8^+$ T cells, as well as MHC class I molecules, contribute minimally to granulomatous inflammation during schistosome infection.

## 2.3. Concept of Th1/Th2 Dichotomy

Typically, helminth infections of definitive hosts are associated with high levels of circulating and tissue eosinophilia, enhanced mast cell and basophil functional activity, and elevated antigen-specific and polyclonal $IgE/IgG_4$ (human) or $IgE/IgG_1$ (mouse) antibody production (Bell, 1996). Schistosomiasis is no exception and was the first helminth disease to be used as an effective model to study these common immunological characteristics. However, it was not until the mid-1980s that the true mechanistic complexity of the host's immune responses to helminth infections was starting to be appreciated and unravelled. During this time, Mosman and colleagues discovered functional diverse subsets of murine $CD4^+$ T helper cell clones, the most easily identifiable populations of which were called Th1 and Th2 (Mosmann and Coffman, 1989a, b). These clones were distinguished on the basis of the cytokine profiles they produced upon activation: Th1 clones produced interferon (IFN)-γ, tumour necrosis factor (TNF)-β and interleukin (IL)-2; whereas Th2 clones produced IL-4, IL-5, IL-6, IL-13 and IL-10. Human equivalents of murine Th1 and Th2 clones were identified shortly thereafter (Romagnani, 1991a). The subsequent finding that *in vivo* populations of human Th1/Th2 cells were generated during helminth infection and influenced the severity and magnitude of disease susceptibility and resistance strongly suggested that these cells were not just the result of *in vitro* culturing conditions (Romagnani, 1991b). In fact, these important studies represented a watershed in how most host/parasite interactions and immunological investigations would subsequently be interpreted. For example, all helminth infections (including schistosomiasis) are now currently thought of as Th2-dominated diseases, because of the induction of high $IgE/IgG_4/IgG_1$ antibody levels and elevated eosinophil populations, which are the result of chronic, host Th2 cytokine production (mainly IL-13, IL-4 and IL-5). Subsequently, all currently published works investigating the interaction of a host's immune system with a parasite will undoubtedly be dominated by discussions concerning the

Th1/Th2 dichotomy. $CD4^+$ T-regulatory-cells (producing either IL-10, transforming growth factor beta (TGF-β) or a combination of both IL-10 and TGF-β) have recently been identified and may be important in down-modulating or controlling chronic immune responses on both poles (Papiernik, 2001; Read and Powrie, 2001; Roncarolo *et al.*, 2001; Toms and Powrie, 2001). Although it is generally agreed that Th1 cells play a critical role in cell-mediated immunity against intracellular pathogens such as protozoan parasites, and that Th2 cells help in humoral immune responses against extracellular pathogens such as helminth parasites, these cross-regulating and self-amplifying cell populations clearly have additional functional activities. Many of these activities are still being discovered.

A plethora of work has been published describing the initiation, development, cross-regulation, stability and functions of Th cell populations and the interested reader is directed to these for further information (e.g. Abbas *et al.*, 1996). The typical Th2 immunological cascade induced after schistosome infection has also been the subject of intensive research efforts and years of investigation have led to many insights and advances which are also thoroughly reviewed elsewhere (e.g. Pearce and Reiner, 1995; Wynn and Cheever, 1995). However, as this review discusses the deleterious pathological consequences of developing a highly skewed immunological or imbalanced Th response, some time must be spent re-analysing the events that induce, control and regulate the Th2-dominated immunological reactions that typically occur in most schistosome-infected definitive hosts.

## 3. INDUCTION, REGULATION AND CONSEQUENCES OF HOST-MEDIATED TYPE-2 IMMUNE RESPONSES DURING INFECTION WITH *SCHISTOSOMA MANSONI*

### 3.1. Type-2 Immunological Responses Dominate During Helminth Infections

Helminth infections generally induce the production of Th2 cell populations within the infected host. The developmental events leading to Th2 cell differentiation are extraordinarily complex and still incompletely understood. However, two major theories (instruction and selection) have emerged from existing data that could explain how Th cells differentiate, remain stable and influence the evolving immune responses (reviewed in Murphy *et al.*, 2000). According to the instruction model of Th cell differentiation, naive $CD4^+$ Th0 cells (capable of producing both Th1/Th2 cytokines) are 'instructed' to differentiate into a population of $CD4^+$ Th2 cells upon primary activation in the presence of IL-4 (reviewed in Seder and Paul, 1994). These self-amplifying,

IL-4-instructed, $CD4^+$ Th2 cells, producing cytokines that counter-regulate Th1 cells, will eventually predominate during the infection. In contrast, the selection model proposes that naive $CD4^+$ Th0 cells develop into a population of $CD4^+$ Th2 cells because of the 'selective' outgrowth of precommitted precursors. Evidence exists that the number of cell divisions influences the generation of $CD4^+$ Th2 cells from these precursors (Bird *et al.*, 1998). Currently, there is not enough evidence as yet to argue which model (or another) predominates during helminth infections or even if these models are mutually exclusive. In fact, it is highly likely that both models (and possibly others) influence the development and stabilisation of $CD4^+$ Th2 cell populations from naive $CD4^+$ Th0 cells during helminth infections, albeit probably operating at different times and locations.

Helminth infections drive the differentiation of naive, thymus-selected, $CD4^+$ precursor Th cells ($CD4^+$ Thp cells) into transitory, intermediate $CD4^+$ Th0 cells. $CD4^+$ Th0 cells, capable of producing IL-2, IL-10, IFN-$\gamma$ and IL-4 (mixed Th1/Th2 environment), are now subjected to one or both of the Th differentiation mechanisms described above. According to the instruction theory of Th cell differentiation, IL-4 is the essential cytokine needed to commit precursors down the Th2 pathway during helminth infection. This cytokine exerts its functional activity via binding to the IL-4 receptor (IL-4R) on target cells and signalling through signal transducer and activator of transcription 6 (STAT6) (Takeda *et al.*, 1996). Although this interaction is critical for the maturation, memory, and stabilisation of type-2 immune responses, studies in IL-4R- or STAT6-deficient mice infected with either *Nippostrongylus brasiliensis* or *S. mansoni* have questioned the requirement of IL-4R/STAT6 signalling in the induction of some type-2 immune responses (Jankovic *et al.*, 1999, 2000; Urban *et al.*, 2001). In these studies, infected IL-4R- or STAT6-deficient mice are still capable of producing some parasite-specific type-2 cytokine and antibody responses; however, these responses are much lower than in infected wild-type mice. Furthermore, in a separate study, STAT6 was shown to be necessary and sufficient for IL-4's role in Th2 differentiation and expansion (Zhu *et al.*, 2001). Taken together, these observations imply that type-2 immune response induction proceeds optimally in the presence of IL-4 signalling through the IL-4 receptor and STAT6 pathway. Nevertheless, other mechanisms of type-2 response induction, not yet identified, may also work during helminth infections. These alternative pathways may be influenced by co-stimulatory molecules (King *et al.*, 1996b; Subramanian *et al.*, 1997; Padrid *et al.*, 1998; MacDonald *et al.*, 2002b), dendritic cells (MacDonald *et al.*, 2001; de Jong *et al.*, 2002) parasite molecules (Falcone *et al.*, 1996; Okano *et al.*, 1999; Holland *et al.*, 2000; Whelan *et al.*, 2000; Cuellar *et al.*, 2001; Haisch *et al.*, 2001) or proceed via a default pathway not observed during infections with type-1 immune response-stimulating pathogens (suggested by Abbas *et al.*, 1996).

Pathogen
APCs
(Macs, DCs, B-cells, etc)
CD4+ Thp
IL-2
Antigen/receptor ligation
IL-12
IFN-γ
CD4+ Th0
IL-4
IL-2, IL-10,
IFN- γ, IL-4
?
Pathogen
Mast cells, eosinophils,
basophils, APC,
other cells (CD 4+NK1.1+),
parasite molecules ?
?
IL-4
CD4+ Th1
CD4+ Th2
CD4+ Treg
IL-10
TGF-β
Counter/cross-regulation
IL-12, IL-18, IFN-γ, TNF-α
Classically activated MOs,
NO production,
IgG2 (mouse), IgG1 (human)
IL-4, IL-5, IL-6, IL-13, IL-9, TGF-β, IL-25
Alternatively activated MOs,eosinophilia,
mastocytosis, increase in basophil #s
IgE, IgG1 (mouse), IgG4 (human)
Tc1, Be1, Dc1, Eos1, Mast 1, NK 1, Mac 1
Type 1 polarised response
Intracellular pathogens (viral, protozoan, bacterial)
Tc2, Be2, Dc2, Eos2, Mast 2, NK 2, Mac 2
Type 2 polarised response
Extracellular pathogens (helminths - schistosomes)

Various phenotypically different cell populations have been implicated in providing the early, instructive source of IL-4 during helminth disease (discussed in detail below for schistosomiasis), but scientific support strongly favouring one particular cell population over another is lacking. In addition to the difficulties of identifying which cell populations are producing IL-4, some helminth parasites have evolved clever mimicry strategies. For example, the nematode *Anisakis simplex* is capable of producing and secreting biologically active IL-4-like molecules (Cuellar *et al.*, 2001). What role this 'cytokine mimic' has on the development and regulation of host Th2 immune responses is unclear, but clearly this is an interesting and important area for future investigations. Determining if other parasitic helminths have related cytokine-like molecules and the influence of such molecules on immune function is also an area of developing research interest.

Once Th2 cells are produced, activated and accumulate in sufficient quantities during helminth infections, they are capable of orchestrating the events that lead to and define a type-2 polarised response (Figure 1). Cytokines such as IL-4, IL-5, IL-13, IL-9, TGF-β and IL-6 can be measured during these type-2 polarised responses with each having distinct and overlapping functional

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*Figure 1* The nature of the infective pathogen influences the development of functionally distinct host immune responses: helminth parasites preferentially induce regulated type-2 polarised effector functions. Parasitic infection generally leads to the host producing stable and cross-regulating $CD4^+$ Th subsets. During most intracellular protozoan infections (e.g. *T. gondii*), $CD4^+$ Th1 cells increase in number and co-ordinate effector activities (left-hand side of figure), whereas during extracellular helminth infections, $CD4^+$ Th2 cells orchestrate downstream events (right-hand side of figure). The mechanisms by which these Th subsets become induced, stabilised and counter-regulated during infection are complex and still incompletely characterised. However, during infection with intracellular pathogens, the antigen-presenting cell (APC – macrophages, dendritic cells, B lymphocytes, etc.) is essential for instructing the conversion of $CD4^+$ Th0 into $CD4^+$ Th1 cells via the production of IL-12 (dashed box – left-hand side of figure). Non-B, non-T cells (NBNT) such as mast cells, eosinophils, and basophils participate in an analogous fashion during helminth infection (dashed box – right-hand side of figure), but the parasite molecules that stimulate these cell populations and the exact cellular source of instructive IL-4 are still being investigated (? marks). Autocrine functions (selection model) of $CD4^+$ Th0 cells also participate in the developmental maturation of both Th subsets. $CD4^+$ T regulatory cells ($CD4^+$ Treg) producing IL-10, TGF-β or both may serve an important regulatory role during chronic parasitic persistence and may help to control over-exuberant cellular activites. As Th-mediated immune responses mature, cross-regulatory cytokine biases are readily observed (Th1: IL-12, IL-18, IFN-γ, TNF-α, etc.; Th2: IL-4, IL-5, IL-6, IL-13, IL-9, TGF-β, IL-25, etc.) and are directly associated with the divergent development and functional classification of $CD8^+$ T cells (Tc), B cells (Be), dendritic cells (Dc), eosinophils (Eos), mast cells (Mast), natural killer cells (NK) and macrophages (Mac) (solid boxes in figure). The net accumulation of these cytokine and cellular biases leads to a host response, which eventually becomes polarised either in the type-1 direction (intracellular pathogens) or type-2 direction (extracellular pathogens, e.g. schistosomes).

activities. The presence and role of IL-25 (Fort *et al.*, 2001) in helminth infections has yet to be established. IL-10 seems to not only be secreted by Th2 cells, but also by Th1 cells (and other cells) (Sornasse *et al.*, 1996); therefore, its often cited textbook association with polarised Th2 responses is not completely accurate and needs further revision (Muraille and Leo, 1998). These cytokines can cause numerous physiological effects by directly acting upon target cells expressing complementary receptors. Some of the most common measurable effects of cytokines observed during helminth infections are increases in mature populations and functional activities of accessory cells such as eosinophils, mast cells and basophils. Additionally, these type-2 cytokines can induce the class switching of B cells to produce antibodies of the $IgG_4$ (human), $IgG_1$ (mouse) or IgE (both) isotypes. Type-2-associated immune responses are induced to control the pathogenicity of helminth infections and depend upon appropriate host regulatory mechanisms to limit their potentially destructive nature. Failure to sufficiently induce and control type-2-associated immune responses during helminth infections often leads to the severely detrimental pathological reactions discussed in further detail below. Finally, it must be appreciated that it is not only the Th2 cell population that makes up the type-2 polarised response during helminth infection. The contribution of Tc2 ($CD8^+$ T cells), Be2 (B cells), Eos2 (eosinophils), Mast2 (mast cells), NK2 (natural killer cells), Mac2 (alternatively activated macrophages) and Bas2 (basophils) are often overlooked but are equally important (e.g. Hesse *et al.*, 2001).

### 3.2. Induction of Type-2-associated Immune Responses During *S. mansoni* Infection

How soon after infection can these type-2-associated immune responses be measured, what are the parasite molecules that drive these responses, what are the cellular components of these responses, what activities do the type-2-associated cytokines mediate and how is this type-2 immune response regulated and controlled are some of the questions that schistosome immunobiologists have attempted to answer over the past few decades. Most answers to these questions have come about through investigating schistosome infections in animal models where housing conditions, infectious dose and nutrition can be carefully monitored and controlled. The mouse represents the most extensively studied experimental animal model owing to its fast reproductive nature, the wide availability of transgenic and knockout strains and the susceptible nature of this animal to schistosome infection. In fact, developmental maturation of *S. mansoni* proceeds quite similarly in mouse and man (Armstrong, 1965), although differences in the kinetics and features of immunopathology clearly do exist between these two hosts (Cheever *et al.*, 2000; Fallon, 2000).

In comparison to experimental murine investigations, immunological studies of schistosomiasis in human populations are extremely limited in both study design and outcome measurements. This is mainly owing to ethical constraints, as it is not acceptable to perform longitudinal or invasive studies in infected individuals and, therefore, not permissible to follow the development of chronic morbidity. An additional complication involves setting up field-based laboratories in remote areas of the world, and even when this can be achieved, several factors may potentially confound any relationship between infection status and immune response. Some of these factors include co-infections by other parasites, differences in infection intensity, differences in age and sex, heterogeneous genetic backgrounds and variation in the nutritional status of the study population. However, as schistosomiasis is a major burden on worldwide human populations, it remains imperative to overcome these obstacles and find new ways to make sense of the limited, but very important, information coming from human immunoepidemiological investigations in the field. By combining these data with those from murine experimental studies (and other experimental models), a more thorough understanding of the immunological events induced by schistosome infection will be achieved.

### *3.2.1. Schistosome eggs drive type-2-associated immune responses*

Shortly after Mosman's discovery of functionally diverse subsets of Th cells, Grzych, Pearce and colleagues demonstrated that Th2 cytokines were induced during schistosomiasis at a time consistent with the production of parasite eggs and their deposition into host tissues (Grzych *et al.*, 1991; Pearce *et al.*, 1991). Although the host responded to the developing parasite prior to egg laying, these responses were limited to type-1-associated IFN-γ and IL-2 production. It was only when egg production commenced (~ 5 weeks post-infection) that the authors measured a major boost in the production of type-2-associated cytokines (IL-4 and IL-5) in spleen cells cultured *ex vivo*; importantly, IFN-γ and IL-2 production remained, albeit at lower levels. Even more importantly, high levels of IL-5 and IL-4 (and low levels of IL-2 and IFN-γ) were subsequently found in whole liver and granuloma cell cultures of infected animals also. This finding was subsequently confirmed in additional investigations and most recently by Hayashi *et al.* (1999). Together, these studies formally demonstrated that circumoval granuloma formation during schistosomiasis is associated with the production of type-2 cytokine responses (type-2 granuloma) and is not driven by type-1-mediated delayed-type hypersensitivity reactions (type-1- or tuberculoid-granuloma), as was previously suggested (Boros and Warren, 1970). In addition, these results firmly established that the egg was the major stimulus behind these responses, both systemically (spleen) and locally (liver and granuloma) during schistosomiasis.

This also appears to apply to human infections (e.g. Parra *et al.*, 1992; Williams *et al.*, 1994; El Ridi *et al.*, 1997), although mixed type-1/type-2 anti-egg cytokine responses can be observed in chronically infected individuals (Montenegro *et al.*, 1999).

Additional evidence that confirmed the role of IL-4 in granuloma formation and host type-2 cytokine response induction was presented in the early 1990s when a series of studies investigating the effect of IL-4 depletion in schistosome-infected mice or i.v. egg-injected mice (leads to pulmonary granulomas – pulmonary model) were performed (Yamashita and Boros, 1992; Wynn *et al.*, 1993; Cheever *et al.*, 1994). These related studies all demonstrated that IL-4 depletion had a dramatic effect in reducing egg-induced granuloma inflammation and the production of the type-2 cytokines IL-5 and IL-13. In a related study, administration of recombinant IL-4 to chronically infected animals reversed the down-regulated granulomatous response typically observed in latter stages of infection (Yamashita and Boros, 1992). Moreover, studies in infected IL-4-deficient animals confirmed several of the above findings, although variations among IL-4-deficient mouse genetic backgrounds and experimental procedures produced some divergent results (Pearce *et al.*, 1996). Finally, a careful examination into the kinetics of cytokine production during primary granuloma formation in the pulmonary model thoroughly described the quality and magnitude of the ensuing type-2 dominant response induced by schistosome eggs (Wynn *et al.*, 1993). Together, these findings demonstrated that eggs are the stimulus behind the generation of IL-4, host type-2-mediated immune responses and granulomatous inflammation during infection. Whatever additional roles IL-4 participates in during schistosomiasis, it is clear from the above seminal studies that this cytokine is, in part, responsible for the generation and maintenance of the type-2 immune responses. Important questions arising from this conclusion are how is IL-4 first induced and from which cell population does this cytokine first originate?

### *3.2.2. Possible cell sources of early IL-4 during schistosomiasis*

Because schistosome eggs induce type-2 immune responses during infection, host cells of the innate immune system that probably interact with eggs and their components were examined as possible early sources of IL-4. Williams *et al.* (1993) demonstrated that one such cell population, non-B, non-T cells (NBNT cells) expressing an Fc epsilon receptor, were a major source of spleen-derived, egg-specific IL-4 during murine schistosome infection. The exact identity of these NBNT cells was not certain in this study but basophils or mast cells were the most likely candidates (mouse eosinophils do not express Fc epsilon receptors; de Andres *et al.*, 1997). NBNT cells producing IL-4 were also measured in animals infected with the helminths

*Nippostrongylus brasiliensis* and *Trichinella spiralis* (Conrad *et al.*, 1990; Carman *et al.*, 1992). These studies led investigators to examine carefully the role of NBNT cell populations in providing an early source of antigen-specific IL-4 during schistosome infection. It was postulated that egg/worm antigens could activate NBNT cells to produce IL-4 via cross-linking of IgE molecules and signalling through the Fc epsilon receptor. Indeed, in 1996, Falcone *et al.* demonstrated that egg-stimulated human basophils collected from naive individuals were capable of producing IL-4 (Falcone *et al.*, 1996). This IL-4 response was dependent upon IgE and Fc epsilon interactions, although IgE-independent histamine release from basophils has recently been observed for a recombinantly expressed *S. mansoni* translationally controlled tumour protein ortholog (Rao *et al.*, 2002). Since IL-4 was produced by basophils from non-exposed donors, Falcone *et al.* (1996) concluded that basophils might be responsible for the early source of this cytokine during schistosomiasis. Also during this same year, Sabin *et al.* (1996) identified a pathway of early IL-4 production from eosinophils that was dependent upon mast cell-produced IL-5 in a murine model. However, later studies from these same authors demonstrated that IL-5-deficient and mast cell-deficient mice were still capable of generating a type-2 immune response against schistosome eggs during infection, confounding the role of eosinophils in providing an early source of IL-4 (Brunet *et al.*, 1999b; E.A. Sabin and E.J. Pearce, unpublished data). Nevertheless, a subsequent study confirmed that mouse eosinophils are a significant source of IL-4 and type-2-associated cytokines at later time points during infection (Rumbley *et al.*, 1999). Current and future work in both human studies and animal models will undoubtedly provide additional clues concerning the importance of NBNT cells in the generation of type-2 immune responses.

An interesting recent study provided evidence that Kupffer cells, i.e. macrophages resident in the liver of schistosome-infected mice, could also provide an early source of IL-4 and IL-13 (Hayashi *et al.*, 1999). The authors demonstrated that this Kupffer cell-derived IL-4 (and IL-13) was stimulated by worm antigen, was present 3 weeks after infection (prior to egg deposition) and dramatically increased after egg deposition. How parasite molecules stimulate these hepatic macrophages to produce IL-4 remains uncertain, but clearly these cells have the capacity to manipulate the development of type-2-associated immune responses during schistosomiasis (Hesse *et al.*, 2001). Finally, it must be appreciated that naive T cells, themselves, could contribute to this pool of antigen-specific IL-4 production (Coffman and von der Weid, 1997; Coffman and Reiner, 1999) and, in fact, may be paramount to egg-specific type-2 immune responses occurring in the liver. Complementary to these investigations will be those that define and characterise the components of schistosome eggs that induce the production of IL-4 and type-2-associated immune responses.

### *3.2.3. Egg carbohydrates drive IL-4 production and generation of type-2-associated immune responses during schistosomiasis*

From some of the studies summarised above, it was clear that stable type-2 immune response development during schistosomiasis proceeded at a time concomitant with egg deposition. This led investigators to try and identify/define those egg molecules responsible, with the first clues demonstrating that egg glycoproteins were effective stimulators of granuloma formation in experimental models (Weiss *et al.*, 1987; Jacobs *et al.*, 1999a, b). More recently it was reported that carbohydrates, themselves, on schistosome eggs, play a critical role in the induction of egg-specific type-2 immune responses (Okano *et al.*, 1999). Haisch and colleagues have defined one glycoconjugate of schistosome eggs that drives IL-4 production from human basophils (Haisch *et al.*, 2001). This 'antigen' contains mannose residues attached to a peptide backbone and activates basophils through IgE cross-linking and Fc epsilon receptor signalling. The exact structure of the mannose modification or identity of the protein sequence has yet to be characterised. Okano *et al.* (2001) have also identified a polylactosamine sugar, lacto-*N*-fucopentaose III (LNFPIII) derived from schistosome eggs that can act as a type-2 immune response adjuvant, when coupled to human serum albumin. It also appears that LNFPIII alone can induce B cells from schistosome-infected mice to produce the anti-inflammatory products IL-10 and prostaglandin E2 (Velupillai and Harn, 1994). These anti-inflammatory mediators probably contribute indirectly to the generation of type-2 immune responses by effectively cross-regulating hepatic $CD4^+$ Th1 cells. Additional egg-derived carbohydrate structures that drive type-2-associated immune responses (either directly or indirectly) are currently being identified and studied (Van der Kleij *et al.*, 2002).

### *3.2.4. Antigen-presenting cells and co-stimulatory molecules participate in the induction of egg-specific type-2-associated immune responses*

Macrophages and dendritic cells expressing co-stimulatory molecules have the capacity to potently activate T cells during schistosomiasis (Hernandez *et al.*, 1997; Hayashi *et al.*, 1999; MacDonald *et al.*, 2002b). Kupffer cells, stimulated by worm antigens, can influence the differentiation of type-2 immune responses by secreting IL-4 (Hayashi *et al.*, 1999). Dendritic cells have also been attracting a great deal of attention, specifically regarding their role in T cell differentiation (Banchereau *et al.*, 2000; Moser and Murphy, 2000; Reid *et al.*, 2000). In the case of infectious agents that induce type-1-associated immune responses (*Toxoplasma gondii* and *Leishmania* sp, for example), the functional role of dendritic cells (DC1 cells) has been carefully

investigated (Reis e Sousa *et al.*, 1999). How dendritic cells influence Th differentiation during schistosomiasis is not as clear, but is slowly being unravelled. Current evidence suggests that OX40L, up-regulated on human peripheral blood mononuclear cells (PBMC)-derived dendritic cells primed with schistosome egg antigens, may play an important role in the differentiation of Th2 cells during human schistosomiasis (de Jong *et al.*, 2002). In the murine model, CD40 expression on schistosome egg antigen-pulsed bone marrow dendritic cells ($CD8^-$) and ligation of this co-stimulatory molecule to CD154 on T cells is required for Th2 differentiation (MacDonald *et al.*, 2001). In addition, the signalling pathway facilitated by CD80, CD28 and B7 co-stimulatory molecules also appears to be important for optimal Th2 cell differentiation (King *et al.*, 1996b; Subramanian *et al.*, 1997; Hernandez *et al.*, 1999). Together, these studies demonstrate that antigen-presenting cells and the co-stimulatory molecules they express influence the differentiation and development of Th2 cells in response to stimulation by schistosome egg antigens. The relative importance of this process during schistosome infection in humans and the exact parasite molecules that activate these cells remains to be elucidated.

### *3.2.5. $CD4^+$ T cells and eosinophils sustain egg-stimulated type-2 immune responses during schistosomiasis*

Mast cells, basophils, macrophages, eosinophils and naive T cells all contribute to the induction of type-2-associated immune responses during schistosome infection. However, Vella and Pearce (1992) demonstrated that the maintenance of these responses is highly dependent upon the $CD4^+$ T cell arm. This population of T cells is also critical for the formation of circumoval granulomas. Vella and Pearce used a model of Th2 response induction in which they injected schistosome eggs into the footpads of mice and subsequently measured the *in vitro* parasite-specific cytokine responses of popliteal lymph node cell cultures at 3 and 10 days post-immunisation. By selectively removing $CD4^+$ T cells or $CD8^+$ T cells from the popliteal lymph node populations prior to culturing, they concluded that the type-2 cytokine response (in this model) was almost completely abrogated by $CD4^+$ T cell depletion (Vella and Pearce, 1992). Infected SCID- and $CD4^+$ T cell-depleted mice also fail to generate strong type-2 cytokine responses. These data together with those of Rumbley *et al.* (1998) support the important role of $CD4^+$ T cells in the maintenance of egg-induced type-2 immune responses primed by accessory cells. Recent evidence has also demonstrated that eosinophils are major contributors of type-2 cytokines in the egg-induced granuloma (Rumbley *et al.*, 1999). It seems likely that these two cell populations as well as others all contribute to the continued maintenance of a type-2 cytokine environment in response to egg deposition in the liver (and other tissues) of infected mice.

Secreted egg glycoconjugates
Non-B, non-T cells
Basophils
Mast Cells
IgE receptor
IgE receptor
IL-5
IL-4
IgE receptor (?)
Eosinophils
IL-4
IL-10 (from other sources too)
IL-4
Kupffer Cells/hepatic macrophages
B Cells
Ig receptor (?)
IL-10
PGE2
Th1 Cell
IL-10
egg
Granuloma
egg
Circumoval Fibrosis
IL-4
IL-13
Th0
IL-2, IL-4
Th2 Cells
OX40L
CD40
Dendritic Cells

In summary (Figure 2), existing evidence strongly supports the association of a type-2 immunological response with the development of schistosome granulomas. Glycoconjugated egg antigens have the capacity to induce granuloma formation and drive Th cell differentiation. Various cell populations probably interact with these glycoconjugates and their activation, recruitment and turnover ultimately alters the development and degree of granulomatous immunopathology. These different types of immunopathology are described below with attention given to those severe forms induced by inappropriate cytokine and immune responses.

## 4. DISTINCT FORMS OF SCHISTOSOME-MEDIATED IMMUNOPATHOLOGY CAN BE INDUCED DURING INFECTION

### 4.1. Life-threatening, Egg-induced, Immunopathology does not Develop in most Infected Humans or Experimental Animal Models

In most chronic schistosome infections, where the host is not immunologically compromised, the progression of severe, life-threatening morbidity does not

*Figure 2* The development of tightly regulated circumoval granulomas and tissue fibrosis during *Schistosome mansoni* infection is linked to the development of balanced Th2 cell populations. Many of the eggs laid by sexually mature female schistosomes become deposited in hepatic (photomicrograph illustrated in this figure), intestinal or bladder tissue. Here, the eggs secrete bioactive glycoconjugates that are released into the surrounding milieu and induce a very powerful inflammatory response. This ensuing delayed-type inflammation leads to the formation of circumoval granulomas characterised by the recruitment, maturation and turnover of NBNT cells (basophils, mast cells and eosinophils), B and T lymphocytes, macrophages and dendritic cells. Egg glycoconjugates probably interact with surface IgE receptors on NBNT cells where this receptor/ligand interaction initiates the cellular release of IL-4 and IL-5. Hepatic macrophages and dendritic cells act as powerful antigen (egg glycoconjugate) presenting cells during granuloma formation where they serve as additional reservoirs for IL-4 and IL-10 as well as provide co-stimulation. B lymphocytes are also stimulated by egg glycoconjugates (by an unknown mechanism possibly involving Ig receptor engagement) and this leads to the release of anti-inflammatory IL-10 and prostaglandin E2 ($PGE_2$). The additive contribution of immunological mediators provided by these cell populations (and others mentioned in the text) ultimately leads to an environment rich in Th2 cytokines and favours the preferential expansion of Th2 $CD4^+$ T cells. Fibrotic lesions begin to develop in these tissues as the result of increased pro-fibrotic IL-13 and IL-4 transcription. The inhibition of $CD4^+$ Th1 cell expansion and the prevention of imbalanced $CD4^+$ Th2 cellular responses are, in part, actively tempered by IL-10. Top photomicrograph: haematoxylin- and eosin-stained liver granuloma (original magnification, × 40). Bottom photomicrograph: picrosirius red-stained liver granuloma (original magnification, × 40).

usually occur. However, type-2-associated inflammation induced by the egg does initiate clear histological changes within an infected host. One of these easily measurable histological changes is the development and accumulation of hepatic, periportal and intestinal fibrosis. The development of fibrotic lesions and the immunological mechanisms responsible have been most extensively studied in the murine model of *S. mansoni* infection. Here, it has been hypothesised that these lesions (often found within or near granulomas; see Figure 2) are responsible for maintaining a host-protective barrier that prevents potentially hepatotoxic egg antigens from being released (evidence from SCID mice, etc.). This can't be the whole story as *S. japonicum*-infected SCID mice, which cannot form fibrotic circumoval lesions live just as long as infected wild-type animals that do form fibrotic lesions (Cheever *et al.*, 1999). Although this model implies that hepatotoxic egg antigens are not expressed by *S. japonicum*, it also raises the question: why do fibrotic lesions develop during infection if there is nothing to protect the host from? Mice infected with *S. mansoni* by vaccination with *S. mansoni* eggs/IL-12 also fail to develop fibrotic lesions around hepatic eggs, yet live despite the presence and release of hepatotoxic antigens (Wynn *et al.*, 1995). It is argued in both of the above studies (and others not mentioned here) that the development of collagen-containing fibrotic lesions around deposited eggs probably occurs as an inevitable consequence of the type-2 granulomatous response. This function requires the action of profibrotic cytokines induced during the response. It could also be debated that the balanced, inflammatory type-2 immune response (leading to fibrotic lesions) might be an evolutionary adaptation, mutually induced by both host and parasite to ensure that minimal host mortality coincides with maximal egg transmission. Finally, the fibrotic lesions may serve a secondary function in preventing the escape of hepatotoxic egg antigens (although host antibodies are also capable of protecting from hepatocellular damage; Dunne *et al.*, 1991) that is not necessary for the survival of all schistosome-infected hosts.

The development of fibrotic lesions during schistosomiasis has, thus far, been directly attributable to the activities of the pro-fibrotic mediators IL-4, IL-13 and TGF-β. Studies published during the late 1990s and shortly after the turn of the millennium conclusively demonstrated that IL-13 was the most important cytokine directly responsible for this tissue fibrosis during murine schistosomiasis (Chiaramonte *et al.*, 1999; McKenzie *et al.*, 1999). Chiaramonte *et al.* (1999) further provided evidence for IL-13's mechanism of action by showing that this cytokine directly stimulates *in vitro* cultured fibroblasts to produce the building blocks of fibrosis, i.e. collagen. Therefore, it is likely that IL-13, acting through the IL-4 receptor (Jankovic *et al.*, 1999) and STAT6 (Kaplan *et al.*, 1998) signalling pathway directly stimulates hepatic-resident fibroblasts (developmentally mature ITO cells/myofibroblasts) or recruited via chemotaxis (Wyler and Postlethwaite, 1983) circulating fibrocytes (Bucala *et al.*, 1994) to produce collagen. The mechanism of collagen

production in these cells may involve the TGF-β pathway (Czaja *et al.*, 1989; Farah *et al.*, 2000; Lee *et al.*, 2001) and rely upon the production of proline molecules supplied by alternatively activated macrophages (Hesse *et al.*, 2001). Although it is difficult to measure the mechanisms leading to fibroblast activation during human schistosomiasis, a similar pathway of collagen production leading to periportal and hepatic fibrosis presumably occurs.

In summary, the development of fibrotic lesions in combination with circumoval granulomas is likely to be an inevitable consequence of generating type-2 immune responses against tissue-trapped schistosome eggs. This host response ultimately leads to a disease state characterised by chronic morbidity but, most importantly, seldom induces the detrimental cascade of events leading to death. Investigating the immunological participants and inappropriate mechanisms associated with enhanced morbidity and mortality rates during schistosomiasis will help our understanding of appropriate strategies induced by the host to control chronic type-2-associated immune responses.

## 4.2. Development of Severe Immunopathology During Schistosomiasis is Linked to IL-10 and Associated with Inappropriate, Highly Skewed or Uncontrollable Immune Responses

### *4.2.1. Unregulated, polarised type-1-associated immune responses during schistosomiasis lead to increased mortality*

The host response to schistosome eggs should be one that recognises the destructive nature of these agents and responds appropriately in a controlled manner to limit severe pathology. Indeed, most experimental infections of animals and natural infections of humans are associated with the development of tightly regulated type-2 immune responses directed against the egg. As mentioned above, these responses can lead to fibrotic tissue lesions, but generally do not contribute to or result in mortality. Deviation away from this tightly regulated, egg-associated, type-2 immune response leads to pathologies that are severe and often life-threatening.

One of the first reports of atypical immunopathology leading to schistosome-induced death was made in the mid-1990s when schistosome infection studies were being performed in IL-4-deficient mice (Brunet *et al.*, 1997). Brunet *et al.* showed that IL-4-deficient mice responded to schistosome infection by generating strongly polarised type-1, as opposed to type-2 inflammatory reactions. This type-1 inflammatory response reported by Brunet *et al.* was observed shortly after egg deposition and was defined by egg-specific IFN-γ, TNF-α, and inducible nitric oxide (iNO) production as well as a rapid rate of weight loss (Brunet *et al.*, 1997). The granulomas that formed around tissue-deposited eggs in IL-4-deficient animals were of a similar size to

control, infected wild-type mice but hepatic fibrosis was diminished. The authors concluded from these studies that the pronounced type-1-mediated inflammatory reactions that developed in the absence of IL-4 in combination with increased systemic endotoxin levels induced a state of cachexia that led to rapid mortality (Brunet *et al.*, 1997). Subsequently, work from the same laboratory demonstrated that this rapid mortality (in the absence of IL-4) was IL-12 and IFN-γ independent (La Flamme *et al.*, 2001b; Patton *et al.*, 2001), associated with inducible nitric oxide synthase (iNOS) dysregulation (Brunet *et al.*, 1999a) and linked to damaging peroxynitrite production (La Flamme *et al.*, 2001a). However, IL-4-deficient mouse infection studies performed in other laboratories (Metwali *et al.*, 1996) or on a different genetic background (Pearce *et al.*, 1996) did not result in a strong default towards the type-1 direction or enhanced mortality.

It is important to note that cachexia and elevated death rates observed in IL-4-deficient mice are directly related to infection intensity. Decreasing the infectious dose will induce fluctuating weight loss in IL-4-deficient mice but will not increase mortality in comparison to infected WT animals (Metwali *et al.*, 1996; Brunet *et al.*, 1997; Hoffmann *et al.*, 2000). Together, these important investigations in infected IL-4-deficient mice provided the first real evidence that deviation away from properly regulated type-2 immune responses during schistosomiasis could be lethal. Immunological factors, in addition to IL-4, are now being investigated in order to ascertain their protective role during schistosomiasis.

An interesting finding revealed during Brunet and colleagues' studies in infected IL-4-deficient mice was in the level of IL-10 produced from restimulated spleen cells *ex vivo* (Brunet *et al.*, 1997). Infected IL-4-deficient animals, suffering from increased mortality rates, had significantly less antigen-specific IL-10 production in comparison to control infected animals. Could a decrease in IL-10 production be responsible for the increased immunopathology observed in IL-4-deficient animals? Shortly after IL-10 was identified (Mosmann *et al.*, 1990) as a soluble factor responsible for inhibition of cytokine synthesis by Th1 cells, Sher *et al.* (1991) investigated the kinetics of spleen-derived IL-10 production during schistosomiasis.

Sher *et al.* (1991) demonstrated that IL-10 production was rapidly produced at a time concomitant with egg deposition and, furthermore, that this cytokine remained elevated into the chronic stages of disease. A conclusion from this study and of others (Flores-Villanueva *et al.*, 1996; Reiser and Stadecker, 1996) was that IL-10 counter-regulated, or made anergic, inflammatory Th1 cell populations and allowed for the development of stable, egg-induced, type-2 immune responses. This finding, in addition to defective IL-10 production during the study by Brunet *et al.* (1997) of IL-4-deficient mice, led investigators to believe that IL-10 was induced as a host-protective cytokine during schistosomiasis. Defects in IL-10 production during

schistosomiasis were, therefore, hypothesised to correlate with a host's increased risk of developing severe disease. A series of important studies, in both mouse and man, confirmed this hypothesis and conclusively demonstrated the important regulatory role of IL-10 during schistosomiasis.

Bosshardt *et al.* (1997) investigated *S. mansoni* infection in CBA/J mice. This mouse model is actually quite unique in that 80% of infected animals develop a moderate disease state after infection (moderate splenomegaly or MSS) whereas the remaining 20% go on to develop severe hepatosplenomegaly (HSS). These percentages of moderate to severe disease states seen in infected CBA/J mice are reminiscent of the human situation (larger percentage of infected humans suffering from moderate disease than severe disease states). The important finding from the studies of Bosshardt *et al.* (1997) was that the MSS mice produced significantly more antigen-specific IL-10 than the HSS mice especially during chronic stages of disease. Schistosome-specific cross-reactive idiotypes (Id) appear to participate in the modulation of these murine pathologies, although the timing of Id exposure seems to be critical (Montesano *et al.*, 1999, 2002). Together, these data support the contention that deficiencies in IL-10 production and Id regulation during schistosomiasis are indeed related to increases in the severity of host immunopathology. Mechanisms that regulate IL-10 production, such as co-stimulation during antigen presentation, are being investigated in order to ascertain their role in the development of lethal immunopathology (King *et al.*, 1996b; Hernandez *et al.*, 1999; MacDonald *et al.*, 2002a).

Another role of IL-10 in preventing severe disease was subsequently confirmed in a murine study where IL-10 administration to infected animals dramatically decreased the size of circumoval granulomas during the acute stages of disease (Flores-Villanueva *et al.*, 1996). Later, Wynn *et al.* (1998) demonstrated that infected IL-10-deficient mice displayed significantly larger circumoval granulomas than control-infected WT animals, thereby confirming the anti-inflammatory role of this cytokine during acute schistosomiasis. Finally, a detailed examination of the immunological characteristics in these infected IL-10-deficient mice demonstrated that they develop increases in both type-1 and type-2 immune responses and do indeed suffer enhanced mortality (Wynn *et al.*, 1998). Together, these studies provided convincing evidence that increased disease states develop in the absence of IL-10 during murine infections, and suggest that IL-10 induction is essential for the development of controlled and regulated type-2 immune responses during schistosomiasis. Is this the case for human schistosomiasis?

A recent study has elegantly confirmed the role of IL-10 in urinary tract morbidity during *S. haematobium* infection (King *et al.*, 2001). In this investigation, the authors measured IL-10 (and TNF-$\alpha$) release from egg-stimulated PBMC cultures obtained from infected children and adolescents suffering from moderate to severe bladder wall pathology. In a comparison with age- and infection

intensity-matched controls, the authors reported a significantly lower ratio of egg-specific IL-10/TNF-α production from PBMC cultures obtained from the patients with severe bladder wall pathology. This data suggests that low IL-10 and high TNF-α correlates with an increased risk of developing severe disease during schistosomiasis (see also Brunet *et al.*, 1997; Hoffmann *et al.*, 2000; Bosshardt *et al*, 1997). Furthermore, a recent study, examining the correlates of developing severe fibrosis in adult male populations living in Uganda who were chronically infected with *S. mansoni,* demonstrated that a deficiency in IL-10 was partly responsible (M. Booth and D. W. Dunne, personal communication). Therefore, these studies, in both mouse and man, have now demonstrated that IL-10 regulates the development of severe pathology during schistosomiasis. This cytokine presumably exerts its activity by inducing a state of temporary hyporesponsiveness or reversible anergy during infection and, therefore, prevents the development of severe pathological reactions caused by exaggerated or uncontrollable immune hyperactivity (King *et al.*, 1996a; Malaquias *et al.*, 1997; Montenegro *et al.*, 1998). Hyporesponsiveness associated with IL-10 has also been observed for other helminth infections (Mahanty *et al.*, 1997) suggesting a commonly employed host regulatory strategy induced during chronic helminth infections. Interestingly, a beneficial side effect of prolonged, helminth-induced, IL-10 production in chronically infected individuals (not normally observed in people from developed countries) is the ability of this cytokine to suppress atopy (van den Biggelaar *et al.*, 2000). The role of other IL-10 family members (Fickenscher *et al.*, 2002) during schistosomiasis remains to be determined

There has been extensive debate concerning the 'protective versus pathological' role of type-2 immunological responses during helminth infection (e.g. Abbas *et al.*, 1996; Finkelman and Urban, 2001). This debate stems from several findings in which severe immunopathology is induced when the infected host inappropriately responds to infection with a type-1 polarised immune response. High-dose infection of IL-4-deficient mice (Brunet *et al.*, 1997), infection of IL-10/IL-4 doubly deficient mice (Hoffmann *et al.*, 2000), infection of mice made tolerant to egg antigens (Fallon and Dunne, 1999), immunisation of mice with egg antigens and complete Freund's adjuvant (Rutitzky *et al.*, 2001), infection of $CD4^+$ T cell-depleted mice (Fallon *et al.*, 2000a) and co-infection of *S. mansoni*-infected mice with *Toxoplasma gondii* (Marshall *et al.*, 1999) all lead to increased mortality and polarised type-1 immune responses against the schistosome and its egg. In each of these experimental models, the host deviates away from controlled and regulated Th2–associated immune responses. The pathologies that develop under these conditions are quite severe and range from extensive liver damage owing to impaired circumoval granuloma formation, cachexia, exaggerated iNOS activity, detectable serum IFN-γ and TNF-α, to eventual death. Increases in hepatic neutrophil and macrophage recruitment and activation also correlate with

severe pathology (Hoffmann *et al.*, 2001). Interestingly, when measured, all of the above-described atypical pathologies were also associated with a defect in IL-10 production. Therefore, it is not just the development of polarised type-1 associated immune responses that predisposes an infected host to enhanced morbidity and mortality. These immune responses must also be associated with a deficiency in IL-10 production. This may explain why egg/IL-12-immunised mice (Wynn *et al.*, 1995) or some IL-4-deficient mice (Chiaramonte *et al.*, 1999), which develop type-1 immune responses after *S. mansoni* infection, do not die. In both of the models described above, antigen-specific IL-10 is continuously produced during infection, which counteracts the potentially lethal pathology of an overwhelming type-1 dominant immune response. Egg/IL-12-immunised IL-10-deficient mice quickly succumb to lethal pathological reactions after infection (T.A. Wynn and M. Hesse, personal communications). However, it is important to note that none of these atypical murine pathologies, associated with type-1 immune polarisation, are homologous to morbidity measurements described for infected humans (Cheever *et al.*, 2000).

Nevertheless, there is evidence from at least one study of hepatosplenic patients that indicates increases in type-1 immune responses do correlate with a unique form of severe hepatosplenic disease in human populations (Mwatha *et al.*, 1998). In this study, the authors described immunological responses in groups of *S. mansoni*-infected children and adolescents, with and without hepatosplenomegaly. Importantly, there was little or no apparent periportal or hepatic fibrosis (clinical and ultrasound measurements, unpublished observations) in either study group. Both of these groups of individuals were carefully matched for age, prevalence and intensity of infection and originated from the same ethnic tribe (Akamba people). Therefore, the purpose of this carefully controlled study was to identify the immunological differences between patients displaying severe hepatosplenomegaly (minimally related to fibrosis) and those who did not. A striking finding uncovered in this investigation was that those children/adolescents who suffered severe enlargement of liver and spleen (assessed by palpation) had significantly more type-1-associated IFN-$\alpha$ and TNF production from antigen-stimulated PBMCs in comparison to controls. Additionally, high plasma levels of sTNFR-I, sTNFR-II and ICAM-1 were all significantly associated with hepatosplenomegaly. Interestingly, the type-2-associated cytokine IL-5 was negatively associated with hepatosplenomegaly. IL-10 production was not assayed in this study. These observations indicate that hepatosplenomegaly, in the absence of severe fibrosis, can be associated with elevated type-1-associated immune responses under certain conditions. Other possible explanations for these differences in hepatosplenomegaly, such as environmental factors and co-infections with other pathogens (Fulford *et al.*, 1991), are currently being examined.

Another recent study of acute schistosomiasis also reported the association

of elevated type-1 cytokine production to severe disease (de Jesus *et al.*, 2002). In this study, the authors studied PBMC cytokine responses from 31 Brazilians who had recently acquired a *S. mansoni* infection. Blood was drawn from these individuals shortly after infection and prior to egg deposition so that the immune correlates of toxaemic (Gazzinelli *et al.*, 1985; Lambertucci *et al.*, 2000), acute schistosomiasis could be compared to those immune responses associated with chronic schistosomiasis (de Jesus *et al.*, 2002). Whereas chronic schistosomiasis was characterised by worm-specific IL-5 production, acute disease was associated with worm-specific IFN-γ responses. Additionally, significant elevations in the pro-inflammatory cytokines IL-6, IL-1 and TNF-α were only observed in PBMC cultures stimulated with worm antigens collected from acute schistosome-infected patients. In a similar investigation, Montenegro *et al.* (1999) also found dominant Th1 responses in patients with acute schistosomiasis.

Evidence is mounting from studies like these that shows inappropriate induction of polarised type-1 immune responses (in the absence of IL-10) at any stage during schistosomiasis can lead to severe pathological consequences. The effect of type-1 cytokine and antibody responses on the development of immunopathology during other helminth infections has also recently started to attract attention. For example, *Trichuris muris* infection of IL-10-deficient or IL-10/IL-4-deficient mice, where polarised type-1-associated immune responses develop, leads to devastating pathological changes and 100% mortality (Schopf *et al.*, 2002). While the above example illustrates that type-1-mediated immunopathology can occur during infection with helminths other than schistosomes, the mechanism by which infected hosts deviate away from a controlled Th2 response towards Th1-dominant reactions remains an important area of research. As demonstrated by several studies, co-infection with other pathogens clearly manipulates the host immune responses to helminths (Fulford *et al.*, 1991; Curry *et al.*, 1995; Marshall *et al.*, 1999; Mwinzi *et al.*, 2001) and may be partly responsible for this immune imbalance. Areas of the world where schistosome infection is endemic are also hot-spots for the transmission of geohelminths and there is a clear potential for interactions among parasites occupying overlapping niches (Booth *et al.*, 1998). Additionally, viral, bacterial and protozoan co-infections may affect maintenance of type-2-associated immune responses to helminths and the development of immunopathology. Environmental and social factors may also alter the development, maintenance and stability of helminth-induced type-2 immune responses. Host factors such as age (Smith *et al.*, 2001) and genetics may also play influential roles. Investigations into these topics should provide additional insight into the mechanisms responsible for inappropriate type-1 immune response development during schistosomiasis.

### *4.2.2. Unregulated, polarised type-2-associated immune responses during schistosomiasis also lead to elevated mortality and morbidity*

From the above discussions, it is clear that uncontrolled, polarised type-1 immune responses against schistosome parasites lead to increases in host immunopathology. Although it is argued that induction of chronic type-2-associated immune responses during schistosomiasis serves a 'host-protective' role, recent data seriously question this often-stated, but over-simplified, statement. Specifically, what happens when the host fails to control the chronic type-2 immune response during schistosome infection? Alternatively, what happens if a host responds too vigorously, without proper control or temperament, in the direction of exacerbated type-2 immune responses during schistosomiasis?

One of the first clues demonstrating that not all schistosome-mediated, type-2-associated immune responses were host-protective was obtained in an experimental animal model using several different mouse knockout strains. In this investigation, Hoffmann *et al.* (2000) demonstrated that two distinct, but equally lethal, *S. mansoni*-mediated immunopathologies could develop in the context of both type-1 and type-2 polarised cytokine and antibody responses. The first part of this study confirmed previous murine investigations and demonstrated that death increases when the host develops inappropriate polarised type-1 immune responses against parasite antigens. Hoffmann *et al.* (2000) also provided convincing evidence in their study demonstrating that the type-1 induced mortality occurs only in the absence of IL-10. Indeed, in this study, infected IL-4-deficient mice (displaying type-1 immune responses) lived just as long as infected WT animals (both groups of animals made similar levels of IL-10), whereas animals deficient in both IL-10 and IL-4 (IL-10/IL-4 doubly deficient mice) suffered 100% mortality by week 8 post-infection. Rapid cachexia, abundantly produced IFN-$\gamma$ and TNF-$\alpha$, elevated hepatotoxicity, minimal fibrosis and increased splenic iNO production were all easily detectable in the IL-10/IL-4 doubly deficient, type-1 polarised mice. Hoffmann *et al.* (2000) also reported results in this study suggesting that extremely polarised type-2 immune responses induced during schistosomiasis were also highly detrimental to the host.

Infected animals deficient in both IL-10 and IL-12, compared to WT mice, produced 10 times the amount of antigen-specific type-2-associated IL-4, IL-5 and IL-13 during the acute stages of schistosomiasis. All of the type-2 polarised, IL-10/IL-12 doubly deficient animals also lost weight at the onset of schistosome oviposition (~ 5 weeks post-infection) and over 50% succumbed to death at 12 weeks post-infection. The increased mortality was directly related to overproduction of the pro-fibrotic cytokines IL-4 (Kovacs, 1991) and IL-13 (Chiaramonte *et al.*, 1999) as well as to diminished levels of the anti-fibrotic cytokine, IFN-$\gamma$ (Czaja *et al.*, 1993). Therefore, this increased ratio of

pro-/anti-fibrotic mediators resulted in the deposition of significantly more hepatic collagen in the livers of infected IL-10/IL-12 doubly deficient mice and ultimately death, in a high proportion of animals. The type-2-associated death, during the chronic stages of infection, was presumably due to rupturing of gut varices caused by fibrosis-induced hypertension and increased portal vein pressure as evidenced by pools of blood found in the caecum and intestinal cavities at autopsy (Hoffmann *et al.*, 2000, 2001). A definitive link between the development of unregulated type-2 immune responses and severe, fibrosis-related immunopathology contributing to enhanced mortality rates during schistosomiasis was thereby established in this investigation.

A more recent study using cDNA microarray expression profiling provided additional evidence that type-2-associated immune responses actively induced the transcription of several genes involved in the fibrotic machinery (Hoffmann *et al.*, 2001). The contribution of these genes in hepatic fibrosis-related immunopathology seems probable, as they were preferentially induced only in the infected IL-10/IL-12 animals. The type-1 polarised, IL-10/IL-4 doubly deficient animals (minimal development of fibrosis) displayed minimal induction of these genes. Interestingly, the IL-10/IL-12 doubly deficient animals also had high detectable levels of systemic TNF-α as well as increased recruitment of hepatic neutrophils (also observed in infected type-1 skewed, IL-10/IL-4 doubly deficient mice). Both TNF-α and activated neutrophils probably affected the development of severe fibrosis-related immunopathology (Hernandez-Pando and Rook, 1994; Louis *et al.*, 1998). Therefore, the studies by Hoffmann *et al.* (2000, 2001) suggest that while balanced Th2 responses are clearly host protective, when uncontrolled, these responses can also cause significant damage to the host. In fact, these reports formally demonstrate that generation of highly skewed or uncontrolled type-2 polarised responses are as pathogenic as the generation of type-1 immune responses.

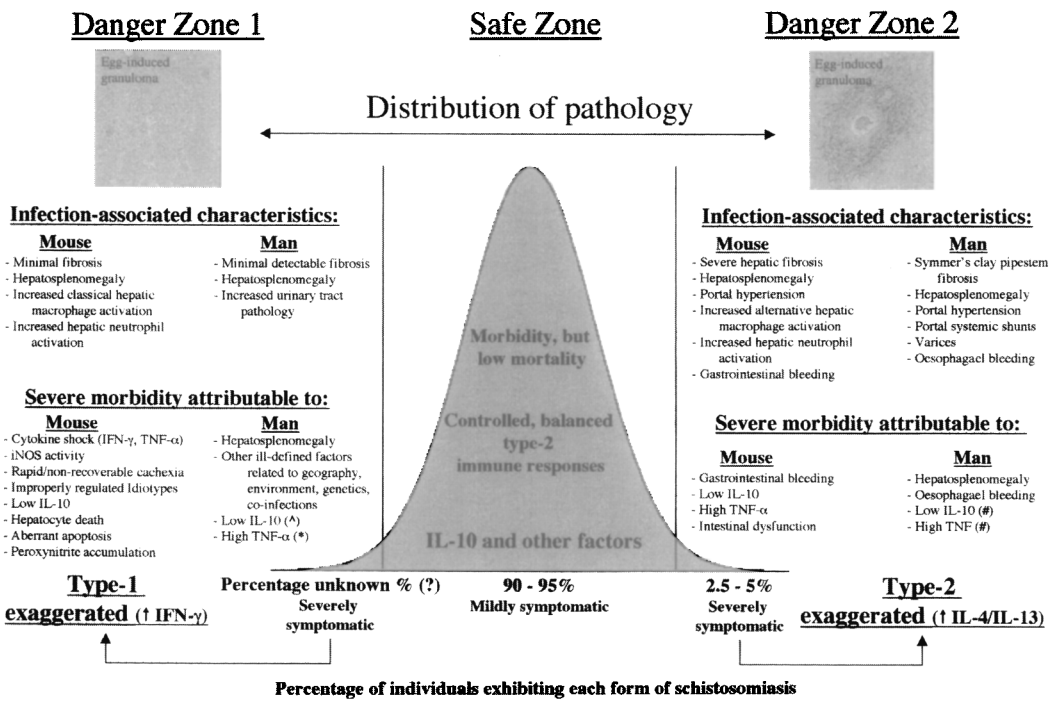
Additional evidence that supports the pathogenic role of uncontrollable type-2 immune responses during murine schistosomiasis was recently reported (Fallon *et al.*, 2000b). Here, the authors infected a transgenic mouse strain that constitutively expresses IL-9 with *S. mansoni*. Although no differences in parasitological burdens or fecundity were measured in this study, the infected transgenic mice developed enhanced type-2 immune responses and suffered higher mortality rates in comparison to control infected WT mice. Unlike the fibrosis-related pathology observed in infected IL-10/IL-12 doubly deficient animals, the infected IL-9 transgenic mice produced normal (equivalent to controls) levels of hepatic fibrosis. Here, the cause of death was attributable to enteropathy. Therefore, in the murine model of schistosomiasis, unregulated type-2 immune responses can lead to severe pathological consequences related to overexpression and action of profibrotic cytokines and/or factors that induce intestinal dysfunction. Is there evidence that

unregulated type-2-associated immune responses induce severe pathology in infected human populations?

In the setting of chronic type-2 immunological responses, the vast majority of all schistosome-infected humans do not develop severe hepatic morbidity, but a minority (2.5–5%) of individuals will evolve quite severe hepatosplenomegaly and immunopathology related to fibrosis. Specifically, these hepatosplenic, fibrotic individuals develop disease states that can include hepatic and periportal fibrosis, which leads to portal hypertension, portal-systemic shunting of blood vessels around the liver, oesophageal varices, abdominal ascites and haematemesis. Hepatosplenomegaly, associated with this tissue fibrosis, develops as a result of increased hepatic portal and splenic vein pressures, reticuloendothelial hyperplasia and hepatic inflammation due to circumoval granulomas. These severely ill individuals are not necessarily the most heavily infected (peak levels of infection occur around puberty), but have usually harboured the disease for the longest period of time. Ultrasound measurements have confirmed that the progression of hepatic and periportal fibrosis develops with age. This has been well documented in *S. japonicum*-infected patients where more severe fibrosis (Grade 3) was observed in older individuals as compared to young individuals (Grade 1) (Olds *et al.*, 1996). Our cross-sectional studies in Uganda and other investigators' studies in *S. mansoni* endemic areas confirm this age/fibrosis relationship. These findings seem to suggest that the individuals who go on to develop severe fibrotic-related hepatosplenomegaly later in life are a subgroup of the infected population that fail to resolve their initial low-grade hepatic lesions. Why?

It is possible that these people have defects in their ability to counter-regulate the detrimental effects of prolonged production of IL-4, IL-13 and other type-2-associated immune responses. This would certainly increase the risk of developing health complications due to fibrosis. Existing evidence from human genetic segregation analyses conducted in Sudan suggests that this is a very likely hypothesis. Dessein and colleagues have elegantly demonstrated a link between a major gene mapped to human chromosome 6q22–q23 and to those individuals who suffer from hepatosplenomegaly related to severe hepatic and periportal fibrosis (Dessein *et al.*, 1999). A candidate gene mapped close to this chromosomal location is one that codes for the IFN-γ receptor alpha chain. From this study, it is easy to construct a hypothesis that links defects in IFN-γ signalling to the people suffering from severe fibrosis. As IFN-γ is a potent anti-fibrotic cytokine (Czaja *et al.*, 1993), segregation of 6q22–q23 among severely fibrotic individuals suggests that these infected people cannot counter-regulate the pro-fibrotic activities of IL-4 and IL-13.

A more recent study examining immune correlates related to immunopathology in chronically infected Ugandan populations has provided firm evidence linking high IL-4 and IL-13 levels with those adult male and


Danger Zone 1
Safe Zone
Danger Zone 2
Egg-induced granuloma
Distribution of pathology
Egg-induced granuloma
Infection-associated characteristics:
Mouse
- Minimal fibrosis
- Hepatosplenomegaly
- Increased classical hepatic macrophage activation
- Increased hepatic neutrophil activation
Man
- Minimal detectable fibrosis
- Hepatosplenomegaly
- Increased urinary tract pathology
Severe morbidity attributable to:
Mouse
- Cytokine shock (IFN-γ, TNF-α)
- iNOS activity
- Rapid/non-recoverable cachexia
- Improperly regulated Idiotypes
- Low IL-10
- Hepatocyte death
- Aberrant apoptosis
- Peroxynitrite accumulation
Man
- Hepatosplenomegaly
- Other ill-defined factors related to geography, environment, genetics, co-infections
- Low IL-10 (^)
- High TNF-α (*)
Morbidity, but low mortality
Controlled, balanced type-2 immune responses
IL-10 and other factors
Infection-associated characteristics:
Mouse
- Severe hepatic fibrosis
- Hepatosplenomegaly
- Portal hypertension
- Increased alternative hepatic macrophage activation
- Increased hepatic neutrophil activation
- Gastrointestinal bleeding
Man
- Symmer's clay pipestem fibrosis
- Hepatosplenomegaly
- Portal hypertension
- Portal systemic shunts
- Varices
- Oesophagael bleeding
Severe morbidity attributable to:
Mouse
- Gastrointestinal bleeding
- Low IL-10
- High TNF-α
- Intestinal dysfunction
Man
- Hepatosplenomegaly
- Oesophagael bleeding
- Low IL-10 (#)
- High TNF (#)
Type-1 exaggerated (↑ IFN-γ)
Percentage unknown % (?)
Severely symptomatic
90 - 95%
Mildly symptomatic
2.5 - 5%
Severely symptomatic
Type-2 exaggerated (↑ IL-4/IL-13)
Percentage of individuals exhibiting each form of schistosomiasis

female individuals, respectively, suffering from severe fibrosis (M. Booth and D.W. Dunne, personal communication). Data from murine models also demonstrate that without proper counter-regulation mediated by IFN-γ, alternatively activated macrophages (stimulated by IL-4 and IL-13) producing collagen precursors (proline) can contribute to the process of fibrosis (Hesse *et al.*, 2001). The counter-regulatory role of IFN-γ has most recently been alluded to in the human setting (Henri *et al.*, 2002). These data, therefore, suggest a probable mechanism by which severe fibrosis can occur in chronically infected human populations. The combination of defective IFN-γ signalling with improperly balanced type-2 cytokine regulation leads to an environment suitable for collagen synthesis, hepatic and periportal fibrosis, and elevated morbidity and mortality rates. From existing evidence, this type of immunopathology becomes manifested fully only after years of exposure to schistosome parasites and/or abnormal host responses, which may be more often observed during the chronic stages of disease. It is envisaged that additional investigations into the immunopathology of chronically infected individuals living in endemic areas will provide novel genetic and environmental links associated with the development of severe hepatosplenomegaly related to fibrosis. Experimental animal models will also continue to be invaluable in our understanding of the processes leading to dysfunctional immune response regulation resulting in the accumulation of fibrosis-related pathology.

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*Figure 3* Successful, long-term host/schistosome interexistence depends upon the induction and maintenance of controlled/balanced type-2 immune responses: increased morbidity and enhanced mortality strongly associate with immune polarised/exaggerated states. As described in this review, the vast majority of schistosome-infected individuals living in endemic areas generally harbour disease states that are mildly symptomatic and are often not associated with life-threatening conditions (safe zone: 90–95% of all infected individuals). Although these individuals present with some morbidity, they are able to control properly chronic type-2-associated immune responses and, therefore, seldom succumb to infection-induced death. Studies in both animal models and man have demonstrated that the induction and maintenance of IL-10 during schistosomiasis is critical for suppressing the development of severe egg-associated pathologies, although other immunological factors are certain to participate. Current evidence suggests that individuals presenting with severely symptomatic forms of schistosomiasis fall into two major subgroups: those that fail to control the downstream effects of chronic type-2-associated immune responses (danger zone 2: approximately 2.5–5% of infected individuals) or those that develop egg-associated immune responses that are type-1 in nature (danger zone 1: percentage uncertain). Infection-associated characteristics and factors that probably contribute to severe morbidity in diverse mouse models and man are summarised here (photomicrograph of liver granuloma: picrosirius red stain, × 40), ^, observations obtained in *S. haematobium*-infected humans; *, observations obtained in *S. haematobium*- or *S. mansoni*-infected humans; #, observations obtained in *S. mansoni*-infected humans (M. Booth and D.W. Dunne, unpublished data and Henri *et al.*, 2002).

## 5. CONCLUSIONS

In the vast majority of infected individuals, schistosomes and their eggs generally induce a tightly regulated and controlled host inflammatory response characterised by the production of type-2-associated cytokines and antibodies directed against parasite antigens. Individuals who develop this type of immune response maintain chronic infections that often present with mild symptoms and morbidity but do not correlate with life-threatening immunopathologies. Improper host regulatory or control mechanisms may induce a shift in this response that instigates increased morbidity and mortality rates among infected individuals. The majority of these improperly controlled host mechanisms are caused by inappropriate regulation of cytokines and their effector mechanisms. Evidence points to extreme immune response polarisation as a major contributing factor to some of the most lethal schistosome-associated immunopathologies. As illustrated here, unchecked Th1- and Th2-mediated immune responses against schistosome parasites can lead to devastating consequences (Figure 3). Therefore, as elegantly summarised and perhaps even understated by Rhodes and Graham, when discussing *Onchocerca* infection of cattle, 'a prolonged cytokine bias, indicating a lack of immunological control, is not desirable' (Rhodes and Graham, 2002). In terms of schistosomiasis, our review has expanded upon this topic and offered evidence that suggests prolonged cytokine biases are not only undesirable, but are, in fact, also lethal. Studying the development of severe immunopathology in other helminth infections will determine if this is a widespread and common phenomenon.

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