# Impact Assessment of Non-Native Parasites in Freshwater Fisheries in England and Wales

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by

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## Declaration

I declare that this thesis has been compiled by myself, and is the result of my own investigations. It has not been submitted for any other degree and all sources of information have been duly acknowledged.

Chris Williams

**Abstract**

Non-native parasites pose a significant threat to aquatic bio-diversity and fishery development. Many factors have facilitated the introduction of non-native parasites into England and Wales. Identifying the effects of these parasites and the importance of any changes to wild fish populations represents a considerable challenge. However, in order for the Environment Agency to identify future disease threats, effectively manage resources and implement practical and sustainable control measures, the risks posed by these parasites to fisheries must be better understood.

A structured, risk-assessment process for non-native freshwater parasites is proposed. This approach includes procedures for hazard identification, impact assessment, risk management and communication. A quantitative approach to hazard identification provides an initial prediction of impact at the time of introduction to inform decision-making and guide preliminary control measures. This is followed by a more comprehensive framework for impact assessment that promotes understanding of changes at host, population and fishery levels. These are placed into context with the economic and ecological value of native resources. An independent 'non-native parasite review group' has been convened to ensure consistency of policy decisions and clear communication of disease risks to interested bodies. It is hoped that this will assist the management of future invasions and provide a scientifically robust foundation on which to base proportionate control measures.

The Category 2 parasites are defined as "species having a significant disease potential when introduced into waters where they do not already exist, or are non-indigenous species with unknown pathogenicity and distribution". Understanding of the dangers

posed by these parasites varies considerably. *Ergasilus sieboldi, Anguillicola crassus, Bothriocephalus acheilognathi* and *Lernaea cyprinacea,* are well recognised non-native fish pathogens that pose a considerable threat to fisheries. Conversely, the effects of other introduced parasites remain poorly understood. In many cases, a paucity of published literature at the time of introduction has limited a reliable assessment of impact.

Four non-native parasites were considered a priority for further study. These were *Paraergasilus longidigitus* (Copepoda: Poecilostomatoida), *Ergasilus briani* (Copepoda: Poecilostomatoida), *Atractolytocestus huronensis* (Cestoda: Caryophyllidae) and *Philometroides sanguinea* (Nematoda: Philometridae). Pathological, epidemiological and experimental investigations were undertaken to assess the effects of these parasites at host and population levels.

*P. longidigitus* can cause pronounced pathological changes to the olfactory epithelium of infected fish. Although this damage provides the potential for disruption to spawning through reduced sensitivity to reproductive chemical cues, experimental observations combined with seasonality studies suggest that the parasite is unlikely to disrupt reproduction within infected cyprinid fisheries. Similarly, studies conducted on the caryophylidean cestode *A. huronensis* provide little evidence to suggest that the parasites poses a threat to carp fisheries. Damage within the intesinal tract was characterised by relatively mild mechanical and inflammatory changes. Infections of up 213 parasites had no adverse affect upon the conditon of common carp. Based on these findings and the application of the aformentioned risk model, *P. longidigitus* and *A. huronensis* are considered to be of low disease risk to fisheries.

The pathology of *E. briani* and *P. sanguinea* are described for the first time. Literature suggests that both parasites are pathogenic to juvenile fish. *E. briani* causes a number of pathological changes within the gills of juvenile cyprinids, although these remain localised due to the very strict site specificity of the parasite. Migrations of the nematode *P. sanguinea* causes damage to the fins and caudal musculature of juvenile crucian carp. For both parasites, the extent and severity of pathological changes are inversely proprtional to host size. Further studies at the population level are needed before an assessment of impact to fisheries can be made. The difficulties associated with studying the effects of introduced parasites to juvenile fish populations in the wild are recognised. Recommendations for further study are given.

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**Definitions of terminology**

For the purpose of this study the ecological terms for prevalence and intensity are given according to Margolis *et al.*, (1982). The term non-native will be used rather than alien or bio-invader to describe introduced species. The term epidemiology is used rather than epizootiology as stated by Thrusfield (1995). The following ecological terms used to describe biological invasions are taken from Manchester & Bullock (2000), Richardson *et al.*, (2000) and Copp *et al.*, (2005a).

**Colonisation** – when an organism of a founding population reproduces, allowing the species to increase in numbers to form a colony that is self-perpetuating.

**Intensity** – the number of individuals of a particular parasite species in each infected host.

**Introduction** – the deliberate or unintentional transfer and/or release of an organism into the wild, or into locations not completely isolated from the surrounding environment, by humans in geographical areas where the taxon is not native.

**Invasive** – Native or alien species that spread, with or without the aid of humans, in natural or semi-natural conditions, producing a significant change in the composition, structure or ecosystem processes, or cause severe economic losses to human activities.

**Mean intensity** – the total number of individuals of a particular parasite species in a sample of a host species, divided by the number of infected individuals of the host species in the sample.

**Native** – Refers to a species or taxon that occurs naturally in a geographical area, with dispersal occurring independent of human intervention. Also includes a characteristic of or existing by virtue of geographic origin.

**Non-native** – Refers to a species, sub-species, race or variety that does not occur naturally in a geographical area.

**Prevalence** – the number of individuals of a host species infected with a particular parasite species, divided by the number of hosts examined (expressed as a percentage)

**Translocation** – the introduction of a species from one part of a political entity (country) in which it is native to another part of the same country in which it is non-native.

**Wild** – a condition in which an organism can disperse to other sites or can breed with individuals from other populations. Also to include self-sustaining populations in discrete water bodies.

## Chapter 1. Introduction – Rationale of study

### 1.1. Current problems with the management of non-native parasites

The introduction and dissemination of non-native parasites represent significant threats to the development of freshwater fisheries in the British Isles. The number of non-native parasites detected within England and Wales has increased considerably in the last 30 years (Kennedy, 1974, 1993, 1994; Fryer & Andrews, 1983; Gibson, 1993; Chubb & Yeomans, 1995; Kirk, 2000a; Kirk et al., 2003a; Gozlan et al., 2005). This has raised concern over the spread of disease to fish populations and placed increasing pressure upon Government agencies that have responsibilities for environmental protection and sustainable fishery development (Hickley & Chare, 2004). Within England and Wales, the Environment Agency regulates fishery stocking under its remit to maintain, improve and develop fisheries. Movement of fish infected with non-native parasites is restricted where the risk of disease to wild fish populations exists (Environment Agency, 1999). In order for the Environment Agency to meet these duties, there is need to balance the social, economic and environmental benefits of fish stocking against the risks of disease transfer. This can be extremely difficult when the pathogenicity, distribution and colonisation potential of parasites are poorly understood.

The management of non-native parasites in England and Wales has long employed a precautionary approach (Environment Agency, 1999; Hickley & Chare, 2004). The assumption underlying this approach is that it takes the introduction of only one pathogenic species to have a serious and irreversible effect upon fish populations (Kennedy, 1993, 1994; Mo, 1994; Kirk, 2003). Although precaution is an important consideration when managing non-native species introductions (Bartley & Minchin, 1996; FAO, 1996; Minns & Cooley, 1999; ILGRA, 2002a,b; Bartley, 2004), its

application alone has led to restrictions placed on growing number of parasite species with unknown or unclear impacts (Environment ·Agency, unpublished). These restrictions can be expensive to manage, difficult to enforce and exert their own impacts upon the economic value of the fish movement trade. For some parasites, controls have been implemented for over two decades, with little progress in understanding of the extent and severity of impact to fisheries. In some cases, poor understanding of these parasites has also limited the efficacy of control measures, hindered effective management of infected fisheries and made it increasingly difficult to justify long-term fish movement restrictions. Growing recognition of these problems has highlighted the need for better evaluation of the risks posed by these parasites to fisheries (Brewster *pers. comm*). Furthermore, a shift towards more evidence-based regulation has prompted the Environment Agency to review how both current and future invasions are managed (Pollard, 2001; Chare *et al.,* 2002).

## 1.2. Dangers posed by non-native species introductions

The threats posed by non-native species, also termed 'exotics', 'alien-species' or 'bioinvaders' are well recognised within both terrestrial and aquatic environments (Kluge *et al.,* 1986; Arthington, 1991; Bullock *et al.,*1996; Cunningham, 1996; Belkessam *et al.,* 1997; Brancato & MacLellan, 1999; Leppakoski *et al.,* 2002a; Yap & Sodhi, 2004). Literature provides many examples of environmental impacts from non-native organisms, including exotic fish species (Barel *et al.,* 1985; Ochumba *et al.,* 1994; Van Der Velde *et al.,* 2002; Townsend, 2003; Gozlan *et al.* 2002), invasive plants (Zedler & Rea, 1998; Toft *et al.,* 2003), aquatic invertebrates (Karatayev *et al.,* 2002) and disease causing organisms (Hoffman, 1970; Bauer & Hoffman, 1976; Johnsen & Jensen, 1988; Bauer, 1991; Kennedy, 1993; Leberg & Vrijenhoek, 1994). Such introductions are not only regarded as a threat to native aquatic biodiversity, but also as

a concern for economic fishery development (Williams *et al.,* 1988; Halvorsen & Hartvigsen, 1989; Bakke, 1991; Hall & Mills, 2000).  ·

The spread of non-native parasites has caused concern to fishery scientists throughout the world (Andrews *et al.,* 1981; Hoffman & Schubert, 1984; Boxshall & Frear, 1990; Leberg & Vrijenhoek, 1994; Heckmann *et al.,* 1995; Yeomans *et al.,* 1997; Evans & Lester, 2001; Hayward *et al.,* 2001; Kirk, 2003; Zyll de Jong *et al.,* 2003; Salgado & López, 2003; Gozlan *et al.,* 2005, 2006). This has stemmed from many well-documented examples of impact, including the introduction of the protozoan parasite, *Myxobolus cerebralis* Hofer, 1903, the causative agent of whirling disease of salmonids to North America (Hoffman, 1970; Tietz, 1998; Heckmannn, 2001); the spread of the pathogenic tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 with international trade in cyprinids (Korting, 1974, 1975; Hoffman & Schubert, 1984; Hoole, 1994); losses of native sturgeon populations in the Aral Sea from the monogenean, *Nitzschia sturionis* (Abildgaard, 1794) (Bauer, 1991); and the decline in native salmon stocks following the introduction of *Gyrodactylus salaris* Malmberg, 1957 to Norway (Malmberg & Malmberg, 1987; Johnsen & Jensen, 1988; Mo, 1994; Bakke *et al.,* 2004). These events have raised awareness of the impacts of parasite translocations and emphasised the importance of national control measures to minimise disease risks to fisheries (Boxshall & Fear, 1990; Lumanlan *et al.,* 1992; Scholz & Di Cave, 1992; Kennedy, 1993; Yeomans *et al.,* 1997).

**1.3. Introduction and dissemination of non-native parasites within the British Isles**

The geographical isolation of the British Isles from mainland Europe provides a barrier to the natural colonisation of many freshwater fish parasites (Kennedy, 1994). Historically, this has provided protection against many fish diseases experienced in

other parts of the world. However, growing demands for fish to stock fisheries, increasing illegal fish movements, implementation of the single European Market, improved transport links and expanding global trade in fish for aquaculture and ornamental industries, have facilitated the dissemination of many parasites far beyond their natural geographical ranges (Kennedy, 1975, 1976, 1993, 1994; Esch *et al.,* 1988; Stewart, 1991; Environment Agency, 1999; Ariel & Olsen, 2002; Vincent & Font, 2003). These factors have resulted in the introduction of exotic fungal pathogens (Lilley *et al.,* 1997; Edgerton, 2002), viruses (Bucke & Finlay, 1979; Paisley, 1988; Snow *et al.,* 2003; Way, 2004), bacteria (Lincoln, 2001) and almost every major taxonomic group of parasite into the British Isles (Kennedy, 1994; Kirk, 2000a). The increase in both number and diversity of introduced species highlights the growing potential for disease outbreaks and the difficulties in predicting future invasions.

Most parasites are spread by the anthropochore movement of infected hosts (Kennedy, 1975; Fryer, 1982; Hedrick, 1996; Majoros *et al.,* 2003). Recent trends in fish stocking activity therefore serve to increase the risks of future parasite introductions. Growing demand for non-native fish species like wels catfish *Siluris glanis* L., sturgeon *Acipenser* spp., golden orfe *Leuciscus idus* (L.) and carp variants including silver carp *Hypophthalmichthys molotrix* (Valenciennes) and bighead carp *Aristichthys nobilis* (Richardson) has led to increasing occurrence of these species within fisheries (Hickley & Chare, 2004: Britton & Davies, 2007; Environment Agency, unpublished). Demand for specimen fish has also fuelled illegal introductions of many species from abroad, where health status is often a much less important consideration than availability, size and cost. The increasing occurrence of ornamental fish in wild fisheries provide further avenues for the spread of exotic pathogens (Lester & Evans, 1999; Gozlan *et al.,* 2002, 2005; Haenfling *et al.,* 2005; Jeffery, 2006). Records of African cichlids in the drains of

East Anglia, Red-tailed catfish *Phractocephalus hemioliopterus* (Bloch & Schneider) in the River Ouse catchment and a thriving population of *Catastomus* species in a southern river are just a few examples substantiating these risks (Environment Agency, unpublished). Similarly, the spread of exotic 'pest-species' like topmouth gudgeon *Pseudorasbora parva* (Temmick & Schlegel), bitterling *Rhodeus sericeus* (Pallas), sunbleak *Leucaspius delineatus* (Heckel), fathead minnow *Pimephales promelus* Rafinesque, and pumpkinseed *Lepomis gibbosus* (L.) throughout England and Wales, have raised growing concerns of disease threats to native fish populations (Wheeler, 1991; Beyer, *et al.,* 2005; Pinder *et al.,* 2005; Williams *et al.* 2005; Gozlan *et al.,* 2006).

## 1.4. Placing non-native introductions into context with the value of fisheries

A recent review of fishery development in England and Wales estimated that angling contributes over £3.4 billion to annual GDP (Environment Agency, 2004). There are an estimated 30,000 stillwaters and over 40,000 km of waterways in England and Wales, fished by over 4 million anglers (Environment Agency, 2004). On average, each of these anglers spend £1,000 annually on the sport. In order to support these angling interests, fish stocking represents a valuable component of fishery management (Leonard, 2001; Hickley & Chare, 2004). Approximately 6,000 legal fish movements take place annually for the stocking of inland waters. These movements comprise an estimated 8 million fish with a value exceeding £23 million (Environment Agency, unpublished). In view of this, the introduction of a pathogenic parasite has the potential to cause serious ecological and economic impacts to fisheries. However, excessive or inappropriate disease controls can also be damaging, placing unnecessary pressures upon the fish movement trade. In such cases, it is important to recognise that the risks associated with the introduction of fish diseases arise as a by-product of the fish movement process, thus need to be placed into context with the benefits of such

activities (Cowx, 1997; Pollard, 2001). This is why it is important to understand the disease threats posed by any introduced parasite and balance these changes with the environmental and economic value of fisheries (Kennedy, 1994).

## 1.5. The Category 2 parasites

The Category 2 parasites / diseases are species considered by the Environment Agency to pose a potential threat to fisheries within England and Wales. The Category 2 parasites / diseases are defined by the Environment Agency to either:

1. have a significant disease potential when introduced into waters where the disease or parasites do not already exist; or

2. be novel, non-indigenous or have unknown pathogenicity and distribution (Environment Agency, 1999).

The Category 2 parasite list as at 2003 comprised a total of 15 macroparasite species (Table 1.1) (Environment Agency, 1999). The bacterium *Lactococcus garvieae* and Koi Herpesvirus (made a notifiable disease in April 2007) are also recognised as novel pathogens warranting control. Within the last 20 years, the number and composition of species on the Category 2 list has varied, following the detection of new species and de-regulation of others.

For some of the Category 2 parasites, their introduction was supported by a wealth of literature detailing disease risks and colonisation potential. Examples include *Ergasilus sieboldi* von Nordmann, 1832 and *Bothriocephalus acheilognathi,* both important pathogens of wild and cultured fish populations in many parts of the world (Lahav & Sarig, 1967; Fryer, 1969; Heckmann *et al.,* 1987; Abdelhalim, 1990; Alston & Lewis,

1994; Lester & Roubal, 1995; Hoole *et al.*, 2001). Conversely, for many species, a paucity of published literature has prevented a reliable assessment of disease potential. With such limited understanding, it is difficult to develop, prioritise and justify effective control strategies.

Table 1.1. List of Category 2 and novel parasites (2003) (*species considered by the Environment Agency to be 'novel')

| Taxa | Category 2 Parasite |
|---|---|
| Cestoda | *Atractolytocestus huronensis* (Anthony, 1958)<br><br>*Bothriocephalus acheilognathi*, (Yamaguti, 1934)<br><br>*Monobothrium wageneri* (Nybelin, 1922) |
| Crustacea | *Ergasilus gibbus* (von Nordmann, 1832)<br><br>*Ergasilus briani* (Markewitsch, 1933)<br><br>*Ergasilus sieboldi* (von Nordmann, 1832)<br><br>*Paraergasilus longidigitus* (Yin, 1954)<br><br>*Neoergasilus japonicus* (Harada, 1930)<br><br>*Tracheliastes polycolpus* (Nordmann, 1832)*<br><br>*Tracheliastes maculatus* (Kollar, 1836)*<br><br>*Lernaea cyprinacea* (L., 1758) * |
| Monogenea | *Pellucidhaptor pricei* (Gussev & Strijak,1972) * |
| Acanthocephala | *Pomphorhynchus laevis* (Muller, 1776) |
| Nematoda | *Philometroides sanguinea* (Rudolphi, 1819) *<br><br>*Anguillicola crassus* (Niimi & Itagaki, 1974) |
| Bacteria | *Lactococcus garvieae* * |
| Virus | Koi Herpes Virus (KHV) |

## 1.6. Fish disease controls within England and Wales

Disease controls in the British Isles are designed to balance the promotion of trade and socio-economic development, whilst minimising the adverse consequences of disease transfer (MAFF, 1995; Hill, 1996; Environment Agency, 1999). Current disease controls in England and Wales can be broadly categorised as European legislation controlling fish imports, fish farms and introductions of notifiable diseases, and internal regulations to prevent the introduction and spread of disease to inland fisheries and wild fish populations. The following overview divides these accordingly and highlights current problems surrounding exotic disease introductions.

## 1.6.1. Importation of fish into England and Wales

The responsibility for the control of fish imports into England and Wales lies with the Department for Environment, Food and Rural Affairs (Defra) and the National Assembly for Wales (NAW). These controls stem from the European Directive 91/67 and the Diseases of Fish Act, 1983, as amended. Following the formation of the single European market in 1991, the controls on trade of fish were relaxed (Howarth, 1992; Minchin & Gollasch, 2002). This has led to the restriction of only very serious and well-recognised pathogens, known as notifiable or Category 1 diseases. With the exception of the monogenean parasite *Gyrodactylus salaris*, the Category 1 diseases of fin-fish are either viral or bacterial pathogens, most of which are associated with economic losses in farmed salmonids (Haenen *et al.,* 2004). Monitoring programmes for the notifiable diseases involve annual screening of all registered fish farms in England and Wales. The detection of a notifiable disease may lead to eradication, restriction of fish movements for 3 years pending further tests or changes to the status of approved zones.

Despite historic outbreaks of notifiable diseases within the British Isles (Paisley, 1988; Raynard *et al.,* 2001; Anon, 2006) current import controls generally provide effective protection to fish farms against Category 1 diseases. This is aided by legislative restrictions on the importation of live salmonids into the British Isles. However, these controls do not prevent introduction of non-notifiable pathogens, emerging diseases (*e.g.* Koi Herpesvirus) or parasites with unknown pathogenicity. Furthermore, routine parasitological examinations are not routinely conducted on fish imported into the UK. Consequently, non-native parasites and disease-causing organisms have, and continue to be introduced into England and Wales from all over the world. This has long raised concern over the adequacy of such measures to protect fisheries (Andrews *et al.,* 1981; Andrews, 1984; Crawshaw & Sweeting, 1986; Boxshall & Frear, 1990; Kennedy, 1993; Yeomans *et al.,* 1997; Lester & Evans, 1999; Evans *et al.,* 2001; Moore & Kirk *pers. comm*). This emphasises the importance of effective management of exotic introductions, justification of controls and a structured approach to impact assessment.

### 1.6.2. Fish movements within England and Wales

Within England and Wales, the control of fish disease, other than the notifiable pathogens, is carried out by the Environment Agency under Section 30 of the Salmon and Freshwater Fisheries Act, 1975 (Anon, 1975). These controls do not form part of European legislation, but represent internal policies designed to protect fisheries from spread of fish disease, including introduced exotic parasites. These measures are designed to protect wild fish populations and are flexible to accommodate new and emerging pathogens. However, restrictions are placed only where there is a risk of disease to wild populations. The health status of fish stocked into fully enclosed waters remains largely uncontrolled. Furthermore, current legislation makes no provision for

eradication of any non-notifiable disease once introduced. These factors, in addition to a growing number of illegal fish introductions, have resulted in the progressive dissemination of Category 2 parasites rather than complete prevention of spread (Environment Agency, 1999). As a consequence, certain parasites with high colonisation potential, cryptic life stages or long-standing presence in the British Isles have become widely distributed. This dissemination not only increases disease risks to a growing numbers of fisheries, but also increases the reservoirs of infection facilitating further spread through fish stocking activity. Furthermore, as the number of infected waters increases, so have the restrictions placed on fish movement activities.

## 1.7. Summary

The potential for disease surrounds any exotic parasite introduction, with serious and irreversible effects upon fish populations (Kennedy, 1994). The growing scale and frequency of fish movements, into and within England and Wales, involving a widening diversity of species is increasing the likelihood of such impacts occurring. However, the 'potential' for disease cannot alone support long-term policy decisions. Understanding the risks posed by these parasites is essential for effective and sustainable management of fisheries. Efforts are urgently required to improve understanding of the Category 2 parasites and develop a structured and transparent approach to assess disease threats from future parasite invasions.

The specific aims of this project were:

1. To reduce future reliance upon the precautionary principle through improved understanding of the Category 2 parasites within England and Wales.

2. To develop a structured, transparent and consistent process for evaluating current and future disease threats from non-native parasites to fisheries in England and Wales.

3. To undertake studies to progress understanding of parasite impacts to fisheries, thus providing a foundation of information on which to support policy decisions and base future investigations.

Chapter 1.

In order to clarify the work undertaken to satisfy these objectives, the structure of this

thesis and studies conducted are shown in Fig 1.1.

Fig 1.1. Thesis structure and studies undertaken during the project.

| **Introduction and rationale of study** <br> • Background information <br> • Rationale for the study <br> • Study aims and objectives | **Chapter 1. pg 1** |
|---|---|
| **Risk assessment for non-native parasite introductions** | **Chapter 2. pg 13** |
| **Application of impact assessment and prioritisation of further studies** | **Chapter 3. pg 58** |
| *Atractolytocestus huronensis* <br><br> • Importance of *A. huronensis* to common carp fisheries in England and Wales. | **Chapter 4. pg 84** |
| *Ergasilus briani* <br><br> • Impact of *E. briani* to rudd and juvenile common bream populations. | **Chapter 5. pg 138** |
| *Paraergasilus longidigitus* <br><br> • Effect of *P. longidigitus* upon the olfactory sensitivity of common carp. | **Chapter 6. pg 203** |
| *Philometroides sanguinea* <br><br> • Pathogenicity and epidemiology of *P. sanguinea* in crucian carp fisheries. | **Chapter 7. pg 261** |
| **Summary and Recommendations** <br> • Review of current understanding <br> • Recommendations for future management of non-native parasites | **Chapter 8. pg 327** |

## Chapter 2 – Risk assessment for non-native parasite introductions

### 2.1. Introduction

The global spread of non-native organisms is recognised as one of the largest threats to aquatic bio-diversity (IUCN, 1987; Courtenay & Robins, 1989; Bullock *et al.*, 1996; Hodder & Bullock, 1997; Smith *et al.*, 1999; UK Defra, 2003; Ciruna *et al.*, 2004; Copp *et al.*, 2005b,c). Disease is amongst the greatest, if not *the* greatest impact that can result from the introduction of any non-native organism (Hoffman, 1970; Fletcher, 1986; Bauer, 1991; Ganzhorn *et al.*, 1992; Kennedy, 1993, 1994; Goodchild, 1999; Copp *et al.*, 2005b; Gozlan *et al.*, 2006). Such invasions can cause more damage to aquatic than terrestrial environments due to the relative geographic isolation and vulnerability of these ecosystems (IUCN, 1987; Sala *et al.*, 2000; Ciruna *et al.*, 2004). In view of this, the introduction of non-native parasites to freshwater fisheries represents a serious threat to the ecology and socio-economic development of aquatic resources in England and Wales (Kennedy, 1993, 1994; Kirk, 2003; Gozlan *et al.*, 2006). In the last decade, growing recognition of these threats has highlighted the need for greater awareness of the dangers posed by introduced parasites and greater efforts to manage these invasions (Kirk, 2003; Gozlan *et al.*, 2005; 2006).

Risk assessment has proven a useful tool in managing potential hazards of non-native species introductions (Pheloung *et al.*, 1999; Champion & Clayton, 2001; Nz MAF, 2002; Hewitt & Hayes, 2002). This has become a particularly well developed discipline for international weed control and for protection of agriculture and marine environments, where examples of impact are numerous (Kluge *et al.*, 1986; Sinderman, 1988, 1993; Pheloung *et al.*, 1999; Pheloung, 2001; Panetta *et al.*, 2001; Reichard, 2001; Hewitt & Hayes, 2002; Kolar & Lodge, 2002; Copp *et al.*, 2005b). Risk

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assessments have been recently developed in the UK to identify threats posed by a range of non-native taxa (UK Defra, 2003, 2005; Copp *et al.,* 2005b). This followed a key recommendation from a government review of non-native species, which emphasised the need for comprehensive risk-assessments to assess the threats posed by future invasions to the UK (UK Defra, 2003). This is consistent with guidelines given by The Convention of Biological Diversity (CBD, 2001) that stated risk assessment should be used to justify any actions taken against threats to bio-diversity.

Within the UK, current responsibilities for dealing with non-native species are spread across a number of government departments and involve a range of legislative measures (Bullock *et al.,* 1996; UK Defra, 2003). Current import risk assessments (IRAs) for aquatic animal health in the UK provide guidelines for the control of diseases listed by the Office International des Epizooties (OIE). These measures, based upon the earlier risk models of Covello & Merkhofer (1993), prevent introduction of serious diseases that pose a threat to aquaculture and the economic trade of fish (OIE, 2004; Peeler, *et al.,* 2006a). However, with the exception of *G. salaris,* for which a number of risk-based studies have been conducted (Paisley *et al.,* 1999; Hogasen & Brun, 2003; Peeler & Thrush, 2004, 2005; Thrush & Peeler, 2006; Peeler *et al.,* 2006b) little attention has been given to the use of risk assessment for non-native parasite introductions into the wild. Neither current IRA, nor legislative controls for non-native species introductions define procedures with which to identify, prioritise or control disease risks posed by parasites introduced to fisheries in England and Wales (Bartley & Subasinghe, 1996; Yeomans *et al.,*1997; Gaughan, 2001; Gozlan *et al.,* 2006). The development of a risk assessment process for non-native parasites is therefore necessary in order to identify future disease threats and ensure appropriate and sustainable management of fisheries (Ricciardi & Rasmussen, 1998; Li, *et al.,* 2000; Chare *et al.,* 2002).

In response to a review of non-native species introductions (UK Defra, 2003), a generic

risk assessment scheme was developed to assess dangers posed by any non-native

organism to any species, habitat or ecosystem in all or part of the UK (UK Defra,

2005). This scheme, based upon models for invasive weed control (EPPO 1997, 2000)

included provision for non-native parasites (UK Defra, 2005). However, the absence of

overarching characteristics that accurately satisfy all taxonomic groups (Bullock *et al.,*

1996; Manchester & Bullock, 2000; Kolar & Lodge, 2002; Gollasch, 2002) makes it

difficult to apply this generic risk model specifically to fish parasites. Such limitations

have been recognised with the need for UK taxa-specific risk assessment models (Copp

*et al.,* 2005b). Furthermore, a number of assumptions used in previous risk models to

predict invasiveness and impact have limitations when being applied to parasites. This

stems mainly from the reliance of climate matching (similarity in climatic conditions

between donor and recipient localities) and impact history to predict the dangers of an

organism to a new environment (Ricciardi & Rasmussen, 1998; Pheloung, 2001;

Ricciardi, 2003; UK Defra, 2005; Copp *et al.,* 2005b). Such an approach is useful if the

behaviour of a species in native localities is well understood. However, evidence

suggests that parasite invasions can be unpredictable and are frequently accompanied

by very little information on which to assess disease risk (Harris, 2003; Ricciardi,

2003). This paucity of information can therefore lead to an incomplete estimation of a

parasite's potential for colonisation and impact (Gollasch, 2002). During development

of a risk assessment for non-native fish, Copp *et al.,* (2005b) recognised that predicting

the impact of fish diseases is particularly difficult due to the limited information that

often surrounds these introductions. Whilst the very purpose of risk analysis is to deal

with the unknown (Covello & Merkhofer, 1993; Peeler *et al.,* 2006a,b), the application

of theoretical models without any biological or epidemiological information is

problematic. Lack of information hinders the ability to make informed, evidence-based

judgements of potential impact. Consequently, in order to identify disease threats posed by any introduced parasite, a process is required to define, measure and evaluate impact regardless of the amount of available published information.

A fundamental problem in parasitology is identifying the different ways in which a parasite may affect host populations (Adjei *et al.*, 1986; Barber *et al.*, 2000). Whilst all changes represent a deviation from the normal structure, behaviour or function of the host, extrapolating the significance of these effects at a population level can be extremely difficult (Kennedy, 1993; Gulland, 1995; Grenfell & Gulland, 1995; Hedrick, 1998). This is exacerbated by the large number of factors that can influence the behaviour of a parasite in a new environment, and the practical difficulties of studying diseases in wild populations (Dogiel *et al.*, 1958; Wurtsbaugh & Tapia, 1988; Sinderman, 1993; Bartley & Subasinghe, 1996; Blanc, 1997). These complexities may explain why relatively few comprehensive studies exist on the impacts of parasites on wild fisheries (Grenfell & Gulland, 1995; Blanc, 1997; Feist *et al.*, 1997; Feist & Longshaw, 2005). A frequently documented exception to this concerns the monogenean parasite *Gyrodactylus salaris* in Norway (Johnson & Jensen, 1988, 1991; Mo, 1994). Large-scale mortalities of pre-smolt salmon *Salmo salar* L. are reported to have caused a 95% decline in salmon populations within some rivers. The economic cost of the parasite within Norway has been estaimated to exceed £300 million. Although at the time of the introduction there was little to suggest that *G. salaris* was an important pathogen of fish, extensive studies have since been undertaken in efforts to eradicate the parasite from Norway and identify risks of disease spread to other countries.

Whilst mortality is the most obvious and drastic consequence of parasitism, sub-lethal, chronic and indirect impacts can also have important effects upon fisheries. The

introduction of the dracunculoid nematode *Anguillicola crassus* Kuwahara, Niimi &

Itagaki, 1974 to European eel *Anguilla anguilla* (L.) populations is a well cited example

of such potential (Hedrick, 1998; Kirk, 2003; Gozlan *et al.*, 2006). Although the

parasite has been responsible for mass mortality with significant economic losses

(Molnar *et al.*, 1991; Kirk, 2003; Evans, 2006), the parasite usually exists in outwardly

healthy eels in freshwater. Whilst this suggests limited impact, the parasite causes

considerable damage to the swim-bladder of infected eels, disruption to organ function

and reducing swimming performance (Kirk, 2003). Infections are also known to

increase the susceptibility of hosts to other diseases (Boon *et al.*, 1990). These effects

reduce the likelihood that parasitised eels complete their marine migrations for

reproduction (Sprengel & Luctenberg, 1991; Molnar, 1993; Ashworth & Blanc, 1997;

Kirk, 2003). It has been suggested that the introduction of *A. crassus* from Asia to

Europe may be an important contributory factor in the decline of European eel

populations (Welcomme, 2001; Kirk, 2003; Starkie, 2003). However, due to the

complexities of eel reproductive behaviour, this remains a very difficult impact to

measure and substantiate. This example highlights the difficulties of understanding host

parasite interactions in the wild and emphasises the need to appreciate both host and

population effects before making an assessment of disease risks (Lester, 1984; Grenfell

& Gulland, 1995; Evans, 2006).

Despite clear dangers posed by pathogen tranlocations, there are two important

problems surrounding the current management of non-native parasites in England and

Wales. Firstly, poor understanding of the Category 2 parasites has made it difficult to

identify potential impacts on fisheries, prioritise resources, communicate disease risks

to interested parties and justify existing control measures. According to Parker *et al.*,

(1999) the effective management of non-native species introductions relies upon the

ability to identify potential impacts and understand the importance of these changes. The second problem involves the historic dependence upon the precautionary principle as a means for managing the Category 2 parasites. Precaution has long justified the restriction of many non-native parasites without the immediate need to identify or quantify their effects (FAO, 1996; Environment Agency, 1999). Whilst responsible management of natural ecosystems will always incorporate precaution (Minns & Cooley, 1999; McDowall, 2004; Hickley & Chare, 2004) it cannot be used alone as a foundation for effective and sustainable policy making. This has highlighted the need for a risk-based process to identify, prioritise and manage both current and future disease threats to fish, fish populations and fisheries in England and Wales. In order for current and future policy decisions to be practical, sustainable and proportionate to the risks posed by these parasites, the scientific basis for their development must be better understood (Pullin, 1994; Bartley & Subasinghe, 1996; Hickley & Chare, 2004).

This chapter involved development of a systematic process for assessing the risks posed by non-native parasites to fisheries in England and Wales. This approach allowed progression of understanding for established Category 2 parasites as well as a risk assessment for managing future introductions. A process for hazard identification provided a tool for guiding initial decisions following the detection of parasite introductions. Criteria for measuring impact were identified, including changes as host, populations and fishery levels. The use of an impact matrix, provided a framework to identify what was already known about a parasite and what information was required in order to perform a reliable risk assessment. This approach also enabled gaps in knowledge to be identified and further research prioritised.

## 2.2. Materials and methods

Following a review of the problems associated with the impact assessment of non-native parasites, an approach was developed to provide a structured and consistent method for evaluating disease risks to fisheries. This included scope for dealing with newly introduced parasite species as well as progressing understanding of established Category 2 parasites.

The approach was structured around three key areas, namely current legislative controls for non-native species and fish diseases in England and Wales (Anon, 1975; Hill 1991, 1996; Howarth & McGillivray, 1994; Bullock et al., 1996; Hickley & Chare, 2004), ecological factors effecting the colonisation and spread of parasites (Kennedy, 1993, 1994) and the responsibilities of the Environment Agency for environmental protection and fishery development (Hickley & Chare, 2004). Procedures for hazard identification followed a similar approach to those documented for weed and fish risk assessments (Pheloung et al., 1999, Copp et al., 2005b). However, the current system was simplified and tailored to deal specifically with factors relevant to fish parasites.

The different ways in which parasites may impact upon fisheries were identified from extensive published literature of parasite infections in fish, fisheries and wild fish populations. In order to test and validate the quantitative aspects of the risk assessment, trial assessments were conducted with historic examples of introduced parasites. These included species that are well-described pathogens (e.g. *Anguillicola crassus* and *Ergasilus sieboldi*) as well as parasites which are poorly understood (e.g. *Pellucidhaptor pricei* Gussev & Strijak, 1972 or known to be only weakly pathogenic (e.g. *Skrjabillanus scardinii* Molnar, 1966).

## 2.3. Results

The proposed risk assessment follows a four staged process. This approach includes an initial scoping phase, followed by a process for hazard identification, risk assessment and risk management/communication (Covello & Merkhofer, 1993; Peeler *et al.*, 2006a). For the purpose of clarification, this process is summarised in Fig 2.1.

Fig. 2.1. Summary flow diagram of the proposed risk assessment process for non-native parasite introductions to freshwater fisheries in England and Wales

**Detection of a non-native parasite**

| **Stage 1** **Scoping Process** | **Is there scope to manage the introduction?** *e.g is the parasite non-native or endemic? Is it already restricted by national legislation?* | Decision Tree pg 22 |

| **Stage 2** **Hazard Identification** | **Does impact history and colonisation potential allow prediction of pathogenicity?** *e.g have disease problems occurred in other parts of the world? Is it likely to establish?* | Questionnaire pg 25 |

| **Stage 3** **Impact Assessment** | **What are the effects of the parasite and what are the risks of these occurring?** *e.g what effect does the parasite actually have on fish and fish populations?* | Impact Matrix pg 38 |

| **Stage 4** **Risk Management & Communication** | **What can be done to manage these risks?** *e.g should controls be implemented, how can awareness of the risks be communicated?* | Management options pg 42 |

## 2.3.1. Stage 1 – Scope for managing non-native parasite introductions

Stage one represents a rapid screening process to determine the scope for managing, controlling or eradicating any newly introduced parasite. This process takes the form of a decision tree (Fig. 2.2) and accommodates factors that may limit or hinder the capacity to manage a parasite invasion. These factors primarily focus on resource limitations, ecological characteristics of the introduced species and current legislative controls concerning fish and fish diseases in England and Wales. This simple process serves to highlight parasites that may be uncontrollable, do not warrant control or are already covered by higher legislative measures (e.g. the parasite is notifiable, endemic or freely disseminated with piscivorous birds). This is necessary to ensure that resources are not inappropriate allocated to parasites that could not, or should not be controlled.

Fig. 2.2. Stage 1 - Decision tree for assessing the scope for managing non-native freshwater parasites in England and Wales.



**Confirmed identity of a non-native parasite within England and Wales**

Is the parasite already covered by national legislation (notifiable) or likely to be covered under the remit for new / emerging diseases?

No

Yes

Is the parasite specific to fish that are restricted from movement under national exotic fish legislation?

Support these legislative controls to prevent spread of parasite.

Yes

No

Support these legislative controls to prevent spread. Monitor host range to highlight potential for host shift.

Does eradication of the parasite represent a feasible, cost-effective and practical option to prevent further spread?

No

Yes

Can the parasite be accurately detected through current fish disease examinations and validated diagnostic tools?

Attempt eradication of the parasite from the infected water.

Yes

No

Could susceptible fisheries be effectively protected from the parasite through restriction of infected fish movements?

Could diagnostic tools be developed to allow detection of infected fish prior to the stocking of susceptible fisheries?

Yes          No                    Yes          No

Conduct initial risk assessment to establish potential pathogenicity and colonisation potential
**Progress to Stage 2**

Do not implement control measures Raise awareness, monitor impacts and reduce disease risks through improved fishery management.

## 2.3.2. Stage 2 – Hazard identification

Stage two represents a rapid process for hazard identification (Covello & Merkhofer, 1993). This provides decision-makers with a preliminary indication of a parasite's potential pathogenicity, based upon available information at the time of introduction. This is analogous to a traffic-light system for guiding initial management actions, irrespective of how well a parasite is understood. This process is based upon a quantitative scoring system.

Stage two consists of 10 questions that serve as predictors for parasite colonisation, establishment and impact (Fig 2.3). These questions are divided into three sections, which focus upon the ecological and economic value of native resources, the colonisation potential of the parasite and impact history. This accommodates evidence of disease potential, presence of undesirable traits, biological characteristics of the parasite and similarity of environmental conditions between donor and recipient localities. Each question focuses upon specific attributes of a parasite that together influence colonisation potential and potential impact to fisheries in England and Wales (Kennedy, 1993; 1994; Lambert, 1997). Each question should be answered by a person competent in fish health or parasitology, and should follow a thorough review of available literature. In order to reduce bias and subjectivity, it is proposed that this process may be conducted by a number of suitable persons and the scores averaged.

Stage 2 provides a numerical output that reflects the risk of the parasite colonising and having undesirable impacts (Fig 2.4). Each question is either answered with a yes, no or uncertain response, or a five-step response based upon whether the question has a very low, low, moderate, high or very high likelihood of occurring (Fig 2.3). Each

question is given a score ranging between 0 and 4. Uncertainty or lack of understanding

for any question, defaults to a high-risk output based upon the precautionary approach

(Copp *et al.*, 2005b). Example risk assessments for non-native parasites are shown in

Fig 2.5a-d. This preliminary scoring system may be used to determine whether a

parasite poses a low (0-15 points), medium (16-28 points) or high risk (28-40 points).

The three sections used to divide the questions also enable high-risk attributes for any

parasite to be identified (e.g. high colonisation potential).

Fig. 2.3. Initial risk assessment process for non-native freshwater fish parasites.

| Risk Query | V.low or No | Low | Mod | High | V.high or Yes / ? |
|---|---|---|---|---|---|
| **Score** | 0 | 1 | 2 | 3 | 4 |
| **Value / susceptibility of native resources** | | | | | |
| 1.What is the economic value of the parasites host(s) to fisheries in England and Wales? | | | | | |
| 2.What is the ecological value of the parasites host(s) to fisheries in England and Wales? | | | | | |
| 3.Does the parasite infect a host that is endangered or threatened within England and Wales? | | | | | |
| **Colonisation potential** | | | | | |
| 4. Based upon climatic conditions of donor and recipient localities, what is the likelihood that environmental conditions, or those reasonably expected through global warming, would favour colonisation of the parasite? | | | | | |
| 5. Based upon life-cycle development, host specificity, distribution of intermediate hosts (if needed) and reproductive potential of the parasite, what is the likelihood of successful colonisation of freshwater fisheries? | | | | | |
| 6. How many legal fish movements take place annually within England and Wales comprising host species of the parasite (refer to LFMD records: 0-10= v.low, 10-50=low, 50-250=medium, 250-500=high, >500 v.high) ? | | | | | |
| 7. What is the likelihood that viable life stages of the parasite would allow dissemination in the absence of the fish host? | | | | | |
| **Potential disease risk** | | | | | |
| 8. Is the parasite known to impact upon wild or farmed fish populations in other geographical regions? | | | | | |
| 9. Do experimental observations or pathological descriptions suggest that the parasite may be an important pathogen of fish? | | | | | |
| 10. Does the parasite have pathogenic congeners? | | | | | |

Fig 2.4. Initial predictions of risk obtained from scores generated from Stage 2 of the current risk assessment process. Specific risks are provided given for each of the three sub-sections as well as an overall risk output for a parasite.

| Risk Query | Score | | Risk |
|---|---|---|---|
| **1. Susceptibility of native resource** | 9-12 | points | High |
| | 5-8 | points | Moderate |
| | 0-4 | points | Low |
| **2. Colonisation potential** | 13-16 | points | High |
| | 8-12 | points | Moderate |
| | 0-7 | points | Low |
| **3. Potential pathogenicity** | 9-12 | points | High |
| | 5-8 | points | Moderate |
| | 0-4 | points | Low |
| **Overall risk level** | 29-40 | points | High |
| | 16-28 | points | Moderate |
| | 0-15 | points | Low |

Fig 2.5a. Example risk assessment conducted for *Ergasilus sieboldi* – SCORE 36

| Risk Query | V.low or No | Low | Mod | High | V.high or Yes / ? | Reasons for scoring |
|---|---|---|---|---|---|---|
| Score | 0 | 1 | 2 | 3 | 4 | |
| **Value / susceptibility of native resources** | | | | | | Very wide host range including economically and ecologically important species. Preference for large fish inc. trout, and high value cyprinids. Host range includes ecologically sensitive species (e.g. crucian carp) although these are not currently endangered. |
| Q1. Economic value of the host(s) | | | | | 4 | |
| Q2. Ecological value of the host(s) | | | | | 4 | |
| Q3. Host threatened or endangered. | | | 2 | | | |
| **Colonisation potential** | | | | | | High colonisation potential (low host specificity, high reproductive capacity, direct life-cycle and presence of long lived, free-living infective stages). Present throughout Europe, rapid reproduction in UK climate. Potential spread with stocking activity very high, particularly with cyprinids and rainbow trout. Also known to spread rapidly with water transfer. |
| Q4. Colonisation potential based upon climate matching. | | | | | 4 | |
| Q5. Colonisation potential based upon ecological attributes. | | | | 3 | | |
| Q6. Potential for spread based upon fish stocking activity. | | | | | 4 | |
| Q7. Potential for spread based upon life cycle development. | | | | | 4 | |
| **Potential disease risk** | | | | | | Well-recorded pathogen of fish, losses recorded in wild and farmed populations. Damage at host level well studied, including gill pathology and physiological disruption. Many ergasilid species known to harmful parasites of fish. |
| Q8. Disease potential from historic records in other localities | | | | | 4 | |
| Q9. Potential pathogenicity based upon literature. | | | | | 4 | |
| Q10. Risk of pathogenic congeners? | | | | 3 | | |

| Susceptibility of native resources - 10 | Colonisation potential - 15 | Disease risk - 11 |
|---|---|---|

Fig 2.5b. Example risk assessment conducted for *Skrjabillanus scardinii* SCORE 15

| Risk Query | V.low or No | Low | Mod | High | V.high or Yes / ? | Reasons for scoring |
|---|---|---|---|---|---|---|
| Score | 0 | 1 | 2 | 3 | 4 | |
| **Value / susceptibility of native resources** | | | | | | |
| Q1. Economic value of the host(s) | | | 2 | | | Specialist of rudd, infected the eyes. Not recorded from any other hosts. Rudd have a moderate economic and ecological value to stillwater fisheries but not endangered or threatened within the UK. |
| Q2. Ecological value of the host(s) | | | 2 | | | |
| Q3. Host threatened or endangered. | 0 | | | | | |
| **Colonisation potential** | | | | | | |
| Q4. Colonisation potential based upon climate matching. | | | | 3 | | *S. scardinii* has a high colonisation potential based upon climate matching. Distributed throughout much of Europe. Moderate colonisation potential based upon life-cycle development (indirect). Strict host specificity and need for branchiuran intermediate host. In view of rudd stocking activity, the potential for spread with fish movements is moderate. Risk of dissemination in the absence of fish hosts is low. |
| Q5. Colonisation potential based upon ecological attributes. | | | 2 | | | |
| Q6. Potential for spread based upon fish stocking activity. | | | 2 | | | |
| Q7. Potential for spread based upon life cycle development. | | 1 | | | | |
| **Potential disease risk** | | | | | | |
| Q8. Disease potential from historic records in other localities | | 1 | | | | Pathogenicity of the parasite is poorly understood. No records of disease despite very heavy infestations and widespread distribution. Pathological studies suggest worms cause minimal tissue damage. Related species *S. tincae* is widespread in UK but not a serious pathogen. No pathogenic members of genus. |
| Q9. Potential pathogenicity based upon literature. | | 1 | | | | |
| Q10. Risk of pathogenic congeners? | | 1 | | | | |

Susceptibility of native resources - 4    Colonisation potential - 8    Disease risk - 3

Fig 2.5c. Example risk assessment conducted for *Anguillicola crassus* SCORE 33

| Risk Query | V.low or No | Low | Mod | High | V.high or Yes / ? | Reasons for scoring |
|---|---|---|---|---|---|---|
| Score | 0 | 1 | 2 | 3 | 4 | |
| **Value / susceptibility of native resources** | | | | | | *A. crassus* is a specialist of eels. The commercial fishery for European eels is probably the highest for any fish in the UK. However, declining eel populations have highlighted the ecological importance and vulnerable status of this species. |
| Q1. Economic value of the host(s) | | | | | 4 | |
| Q2. Ecological value of the host(s) | | | | | 4 | |
| Q3. Host threatened or endangered. | | | | | 4 | |
| **Colonisation potential** | | | | | | *A. crassus* has a very high colonisation potential based upon climate matching, ecological parameters and life cycle development. Despite complex life-cycle, the parasite is known to infect copepods that are ubiquitous within aquatic environments. Over 50 species of paratenic host. Very few man-assisted movements of eels take place in England and Wales, although spread has been linked with transportation of eels for trade. |
| Q4. Colonisation potential based upon climate matching. | | | | | 4 | |
| Q5. Colonisation potential based upon ecological attributes. | | | | | 4 | |
| Q6. Potential for spread based upon fish stocking activity. | | | 2 | | | |
| Q7. Potential for spread based upon life cycle development. | | | | 3 | | |
| **Potential disease risk** | | | | | | *A. crassus* is known to be pathogenic to European eels. Heavy parasite infections can cause severe damage to the swim-bladder. Mortalities recorded in wild and farmed fish. Infections also reduce swimming performance and buoyancy of infected hosts. The genus does not consist of many serious fish pathogens. |
| Q8. Disease potential from historic records in other localities | | | | 3 | | |
| Q9. Potential pathogenicity based upon literature. | | | | 3 | | |
| Q10. Risk of pathogenic congeners? | | | 2 | | | |

Susceptibility of native resources - 12    Colonisation potential - 13    Disease risk - 8

Fig 2.5d. Example risk assessment conducted for *Pellucidhaptor pricei* – SCORE 25

| Risk Query | V.low or No | Low | Mod | High | V.high or Yes / ? | Reasons for scoring |
|---|---|---|---|---|---|---|
| **Score** | 0 | 1 | 2 | 3 | 4 | |
| **Value / susceptibility of native resources** | | | | | | |
| Q1. Economic value of the host(s) | | | | 3 | | *P. pricei* is a parasite host specific to common bream. Bream have a high economic value and moderate ecological value. However, bream have a widespread distribution with the UK and do not have a threatened status. |
| Q2. Ecological value of the host(s) | | | 2 | | | |
| Q3. Host threatened or endangered. | 0 | | | | | |
| **Colonisation potential** | | | | | | |
| Q4. Colonisation potential based upon climate matching. | | | | 3 | | The colonisation potential of the parasite is hard to determine based upon climate matching. Due to very sparse literature on many aspects of biology and distribution, many of these attributes default to a high-risk output. Reproduction likely to be more suited to UK climate that native region (Lithuania). Fecundity thought to be low. Moderate colonisation potential in absence of fish. |
| Q5. Colonisation potential based upon ecological attributes. | | | | 3 | | |
| Q6. Potential for spread based upon fish stocking activity. | | | | 3 | | |
| Q7. Potential for spread based upon life cycle development. | | | 2 | | | |
| **Potential disease risk** | | | | | | |
| Q8. Disease potential from historic records in other localities | | | | 3 | | No information exists on the pathogenicity of *P. pricei*. Sparse information on pathology, disease or impact for any species within the genus prevent assessment of impact. For this reason, these questions default to a high risk scoring. Belongs to group containing a number of important fish pathogens. |
| Q9. Potential pathogenicity based upon literature. | | | | 3 | | |
| Q10. Risk of pathogenic congeners? | | | | 3 | | |

Susceptibility of native resources - 5    Colonisation potential - 11    Disease risk - 9

### 2.3.3. Stage 3 - Risk of impact to fisheries in England and Wales

Whilst Stage 2 provides a rapid assessment of disease potential to guide initial decision making, Stage 3 represents a more structured and comprehensive risk assessment process. This is necessary in view of the limited information that often accompanies parasites at the time of introduction. Due to the time and resources necessary to study impacts of parasites in the wild, both stages are needed in order to guide early management measures, yet progress understanding of the disease threats to fisheries. Stage 3 therefore supports the initial hazard identification process and does not replace it.
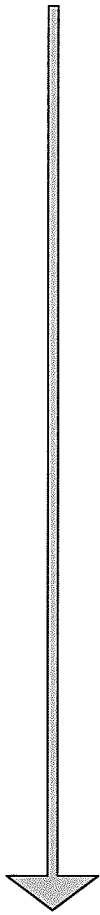
Stage 3 involves the use of defined impact criteria to assess and characterise the potential effects of any parasite to fisheries in England and Wales. This is followed by risk analysis to assess the likelihood of such impacts occurring and causing undesirable economic or ecological outcomes. Stage 3 promotes evidence based-decision making and progression of understanding in the absence of reliable published information.

- **Identification of impact factors for non-native parasites**

Stage 3 identifies the different ways in which a parasite may cause impact (Fig. 2.6) and how these can be measured in the absence of published data (Fig. 2.7a,b,c). This includes consideration of impacts at the individual or host level, increasing in scope to fish populations and ultimately fishery performance. These factors, combined with measures of spread and distribution also provide an assessment of impact at the regional or national level (Table 2.7 a,b,c). These impact criteria include both lethal and sub-lethal changes that may cause ecological or economic impacts to fisheries. This process also provides direction for undertaking further studies, starting with the effect of a

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single parasite on its organ of attachment, before progressing to population and fishery

effects.

Fig. 2.6. Impact factors for determining parasite impact during Stage 3. The arrow shows progression from host level changes to impacts of national importance.

| Level | Impact factor |
|---|---|
| Host level | Pathological changes |
| | Mortality / morbidity |
| | Growth |
| | Condition |
| Population level | Reproduction / Recruitment |
| | Host susceptibility |
| | Distribution of parasites within host populations |
| Fishery level | Productivity |
| | Catchability / Aesthetic value |
| | Profitability |
| Regional / National level | Colonisation potential / rate of spread |
| | Geographical distribution / % waters infected |

Fig. 2.7a. Host level impact factors, how they may be measured and important considerations when making observations.

| Level | Impact Factor | Description | Measurement | Important considerations |
|---|---|---|---|---|
| HOST LEVEL | **Mortality / Morbidity** | Is mortality a realistic potential consequence of parasite infection? | Records / observations of mortality. Examination of moribund fish. Severity of pathology. Experimental studies. | Natural or parasite induced mortality. Role of concurrent infections. Role of environmental conditions. Indirect mortality. |
| | **Growth** | Does the parasite have a negative effect upon the growth of infected hosts? | Scale reading (back calculations of annual growth). Length at age of 0+ fish. Observations of fisheries/farmed pops. | Unknown duration of infection. Need for large sample sizes. Role of environmental influences. Genetic/nutritional influences. |
| | **Condition** | Does parasite infection result in the loss in condition of hosts? | Condition factors from sampled waters. Gross examination of infected hosts. Examination of historic L/W data. | Unknown duration of infection. Need to assess range of infections. Parasites causal or secondary? |
| | **Pathology** | Does the parasite cause pronounced pathological changes in infected hosts? | Histopathology, EM of infected tissues. Observations of gross pathology and clinical disease signs. | Need to observe range of infections. Difficulty with correlating pathology with physiological/behavioural changes to hosts. Host tolerance, role of environment. |
| | **Catchability / Aesthetic value** | Does the parasite affect the catchability (feeding, appetite etc.) or aesthetic value of infected hosts? | Pathogenicity / mode of parasitism. Clinical changes / angler perception. Evidence of angling catch data. | Difficult to interpret, may have to rely upon indicative changes. Differences in angler perception and demand. |

Fig. 2.7b. Population level impact factors, how they may be measured and important considerations when making observations.

| Level | Impact Factor | Description | Measurement | Important considerations |
|---|---|---|---|---|
| **POPULATION LEVEL** | **Reproduction** | Does the parasite have an adverse effect on the ability to reproduce? | Pathology to reproductive organs. Fecundity / experimental studies. Assessment of recruitment in fisheries. Abnormal reproductive behaviour/func. | Not easily detected. Influenced by many env factors. Need for good understanding of reproductive biology of hosts. |
| | **Recruitment (including year class strength)** | Does the parasite have a measurable impact upon recruitment in a fishery? | Fishery surveys. Ageing, year class composition. Host specificity for juvenile hosts. | Hard to quantify. Complex interactions besides parasite. Medium / long term studies needed. |
| | **Host susceptibility** | What is the host range of the parasite and what fisheries are susceptible to impact? | Multi-species sampling infected fisheries. Observations / records of impacts. Experimental studies. | Difference between specificity and host preferences. Varied impacts between hosts. Potential host shifts following intro. |
| | **Population distribution** | What is the distribution of parasites within host populations (prevalence, intensity, dispersal etc)? | Epidemiological studies of infected populations. | Appreciation for seasonality, host specificity etc. Large sample sizes over time. Inclusion of different fishery types. |

Fig. 2.7c. Fishery and national level impact factors, how they may be measured and important considerations when making observations.

| Level | Impact Factor | Description | Measurement | Important considerations |
|---|---|---|---|---|
| **FISHERY LEVEL** | **Productivity of a fishery** | Does the parasite affect the productivity of the fishery? | Surveys of fishery stock Perceived / actual views of fishery owners and anglers. Angler catches. Catch per unit effort. | Variables influencing productivity. Angler perception = impact? |
| | **Catchability / Aesthetic value** | Does the parasite affect the catchability (feeding, appetite etc.) or aesthetic value of infected hosts? | Pathogenicity / mode of parasitism. Clinical changes / angler perception. Evidence of angling catch data. | Difficult to interpret, may have to rely upon indicative changes. Differences in angler perception and demand. |
| | **Profitability of a fishery** | Does the parasite have a measurable effect on the economic development of fisheries? | Economic survey of fisheries. Quantification of lost stock, fishery closure, reduced angling interest. | Perception vs real impact. Considerations for angling effort, fishery types, angling trends. Complex interaction of factors. |
| **NATIONAL LEVEL** | **Geographical distribution** | How widely distributed is the parasite and how many waters are known to be infected? | Parasitological records. Parasite surveys of fisheries in England & Wales. | Resource limitations/sample sizes. Snapshot sampling/seasonality. Sensitivity/specificity of detection Different scales of water bodies. |
| | **Colonisation potential / spread** | What is the risk of the parasite successfully colonising new fisheries and spreading within England and Wales? | Known attributes for colonisation. Records and trends in distribution since first detected (foci/dispersal). Anthropochore movements of hosts. | Spread difficult to establish unless long terms studies. Resources involved with extensive sampling. Influenced by many variables. |

**Stage 3 Continued.**

In order to determine what is known about a parasite and what needs to be known in order to make an assessment of impact, Stage 3 incorporates use of a matrix formed by placing the pre-defined impact factors against each of the parasites (Fig. 2.8). This forms a general framework to confirm information about the effects of a parasite, whether this is known from published literature or epidemiological, experimental or pathological observations. This matrix also provides a means for identifying and prioritising further studies in order to fill gaps in understanding.

- **Risk analysis based upon impact factors**

Once sufficient information is known about the type, extent and severity of harm caused by a parasite, the impact matrix may be used as a guide for undertaking a risk analysis. This deals with the likelihood of pre-defined impacts occurring and having undesirable effects upon aquatic environments and fisheries. Insufficient information pertaining to any parasite may prevent or limit the robustness of the risk analysis process. In such cases, further studies may be required to improve understanding and reduce uncertainty. The ability to conduct a risk assessment on a particular parasite may be indicated as an output in the final column of the impact matrix as RA (risk assess) or FA (further assessment required).

There is no pre-defined level of understanding necessary before a risk analysis may be reliably undertaken. This is because pathogenicity may be determined from a relatively small number of factors, or require understanding of numerous characteristics before disease risks are apparent. The ability to conduct risk analysis for any parasite therefore remains a relatively subjective process and will always be based upon best available information. In order to recognise this, it is suggested that any risk analysis should

include an indicator of uncertainty (low, medium or high) based upon the amount and quality of data (Copp *et al.*, 2005b).

The risk analysis process should be based upon all available information known about the effects of any parasite. A probability score for high risk (0.5), medium risk (0.3), and low risk (0.1) is applied to three main questions, namely the likelihood of the parasite having an undesirable impact at the host level, the likelihood of a parasite having an impact at the population/fishery level and the likelihood of the parasite spreading and colonising new fisheries (Fig. 2.9). These questions are considered in two stages, dealing first with ecological effects of the parasite and secondly with economic impact. The multiplication of these probability values provides two corresponding outputs, one for the economic risks and one for ecological risks. These two values may then be used to distinguish parasites that pose the greatest risk to fisheries (i.e. those with high ecological and economic risks), from species posing a lesser threat (i.e. low economic and environmental risk) (Fig. 2.10).

Fig. 2.8 Impact matrix to identify and prioritise factors in need of investigation and level of current understanding for each parasite.

| Impact Level | Host Level Changes | | | Population Level | | | Fishery Level | | | National Level | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Impact Factor / Parasite | Mort | Cond | Growth | Pathol | Distrib | Reprod | Recruit | Product | Catch | Profit | Spread | Distrib | Output |
| A. huronensis | | | | | | | | | | | | | |
| M. wageneri | | | | | | | | | | | | | |
| B. acheilognathi | | | | | | | | | | | | | |
| E. sieboldi | | | | | | | | | | | | | |
| E. briani | | | | | | | | | | | | | |
| N. japonicus | | | | | | | | | | | | | |
| P. longidigitus | | | | | | | | | | | | | |
| P. laevis | | | | | | | | | | | | | |
| P. sanguinea | | | | | | | | | | | | | |

Key: **Y** – reliable evidence to say parasite can cause this impact.
**N** – reliable evidence to say parasite does not cause this impact
**?** - Uncertain or unknown, requiring confirmation or further study

**Outputs:** **RA** – Enough information known to conduct risk assessment
**FA** - Further assessment needed before able to risk assess

Fig. 2.9 – Risk analysis process for evaluating the probability of a non-native parasite causing undesirable economic and ecological impacts. This is based upon available scientific information summarised within the impact matrix.

| Risk analysis based upon parasite understanding | Probability | | |
|---|---|---|---|
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact**<br><br>1. What is the risk of the parasite having an undesirable effect at the individual level?<br><br>2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level?<br><br>3. What is the likelihood that the parasite will successfully spread and colonise new fisheries | | | |
| **B Economic impact**<br><br>1. What is the risk of the parasite having an undesirable effect at the individual level?<br><br>2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level<br><br>3. What is the likelihood that the parasite will successfully spread and colonise new fisheries? | | | |
| **A Ecological impact risk analysis**<br>What is the risk of the parasite having an adverse ecological effect on fisheries? | = A1 x A2 x A3 | | |
| **B Economic impact risk analysis**<br>What is the risk of the parasite having an adverse economic effect on fisheries? | = B1 x B2 x B3 | | |

Fig. 2.10. Risk analysis matrix to prioritise the potential impact of a parasite as a factor of ecological and economic disease risks.

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low** (0.001 – 0.005) | **Medium** (0.009 – 0.027) | **High** (0.045 – 0.125) |
| **Economic Risk** | **Low** (0.001 – 0.005) | Low | Medium | High |
| | **Medium** (0.009 – 0.027) | Medium | Medium | High |
| | **High** (0.045 – 0.125) | High | High | High |

## 2.3.4. Stage 4 - Risk management and communication

The final stage of the proposed risk assessment deals with risk management and risk communication. Six management options are proposed based upon the risks identified during the earlier stages of the process (Fig. 2.11). These options are intended to ensure that the management of any parasite remains proportionate to the associated risks of disease. It is recognised that whilst management options have been proposed, the implementation of any measures involving the control of non-native parasites remains the decision of the Environment Agency and falls beyond the scope of this project.

Stage 4 also recognises the need for clear communication between all interested and concerned parties (Covello & Merkhofer, 1993; Bullock *et al.,* 1996; Hodder & Bullock, 1997; OIE, 2004). It is proposed that for non-native parasite invasions to freshwater fisheries in England and Wales, this should include fish health professionals, fishery scientists, the fisheries industry (e.g. British Trout Association, Coarse Fish Traders Association), fish movement trade and all government agencies/departments involved in fish health regulation or environmental protection. These interested bodies should be used to communicate new parasite findings, disease threats to fisheries and any management strategies employed to counter these risks. In addition to this, it is recommended that a specific 'Category 2 Parasite Review Group' be set up to act as an independent consultative panel for decisions regarding the status of Category 2 parasites based upon outputs from the current risk assessment. This group should include experts in the fields of pathology, parasitology, epidemiology and fishery science as well as decision-makers and policy representatives. At the time of writing this recommendation has been implemented with the formation of the Environment Agency Category 2 Parasite Review Group.

Fig. 2.11. Stage 4 – Proposed risk management options for the control of non-native parasite introductions into England and Wales.

| Option | Risk Management | Examples of application |
|---|---|---|
| 1 | Do not implement local measures / support wider legislative controls | • Low risk parasite introduction.<br>• Detection of a notifiable disease.<br>• Detection of a parasite specific to ILFA Category 5 species. |
| 2 | Attempt eradication | • Detection of a non-native parasite at import.<br>• Detection of a non-native parasite in discrete and confined water body.<br>• Detection of a highly pathogenic parasite adjacent to a high risk fisheries. |
| 3 | Do not implement control measures unless clinically diseased. Raise awareness, monitor impacts and reduce disease risks through improved fishery management. | • If a parasite cannot be effectively controlled through restriction of infected fish movements. |
| 4 | Implement temporary fish movement controls based upon initial risk assessment, whilst impact studies are conducted. | • As a holding measure following detection of any non-native parasite.<br>• During the initial impact assessment process. |
| 5 | Implement permanent fish movement controls to susceptible or high risk fisheries | • Parasites with medium/high disease risk, but of specific importance to particular fishery types. |
| 6 | Implement permanent fish movement controls to all fisheries. | • High risk parasite introduction posing a significant threat to all fisheries. |

## 2.4. Discussion

### 2.4.1. Stage 1 – Scope for management

The ability to control or manage any non-native parasite relies upon a variety of biological, practical and legal considerations (Bullock *et al.*, 1996; Manchester & Bullock, 2000; Genovesi & Shine, 2003). The first is to establish whether a parasite is truly non-native, or a native species with a discrete or localised distribution. This can be difficult to confirm with certainty, requiring appreciation for detection effort, geographical bias of sampling, understanding of the existing parasite fauna, taxonomic limitations and distribution patterns (Gibson, 1993; Kennedy, 1993; Cone & Marcogliese, 1995; Holland & Kennedy, 1997). Reasons for parasites evading previous detection include the presence of cryptic life stages, unusual location within a host, latency, restricted geographical distribution or presence within a rarely examined host species.

• **Legislative issues**

In the case of *G. salaris*, national legislative controls combined with contingency plans are already in place to manage the parasite if it were ever detected (Peeler & Thrush, 2004; Environment Agency, 2006a). Although this is the only parasite of fin-fish covered by national legislation, EU disease controls allow member states to apply emergency measures to new and emerging diseases if detected. These legislative powers, if implemented, would over-ride local management policies and fish movement restrictions. Similarly, the detection of a parasite within an exotic fish species may negate the need for specific controls due to the existence of national legislation which already restrict movement of certain fish (Import of Live Fish Act, 1980). Depending upon the risk category of the species (ILFA Category 4 and 5), eradication measures

may also be undertaken, depending upon the corresponding risk for dissemination. In such cases, effective management of an introduced parasite may be achieved by the implementation and support of these legislative measures. However, any parasite detected within an exotic fish would require careful monitoring to confirm host specificity. It is well recognised that parasites can adapt within new environments and undergo host shifts, extending threats to native fish populations (Maldonado & López, 2003).

• **Scope for eradication**

Considerable attention has been given to the benefits, problems and limitations of attempting to eradicate non-native species (Bullock et al., 1996; Knapp & Matthews, 1998; Orueta & Ramos, 2001; UK Defra, 2003). International guidelines for non-native species invasions state that measures to prevent introduction and establishment are generally more cost-effective and environmentally desirable than measures taken following successful colonisation (UK, Defra, 2003). Greater efforts are necessary to eradicate non-native species, if possible and appropriate (Dehnen-Schmutz et al., 2004; CBD, 2001). However, eradication is seldom feasible, practical or legal, and may not represent a long-term solution unless the avenues of introduction are controlled (Bullock et al., 2000; Larsen & Buchmann, 2003).

Eradication requires consideration of many different factors, including the characteristics of the infected water body (river, reservoir, farm pond), dissemination of the parasite prior to detection, the frequency and size of the invading propagule and both ecological and socio-economic costs associated with such measures (Kennedy, 1994; Ruesink et al., 1995; Thrush & Peeler, 2006). Furthermore, eradication may simply not be warranted unless a species poses a significant disease threat. Due to the

nature of extensive fisheries, frequency of fish movements and the almost inevitable delay between the establishment and detection of any introduced parasite, eradication attempts are likely to fail more times than they succeed. Using the example of *G. salaris* in Norway, once a parasite is widely established within river catchments eradication attempts can be extremely expensive and problematic (Steinkjer & Bremset, 2004).

Despite these limitations, the eradication of a non-native parasite may represent a realistic option under certain conditions. Local eradication attempts may be considered if a pathogen is detected in, or close to an environment of high ecological or economic importance (e.g. a small stillwater connected to the Lake District). In such cases, eradication should not be automatically discounted due to practical difficulties or evidence of historic failings. This is consistent with current measures employed for the control of non-native fish, where top-mouth gudgeon *Pseudorasbora parva* populations have been successfully eradicated from a number of high risk environments within England and Wales (Britton *pers. comm.*).

- **Detection**

The capacity to effectively control an introduced parasite relies strongly upon the ability to detect it within infected populations (McGladdery, 2000; Cameron, 2002; Culloty *et al.*, 2003). This includes the availability, cost and efficacy of diagnostic methods, as well as the costs of sampling and examining large enough samples of fish to ensure acceptable confidence of detection (Thrusfield, 1995; Kay *et al.*, 1999; McGladdery, 2000; Cameron, 2002; Culloty *et al.*, 2003). The presence of cryptic life stages, latency within host populations, existence of a parasite at a very low prevalence or inhibitively expensive diagnostic methods can represent significant problems for detection and thus

implementation of effective disease controls. Current taxonomic difficulties also makes the identification of species within some parasite groups problematic (Shinn *et al.*, 2001; Harris, 2003; Lom & Dykova, 2006).

- **Ecological considerations**

The ecological characteristics of a parasite can determine whether fish movement restrictions represent effective means for limiting further spread. This would not be feasible if a parasite were naturally dispersed with movements of a piscivorous bird host. Such examples includes the detection of the digenean parasite *Hysteromorpha triloba* (Rudolphi, 1819) (Environment Agency, unpublished) that utilises fish as an intermediate host but has spread widely throughout England with the natural dispersal of cormorants *Phalacrocorax carbo* (L.) which serve as a definitive host (Nasincova *et al.*, 1993). Whilst it may still be necessary to identify the dangers of such parasites and communicate disease threats to interested bodies, little scope would exist for successfully preventing natural dissemination.

### 2.4.2. Stage 2 – Hazard identification

The first step in any risk assessment process is to identify a particular hazard (Covello & Merkhofer, 1993). Effective control of any introduced parasite relies upon this information to enable rapid responses to detection (Ciruna *et al.*, 2004; Peeler *et al.*, 2006a). It is proposed that Stage 2 will aid this initial decision-making process with a transparent and consistent assessment of disease risk and colonisation potential. The use of a scoring system produces an easily interpreted output, based upon pre-defined questions for disease potential. This process is primarily based upon impact history and identification of attributes that favour successful colonisation (Kennedy, 1993, 1994; Lambert, 1997). For well-documented parasites this approach may provide a relatively

robust foundation for predicting initial disease threats to fisheries. However, a paucity

of published information pertaining to any parasite would increase levels of uncertainty.

The automatic default of uncertainty to that of high risk is consistent with previous risk

assessment models and is based upon a precautionary approach (Copp *et al.*, 2005b).

This is considered necessary if the best available scientific information cannot assess

risks with sufficient confidence to inform decision-making (Hickley & Chare, 2004). In

such cases, very poor understanding of a parasite may result in an initial over-

estimation of potential impact. However, as Stage 2 is followed by a more

comprehensive process for impact assessment (Stage 3), this represents a more

desirable outcome than an initial under-estimation of pathogenicity. This is supported

by the 'guilty until proven innocent' approach to non-native species invasions (Ruesink

*et al.*, 1995; Ciruna *et al.*, 2004).


- **Susceptibility and economic value of native resources**

In order to identify the impact of an introduced parasite there is a need to consider the

susceptibility and economic value of native resources. This is gained through

understanding of the parasite's host range and the corresponding importance of these

species to fisheries in England and Wales. The separation of ecological and economic

factors (Q1 & Q2) accommodates hosts that may hold little economic importance to

fisheries, yet may be ecologically sensitive (e.g. crucian carp *Carassius carassius* L.).

In such cases, even relatively small effects of a parasite may have significant ecological

consequences, especially if the host is already threatened or in a stressed state (Q3).

Conversely, a parasite may have massive economic implications yet may be of little

ecological importance to fisheries (e.g. a parasite specific to common carp *Cyprinus*

*carpio* L.). The greatest risk would arise from a parasite with either wide host range (i.e.

potential impact to a wide range of fisheries), or a parasite that is specific to a host with

both high economic and ecological status, e.g. the European eel *A. anguilla* (Starkie, 2003).

- **Colonisation potential and spread**

Colonisation potential is a measure of the ability of a parasite to establish and potentially spread to new environments (Kennedy, 1994). Whilst the attributes of a successful coloniser are numerous, the proposed risk assessment broadly groups these into two for simplicity (Q4 & Q5). The first considers the environmental and climatic conditions of donor and recipient localities. The importance of climate warming is recognised at this stage (Marcogliese, 2001). This follows the widely used 'climate matching' approach applied to other risk assessment models (Pheloung *et al.*, 1999; Copp *et al.*, 2005b). This is based upon the assumption that similarities in environmental conditions will increase the risk of successful colonisation (Kennedy, 1994). However, whilst this is well recognised for non-native weeds (Pheloung *et al.*, 1999) fish (Copp *et al.*, 2005b) and many other taxa (UK Defra, 2005), it should be recognised that for fish parasites, the host itself as well as the aquatic environment forms the environment of the parasite. For this reason, colonisation potential is influenced by many host, parasite and environmental factors. These include the genetic characteristics of both parasite and host (Bakke, 1991; Schrag & Wiener, 1995; Chevassus & Dorson, 1990; Wakelin, 1992; Cunningham, 2002), composition and species richness of the native parasite fauna (Kennedy, 1990, 1994), location and transmission strategy of the parasite (Kennedy, 1976) and presence or absence of available niches (Kennedy, 1993; Parker *et al.*, 1999).

The second component of colonisation potential (Q5) concerns the life-cycle of the parasite, host specificity and reproductive capacity. These attributes strongly influence

the ability of parasites to overcome the many natural barriers to colonisation (MacArthur & Wilson, 1967; Kennedy, 1976; 1994; Kirk, 2003). Whilst detailed information of parasite fecundity may not be known, colonisation potential can be predicted on the basis of broader attributes including complexity of life-cycle, production of infective stages, distribution of intermediate hosts, reproductive strategies and host range (Kennedy, 1976, 1993, 1994; Lambert, 1997). Records of parasite distribution in other geographical regions may substantiate colonisation potential (Korting, 1974; Hoffman & Schubert, 1984; Gozlan et al., 2006).

Live fish movements represent the most important route for the dissemination of non-native diseases (Michel et al., 1986; Kennedy, 1994; Midtlying, 2006; Thrush & Peeler, 2006). These activities can facilitate the rapid spread of parasites to new environments thus increasing disease potential (Sinderman, 1993). This potential is acknowledged in Stage 2 by the number of host movements conducted annually (Q6). This information is collated on the Environment Agency-Cefas, Live Fish Movement Database (LFMD) and includes records of fish farm and fishery stocking activity (LFMD, 2006). The number of fish movements conducted annually is correlated to the economic value of the host species for aquaculture and fishery stocking. Parasites that utilise hosts of high economic value (e.g. common carp -1,600 movements annually, or rainbow trout - 2,200 movements annually, LFMD, 2006) may, therefore, be considered high risk for colonisation and spread. Conversely, hosts that are less frequently moved between waters (e.g. silver bream -7 movements annually) represent a far lower risk for disease transfer.

Understanding the colonisation potential of any parasite requires information on natural dispersal (Q7) as well as anthropogenic translocation. Parasites that can survive for

periods in the absence of a host, infect a ubiquitous water-borne host, or have free-living life stages provide additional avenues for dissemination (Kennedy, 1993). This includes movement with angling equipment, water transfer, aquatic animals and aquatic vegetation. Whilst the risk of such transfer may be far lower than the direct movement of infected hosts (Chubb & Yeomans, 1995; Peeler *et al.*, 2006), such factors also provide potential routes for parasite establishment .

- **Potential disease risks**

Impact history is widely used in biological risk assessments to predict invasiveness of many non-native taxa (Pheloung *et al.*, 1999; Kolar & Lodge, 2002; UK Defra, 2005). This information may be gained from historic records of disease (Q8) as well as observations of pathology or experimental study (Q9). In the absence of published literature, an indication of a parasite's invasiveness and impact may be gained from its congeners (Q10) (Ruesink *et al.*, 1995; Moravec, 1994).

Although impact history can be a reliable predictor of disease potential to new localities, the absence of historic disease records do not confirm a parasite is benign (Kennedy, 1994). The translocation of many herbivorous insects and plant pathogens, benign in their native ranges, have resulted in some of the most devastating examples of non-native species invasions (Cox, 1999; Ricciardi, 2003). Consequently, impact history and pathogenicity of congeners are factors that must be interpreted with caution. The introduction of *G. salaris* to Norway in the 1970s was accompanied by little information to suggest the parasite was pathogenic. At the time of introduction general scepticism surrounded the role of monogeneans as disease agents of wild populations (Harris *pers. comm.*). This emphasises that risk can never be eliminated completely, irrespective of how much information is known about a species prior to introduction

(Sinderman, 1993). Consequently, an assessment of disease risk based solely upon impact history may be misleading and cannot alone be used as a foundation to justify effective and sustainable policy decisions. This requires a more comprehensive and structured process of impact assessment.

### 2.4.3. Stage 3 – Impact assessment

Determining risk depends on specifying a suite of possible effects and then identifying the likelihood and magnitude of these changes occurring (Covello & Merkhofer, 1993; Ruesink *et al.*, 1995). However, it is widely recognised that impacts of non-native species can be very difficult to assess during initial establishment within a new environment (Gilpin, 1990; Moyle, 1999; Smith *et al.*, 1999; Ciruna *et al.*, 2004; Hickley & Chare, 2004). Similarly, defining the criteria with which to assess the negative consequences of a species invasion can be difficult (Copp *et al.*, 2005c). Whilst the attributes for plant 'weediness' or fish 'invasiveness' have received considerable attention (Pheloung *et al.*, 1999; Kolar & Lodge, 2002; Copp *et al.*, 2005b), the factors used to assess parasite impact are less well defined (Kennedy, 1994; Parker *et al.*, 1999; UK, Defra, 2005). Attempts have therefore been made to identify a range of impact factors that may result in undesirable impacts to fisheries. These efforts incorporate both economic and ecological changes and recognise that impacts can occur at different levels of organisation (host, population, fishery) (Kluge *et al.*, 1986; Simon & Townsend, 2002). Efforts to improve understanding of non-native parasites will not only support the management decisions, but promote the wider availability of information should the parasite be moved to other localities (Welcomme, 1988).

Depending upon how well a parasite is understood, considerable time and effort may be needed to measure and evaluate impacts to fish populations. Although specific areas of

investigation may be identified from the impact matrix, this does not represent a rigid process. The relevance of specific impact factors and order with which studies are undertaken will differ between parasites. Similarly, confirming certain impacts may negate the need to understand others. It may not be necessary to identify every impact of a parasite before a reliable risk analysis can be conducted. For example, knowing that a parasite causes severe gill pathology, causes mortality of infected hosts and has rapid colonisation potential may alone justify control measures without the need to identify effects upon host reproduction.

- **Host level changes**

Parasites may cause impacts to fisheries as a result of one or many undesirable effects. These include obvious and drastic changes like mortality (Yamomoto et al., 1984) as well as sub-lethal effects. Poor growth can affect fry survival, year class strength and fishery performance (Paxton & Winfield, 2000; Cowx, 2001; Cowx & Frear, 2004). Condition loss increases susceptibility to disease and natural stressors. This in turn can influence over-wintering survival and reproductive potential of affected fish. Extreme condition loss and gross pathological changes can influence the catchability of fish through reduced feeding. A reduction in aesthetic value, either through deformity or physical marking may also affect the profitability of a fishery as a result of reduced angler satisfaction. Pathological descriptions are necessary to determine the damage of a parasite to host tissues (Ferguson et al., 2006), in turn helping identify host level impacts that may or may not be expressed outwardly. These changes, depending upon their severity can have serious impacts upon fish populations, disrupting fish health, behaviour, population structure and fishery performance (Kennedy & Burrough, 1981; Moore, 1982; Templeton, 1995; Barber et al., 2000; Burdass, 2001; Kirk, 2003; Evans, 2006; Tildesley, 2006).

Theoretically, host level changes are amongst some of the quickest and easiest factors to measure. However, the simple practical task of sampling wild fish in order to measure these effects can alone pose a considerable challenge. Even obvious impacts can be difficult to identify within extensive water bodies. In such environments dead or weakened fish may be quickly removed by predators, water flow and necrophages (Blanc, 1997). Consequently, unless impacts are sudden or severe (Yamamoto *et al.*, 1984) diseases in extensive fisheries may easily go undetected unless long term observations are made (Hedrick, 1998).

The variation in the extent and severity of changes at the host level can create enormous potential for error in estimation of impact (Parker *et al.*, 1999). This problem is exacerbated when changes at the individual level are used to determine population effects. Whilst these observations may be some of the easiest to measure, they are also some of the most important and require careful assessment. For example, obtaining pathological descriptions of a parasite may be relatively straightforward but can be misleading unless observations include a range of host sizes, ages, sexes, intensities of infection and even samples gained from different waters and times of the year (Reimchen & Nosil, 2001; Downing *et al.*, 2002; Roberts, 2004).

## • Population level changes

Population effects caused by parasites can be among the most difficult to measure and interpret (Adjei *et al.*, 1986). Changes that may appear pronounced at the host level may not automatically correlate with important changes at the population level. As an example, mortality is without doubt a drastic host impact. However, mortality of only post-reproductive hosts compared with pre-breeding fish, or loss of only juvenile fish, may have very different consequences to a population or fishery (Lester, 1984). The

impact of a parasite upon host populations therefore requires understanding of host level changes as well as the prevalence, intensity and distribution of parasites within infected populations (Adjei *et al.,* 1986; Hudson & Dobson, 1991). Parasites that effect host reproduction and recruitment also require particular attention as such effects can have significant impacts upon host populations (Hudson & Dobson, 1991; Hudson *et al.,* 2001; Kirk, 2003).

- **Fishery level changes**

Under the remit to maintain, improve and develop fisheries, the Environment Agency has a duty to ensure that all waters in England and Wales are capable of sustaining healthy and thriving fish populations, allowing everyone the opportunity to experience a diverse range of good quality fishing (Environment Agency, 1997, 1998, 2004). Although fishery performance is an important constituent of fishery development (North, 2002; Environment Agency, 2004), it is not easily defined or measured. It is proposed that fishery performance is the success of a fishery to meet the demands and expectations of anglers, as well as fishery owners. This includes an assessment of the number of fish caught, their size and species diversity (North, 2002; Robinson *et al.,* 2003; Environment Agency, 2004). Poor fishery performance can result from both host factors (poor growth, condition, survival) as well as broader population impacts (reduced recruitment, species composition, fish diversity).

Within the current study, fishery performance is determined by the productivity (fish numbers, species composition) and profitability of a particular water (economic success, numbers of fishermen as a measure of angler satisfaction). However, it is recognised that interpreting such factors can be difficult and may vary in complexity depending upon the type of fishery affected and way it is managed. A parasite that

causes undesirable changes in a truly wild population, might not have the same effect to a highly managed commercial fishery. In such waters, chronic fish losses or ecological disturbance resulting from an introduced parasite may be mitigated by the regular stocking activities undertaken as part of routine fishery management (North, 2001, 2002). It is recognised that the current approach to fishery performance may represent an over-simplification of the vast number of factors that influence fisheries. However, the use of two overarching indicators provide consistent and attainable measures by which to assess the impact of a parasite.

- **Regional / national level changes**

The national effects of a parasite may be determined by the population or fishery effects, combined with the distribution of the parasite and potential for spread (Parker *et al.,* 1999). Due to limited resources and the large number of fish movements conducted in England and Wales, confirming the exact distribution of a parasite can be difficult. Current distribution records of parasites in England and Wales generally reflect areas of greatest fish movement activity and thus detection effort (Environment Agency, 1999; LFMD, 2006). However, distribution records alone can provide limited information unless placed into context with the proportion of susceptible fisheries. It is feasible that a parasite present in 20 fisheries may have a greater ecological impact than one in 200 fisheries, if the former parasite infects a greater proportion of a particular fish host. With the growing number and diversity of fisheries in England and Wales, the distribution records of a parasite can be difficult to interpret. However, approximations can be made from fish species distributions (Wheeler, 2000; Environment Agency, 2002; Maitland, 2004), regional angling guides (Environment Agency, 2006b) and fish movement records that current detail species records for over 25,000 fisheries (LFMD, 2006).

## 2.4.4. Stage 3 - Risk analysis

Risk is defined as the probability and consequences of an adverse event occurring, usually within a given time. The current risk analysis process takes what is known about the impact of a parasite and determines the likelihood that such effects will result in undesirable ecological or economic consequences (Williamson, 2001). The separation of ecological and economic impacts allows the specific nature of disease threats to be identified. The assessment of economic and ecological impacts place emphasis upon different impact factors. Primary ecological concerns include species diversity, fry survival, year class strength, natural recruitment and risks to threatened host species. Economic impacts focus more upon financial losses, profitability of fisheries and pronounced host effects like mortality and reduced growth.

By defining the probability of both economic and ecological effects, the overall risk of a parasite may be obtained. This output allows the disease threats of parasites to be identified, compared and prioritised. The proposed risk analysis therefore provides a tool for identifying parasites with the greatest threats to fisheries. This in turn provides a process to justify any fish movement restrictions and ensures that such management measures remain proportionate to the associated risks of a parasite. This provides a transparent and structured process for the categorisation or de-categorisation of parasites based upon evidence of impact and an assessment of risk.

## 2.4.5. Stage 4 – Communication and risk management

Risk management concerns the implementation of appropriate actions to control a pre-defined risk (Covello & Merkhofer, 1993). Stage 4 outlines proposed management options based upon the outputs and disease risks identified in earlier stages of the risk assessment. This promotes a dynamic rather than static process for risk management.

This process also minimises the impact to the fish movement industry through well justified, evidence-based control measures and ensures maximum protection to fisheries by focusing the most stringent controls upon the greatest disease threats.

Risk communication is an essential element of risk analysis (Covello & Merkhofer, 1993; Peeler *et al.,* 2006a). To date, limited understanding of the Category 2 parasites has made it difficult to communicate disease risks, guide fishery owners and raise awareness of the dangers posed by non-native parasite introductions (Environment Agency, 1999). An appropriate level of consultation with stakeholders is a necessity if future controls are to be based upon scientific evidence rather than perception (Robinson *et al.,* 2003). Better education and awareness of threats posed by species introductions were key recommendations from the recent government review of non-native organisms to the UK (UK, Defra 2003). This is recognised in the current system by improving understanding of parasite impacts and the development of a communication network to all interested and concerned parties. The formation of a 'Category 2 Parasite Review Group' also allows the current risk assessment framework to form the foundation for structured, transparent and scientifically justified decision-making.

## Chapter 3 - Application of risk assessment process to the Category 2 parasites

### 3.1. Introduction

Whilst considerable attention has been given to the introduction, colonisation and spread of non-native parasites (Bauer, 1991; Gibson, 1993; Kennedy, 1975; 1993; 1994), relatively little has been given to identifying the impacts of those that have successfully established within the British Isles (Kennedy, 1994; Environment Agency, 1999; Chare *et al.*, 2002). During a review of non-native parasite introductions, Kennedy (1994) suggested that every introduced species must be considered a potential threat, until proven otherwise. However, the same author concluded that it is unreasonable to think that this potential will be realised for every invasion. It is widely accepted within the field of invasion ecology that despite the potential for impact, relatively few introduced species may actually cause serious deleterious effects to native ecosystems. According to Williamson (2001), the introduction of non-native species follows what is termed the 'tens' rule, where only 10% of introductions end with establishment and only 10% of these result in species becoming serious pests. Whilst it is unclear if this applies to parasite invasions, it emphasises the need to evaluate the effects of any introduced species.

Whilst the potential for disease exists for all of the Category 2 parasites, the effects of many species on wild fish populations remain poorly understood (Environment Agency, 1999). Of the 15 macroparasite species currently listed as Category 2, only *Bothriocephalus acheilognathi, Anguillicola crassus, Ergasilus sieboldi* and *Lernaea cyprinacea* L. 1758 are well-recognised pathogens of fish (Kabata, 1970; Andrews *et al.*, 1981; Abdelhalim, 1990; Pike & Lewis, 1994; Haenen *et al.*, 1996; Hoffman, 1999; Kirk, 2003). Whilst a lack of published literature about a parasite does not confirm a

lack of pathogenicity, the fact remains that for over half of the Category 2 parasites considerable efforts are needed to progress understanding of impact. Such efforts are urgently needed if the Environment Agency is to effectively manage future disease risks and develop sustainable policies to protect fisheries.

This chapter was structured around two primary objectives. The first was to review current understanding of the disease threats posed by each of the Category 2 parasites in light of best available information. This involved application of the impact assessment protocol developed in Chapter 2. A foundation of information was collated for each parasite from literature reviews and histopathological investigations. The second objective was to identify and prioritise specific studies necessary to further understand the impact of the Category 2 parasites. This involved a ranking process to determine the parasites most in need of further attention. From this, specific areas of research were identified that would provide the greatest progression of understanding with the time and resources available.

## 3.2. Materials and methods

### 3.2.1. Reducing the number of parasite species studied

At the start of this study, the Category 2 list comprised 15 macroparasites, Koi Herpesvirus (KHV) and the bacterium *Lactococcus garvieae*. This represented too many parasites to effectively review in the time available. In order to narrow the scale of the study, a number of species were omitted from further investigation.

KHV was not included due to the wealth of literature detailing pathogenicity and the clear impact caused by the virus to fisheries (Snow *et al.*, 2003; Way, 2004; Haenen, *et al.*, 2004). *Lactococcus garvieae* was also discounted due to a well-recognised impact to trout populations and detection at only one fishery in England (Royo *et al.*, 2001; Environment Agency, unpublished). The nematode *A. crassus* and copepod *L. cyprinacea* have been the focus of considerable published literature, including reviews of impact, distribution and pathology (Kabata, 1970; Fryer, 1982; Hoffman, 1999; Kirk, 2000b, 2003). Due to this foundation of understanding and active research underway from other workers (Kirk, *pers. comm.*), these parasites were also omitted from the study. *Ergasilus gibbus* von Nordmann, 1832 was not included due to its primary association with estuarine environments (Raibaut & Altunel, 1976; Fryer, 1982). Similarly, *Tracheliastes* spp. were not included due to the very sparse distribution of these parasites (Fryer, 1982; Boxshall & Frear, 1990; Environment Agency, unpublished). Whilst this does not reflect a lack of impact, a localised distribution reduces the restrictions placed upon the fish movement industry. For this reason, parasites with wider distributions were considered of greater priority. The nine parasites that were included in the current study are shown in Table 3.1.

Table 3.1. The Category 2 parasites included within the current study.

| Taxa | Species name |
|---|---|
| Cestoda | *Atractolytocestus huronensis* (Anthony, 1958) <br><br> *Bothriocephalus acheilognathi*, (Yamaguti, 1934) <br><br> *Monobothrium wageneri* (Nybelin, 1922) |
| Crustacea | *Ergasilus briani* (Markewitsch, 1933) <br><br> *Ergasilus sieboldi* (von Nordmann, 1832) <br><br> *Paraergasilus longidigitus* (Yin, 1954) <br><br> *Neoergasilus japonicus* (Harada, 1930) |
| Acanthocephala | *Pomphorhynchus laevis* (Muller, 1776) |
| Nematoda | *Philometroides sanguinea* (Rudolphi, 1819) |

## 3.2.2. Literature reviews

Literature pertaining to each of the Category 2 parasites was collated and reviewed. This included information on identification, morphology, life-cycle development, distribution, epidemiology, pathology, control and management. Areas in need of further investigation were identified and prioritised. Data searches were conducted via internet-based facilities, specifically Cambridge Scientific Abstracts (www.csa.com), Web of Science (www.webofscience.com), Aquatic Sciences and Fisheries Abstracts (ASFA) database, ATHENS (www.athens.co.uk.), Google Scholar (www.scholargoogle.com) and internal databases held at the British Library. Additional information was obtained from angling press, university theses and personal communications.

### 3.2.3. Histopathological studies

Fisheries with historic infections of Category 2 parasites were identified from a database of parasite records held by the Environment Agency, National Fisheries Laboratory, Brampton. Waters with the most recent records of heavy parasite infections were targeted to maximise the chances of gaining infected fish. All fish were killed by immersion in a lethal dose of anaesthetic (benzocaine solution). Fish were weighed and measured and examined for external abnormalities. Small numbers of parasites from each fishery were examined under high power magnification to confirm identification. Tissues with parasites attached were fixed in either 10% Neutral Buffer Formalin (NBF), or Bouins fixative for between 24-72 hours. Further decalcification of hard tissues was performed used 10% formic acid. Samples were trimmed, dehydrated in alcohol series, cleared and embedded in paraffin wax. Sections of between 3-5µm thick were stained using Mayers Haematoxylin and Eosin (H&E). Stained, mounted sections were examined microscopically for pathological changes.

### 3.2.4. Impact assessment

Information gained from the literature reviews and histopathological investigations were used to populate the matrix developed in the aforementioned risk assessment (Chapter 2). Stages 1 and 2 of this risk assessment were not applicable due to the fact that these parasites were already established within England and Wales and were considered to pose a potential hazard to fisheries (Environment Agency, 1999). For parasites supported by sufficient published literature, risk analyses were performed to identify disease potential to fisheries. Less-well studied parasites were prioritised in order to identify those in greatest need of further research.

### 3.2.5. Prioritisation of current Category 2 parasites

A prioritisation process was conducted to identify parasites most in need of further attention. The purpose of this was not to rank parasites in order of disease potential, but to identify species in most urgent need of study due to poor understanding, widening distribution or impact on the fish movement industry. This ranking process involved different environmental, economic and political criteria (Table 3.2). These included parasite distribution (records up to 2004), length of time a parasite had been present within England and Wales, political pressures (e.g. doubt from fish movement industry over justification of controls), current levels of understanding, number of fish movement restrictions enforced annually and estimated cost of these controls upon the fish movement industry. Each parasite was ranked in order of importance either from quantitative values (e.g. number of infected waters) or subjective ranking based upon grey literature, anecdotal evidence or personal communications (e.g. political pressure). A score from 1-9 based upon this rank order was given for each criteria and simply added together to give a total for each parasite. In order to simplify this process, a number of assumptions and generalisation were necessary. These included equal scoring of infected waters irrespective of fishery size or water type (e.g. rivers and stillwaters were both counted as 1 site). Similarly, impact of parasites upon the fish movement trade was assessed from an estimation of the value of fish held up through movement restrictions. This did not take into account more detailed socio-economic values of the fish movement trade.

### 3.2.6. Identification of further studies to progress understanding of impact

In order to improve understanding of the Category 2 parasites, gaps in understanding were identified from the populated impact matrix developed in Chapter 2. Attention was given to studies that were achievable within the allocated time frame, provided the

greatest progression of understanding and contributed the most towards the risk analysis process. However, these investigations were also influenced by opportunities that arose during the study period (i.e. availability of parasite samples, access to infected fisheries).

Table 3.2. Factors used to rank parasites in order of importance for further study.

| Factor | Description / Measurement |
|---|---|
| **Number of recorded waters infected.** | <ul><li>Provides a measure of impact through the number of fisheries infected.</li><li>The more waters infected, the larger the potential impact to both fisheries and fish movement trade.</li><li>Measured as a numerical value from EA distribution records.</li></ul> |
| **Years present within England and Wales** | <ul><li>Provides an assessment of time since introduction and an indicator of how much time has been available for improving understanding.</li><li>The longer time present, the harder it is to justify controls when there is uncertain impact.</li></ul> |
| **Rate of spread** | <ul><li>Indicator of colonisation ability and threat posed to fisheries through dissemination.</li><li>Measured from last 10 years of data (number of waters / time).</li></ul> |
| **Political pressure** | <ul><li>Indicator of pressure from fisheries trade over impact.</li><li>Measurement subjective, based upon internal and external communications (e.g. letters / angling press / *pers. comm.*).</li></ul> |
| **Cost of restriction to fish movement trade** | <ul><li>Indicator of impact of fish movement restrictions upon the fish movement trade.</li><li>Measured as a cost estimate based on health check failures, host specificity and annual fish movements.</li></ul> |
| **Importance to fisheries** | <ul><li>Indicator of importance to different fishery types (e.g. parasite of salmonids would be more important than one of sticklebacks).</li><li>Measured from host specificity and general assessment of fishery importance in England and Wales.</li></ul> |

## 3.3. Results

### 3.3.1. Literature reviews

Literature reviews for each of the Category 2 parasites (Environment Agency, internal documents) were used to populate the impact matrix with a base-line of understanding (Table 3.3). Summary reviews for *A. huronensis, P. sanguinea , E. briani* and *P. longidigitus* are given in each of the following 4 chapters.

### 3.3.2. Histopathological studies

Histopathological descriptions were completed for each of the Category 2 parasites (Environment Agency, internal publication). These studies varied in depth depending upon previous attention and availability of published literature. *B. acheilognathi, E. sieboldi* and *P. laevis* had already been the focus of pathological and pathophysiological studies. Consequently, limited efforts were made to confirm these descriptions. These observations ensured consistency with infections in freshwater fisheries and allowed comparisons to be made in the type and severity of changes between related parasites (e.g. members of the Ergasilidae). Where possible, efforts were made to progress understanding of these parasites through observations in previously unrecorded host species, water types or at different levels of intensity. The pathological changes caused by *M. wageneri, P. sanguinea , E. briani* and *A. huronensis* were described from freshwater fisheries for the first time.

### 3.3.3. Current understanding of the Category 2 parasites

Population of the impact matrix (Table 3.3) confirmed that many of the Category 2 parasites were too poorly understood to provide a reliable assessment of impact. For many of these parasites, literature was very sparse. Exceptions to this included the

ergasilid parasite *E. sieboldi* and the cestode *B. acheilognathi*. These species have prompted considerable amounts of published literature and have been the cause of disease problems following translocation (Andrews *et al.*, 1981; Chubb, 1981; Abdelhalim, 1990; Paperna, 1991; Alston & Lewis, 1994; Lester & Roubal, 1995; Environment Agency, unpublished). This foundation of understanding provided an opportunity to conduct a risk analysis to establish disease potential (Fig. 3.1).

Fig. 3.1. Impact matrix populated with current understanding of the Category 2 parasites. Parasites are listed in order of understanding.

| Impact Level | Host Level Changes | | | | Population Level | | | Fishery Level | | | National Level | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Impact Factor / Parasite | Mort | Cond | Growth | Pathol | Distrib (P / I) | Reprod | Recruit | Catch | Product | Profit | Spread | Distrib | Output |
| A. huronensis | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| M. wageneri | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| P. sanguinea | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| E. briani | ? | ? | ? | ? | Y | ? | ? | ? | ? | ? | Y | Y | FA |
| N. japonicus | N | ? | ? | Y | Y | ? | ? | ? | ? | ? | Y | Y | FA |
| P. longidigitus | N | N | N | ? | Y | N | ? | ? | ? | N | Y | Y | FA |
| P. laevis | ? | Y | Y | Y | Y | ? | Y | ? | ? | ? | Y | Y | ·FA |
| B. acheilognathi | Y | Y | Y | Y | ? | ? | Y | ? | ? | Y | Y | Y | RA |
| E. sieboldi | Y | Y | Y | Y | Y | ? | ? | Y | Y | Y | Y | Y | RA |

**Key:** **Y** – reliable evidence to say parasite can cause this impact.
**N** – reliable evidence to say parasite does not cause this impact
**?** - Uncertainty of evidence requiring confirmation or further study

**Outputs:** **RA** – Enough information known to conduct risk assessment
**FA** - Further assessment needed before able to risk assess

### 3.3.4. Assessment of disease risk posed by well studied parasites

Readers are directed to the literature reviews and histopathological descriptions for a comprehensive summary of information detailing the impact of *E. sieboldi* and *B. acheilognathi*. However, for the purpose of clarity, the following summaries are given for each of these parasites as justification for the subsequent risk assessments.

- *E. sieboldi*

*E. sieboldi* causes severe pathological changes to the gills of infected hosts. This is characterised by loss of gill structure, reduction of respiratory surfaces and disruption to blood flow within the gill. These observations are consistent within both salmonid and cyprinid fish. Heavy parasite burdens are known to cause respiratory distress, disrupt normal blood composition and increase susceptibility to secondary infections. *E. sieboldi* has been the cause of condition loss, reduced growth and mortality in both wild and farmed fish populations. This includes disease of economically and ecologically sensitive species. However, the risk of disease at the population level appears to be influenced by many biotic and abiotic factors. Similarly, it is well recognised that in some water bodies, a considerable delay may occur between the time of introduction and onset of disease.

The parasite has many attributes of a successful coloniser, including a direct life-cycle, wide environmental tolerance, high reproductive potential and low host specificity. The parasite has been recorded from a wide range of water types, including rivers, stillwater fisheries and fish farms. The host level changes combined with high potential for dissemination suggests a relatively high risk of causing undesirable ecological and economic impacts to fisheries (Fig 3.2).

- ### *B. acheilognathi*

*B. acheilognathi* has been the focus of a large amount of literature. The parasite represents one of the best examples of parasite dissemination with the global trade of common carp. Due to the economic importance of the parasite to carp aquaculture, considerable attention has been given to the effects of the parasite at the host level. Pathological changes primarily involve pressure changes within gut, leading to loss of normal gut architecture, thinning of the gut wall and intestinal occlusion. These changes are not restricted to just high intensities due to the inverse relationship that exists between tapeworm size and parasite numbers within the intestinal tract (Read, 1951). *B. acheilognathi* causes disruption to normal gut function, disruption to gut physiology, adverse haematological changes, reduced growth and in extreme cases death. Evidence of host shifts following colonisation of new environments has raised growing concerns of impact to wild fish populations. However, records of disease are more numerous in farmed populations than fisheries.

The parasite has a high natural colonisation potential, assisted by the frequent and large-scale movement of common carp. Although *B. acheilognathi* is predominantly found in juvenile carp, literature confirms a host range exceeding 60 fish species. Due to the growing socio-economic value of carp fisheries, and potential for host shift to native species, *B. acheilognathi* is considered a relatively high-risk parasite to fisheries.

Fig. 3.2. Risk analysis process for evaluating the probability of *Ergasilus sieboldi* causing undesirable economic and ecological impacts to fisheries. This is based upon available published information summarised within the impact matrix. This is based upon the assumption of successful colonisation and establishment within fisheries.

| Parasite species being assessed – *Ergasilus sieboldi* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact** <br><br> 1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | | | 0.5 |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | | 0.3 | |
| 3. What is the likelihood that the parasite will successfully spread and colonise ecologically important fisheries? | | | 0.5 |
| **B Economic impact** <br><br> 1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | | | 0.5 |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | | 0.3 | |
| 3. What is the likelihood that the parasite will successfully spread and colonise economically important fisheries? | | | 0.5 |
| **A Ecological impact risk analysis** <br> What is the risk of the parasite having an adverse ecological effect on fisheries? | **= 0.5 x 0.3 x 0.5 = 0.075** | | |
| **B Economic impact risk analysis** <br> What is the risk of the parasite having an adverse economic effect on fisheries? | **= 0.5 x 0.3 x 0.5 = 0.075** | | |

Fig. 3.3. Risk analysis process for evaluating the probability of *B. acheilognathi* causing undesirable economic and ecological impacts to fisheries. This is based upon available scientific information summarised within the impact matrix.

| Parasite species being assessed – *Bothriocephalus acheilognathi* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact**<br><br>1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | | 0.3 | |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | | 0.3 | |
| 3. What is the likelihood that the parasite will successfully spread and colonise new fisheries | | | 0.5 |
| **B Economic impact**<br><br>1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | | 0.3 | |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | | 0.3 | |
| 3. What is the likelihood that the parasite will successfully spread and colonise new fisheries? | | | 0.5 |
| **A  Ecological impact risk analysis**<br>What is the risk of the parasite having an adverse ecological effect on fisheries? | **0.3 x 0.3 x 0.5 = 0.045** | | |
| **B  Economic impact risk analysis**<br>What is the risk of the parasite having an adverse economic effect on fisheries? | **0.3 x 0.3 x 0.5 = 0.045** | | |

Fig. 3.4. Risk analysis matrix to prioritise the potential impact of *B. acheilognathi* and *E. sieboldi* upon the ecological and economic development of fisheries.

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low** (0.001 – 0.005) | **Medium** (0.009 – 0.027) | **High** (0.045 – 0.125) |
| **Economic Risk** | **Low** (0.001 – 0.005) | Low | Medium | High |
| | **Medium** (0.009 – 0.027) | Medium | Medium | High |
| | **High** (0.045 – 0.125) | High | High | **High** *B. acheilognathi* *E. sieboldi* |

### 3.3.5. Prioritisation of parasites for further study

Prioritisation of the Category 2 parasites indicated that both *P. longidigitus* and *E. briani* were in most need of further attention (Table 3.3, 3.4). This was based upon the need to fill gaps in understanding and not a reflection of potential pathogenicity. For both parasites, considerable impact was being placed upon the fish movement industry as a result of either widespread distribution or long-term presence within the British Isles (Table 3.3). Despite this, understanding of the impacts caused by these parasites remained poor. Conversely, *M. wageneri* and *P. sanguinea* were considered the least important species for further study. This was influenced strongly by their relatively sparse distributions and thus minimal impact upon the fish movement industry.

Table 3.3. Results of the ranking process carried out to identify the Category 2 parasites in most need of further investigation (based on data records up to 2004). (Total rank for each parasite is given, although the rank scores for each criteria are not shown).

| Criteria | E. sieboldi | E. briani | P. longidigitus | N. japonicus | A. huronensis | B.acheilognathi | M. wageneri | P. laevis | P. sanguinea |
|---|---|---|---|---|---|---|---|---|---|
| Number of waters infected in England and Wales | 346 | 232 | 421 | 165 | 35 | 142 | 11 | 40 | 2 |
| Years present in England & Wales (date first detected) | 37 (1967) | 22 (1982) | 10 (1994) | 24 (1980) | 11 (1993) | 25 (1979) | 10 (1994) | (Native) | 21 (1982) |
| Rate of detected spread (within last 10 years) | 9.35/ year | 10.5 / year | 42.1 / year | 6.87/ year | 3.18 / year | 5.68 / year | 1.1 / year | 1 / year | 0.1 / year |
| Political pressure* | 9 | 2 | 1 | 4 | 3 | 8 | 6 | 5 | 7 |
| Cost of controls upon fish movement trade per year (% of total) | 11.36% | 13.62% | 51.2% | 8.57% | 4.88% | 7.99% | 0.8% | 1.47% | 0.1% |
| Potential importance to fisheries | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 2 | 3 |
| RANK | 3 | 2 | 1 | 4 | 6 | 5 | 8 | 7 | 9 |

- **Political pressure** (subjective ranking 1 - 9) – 1 = considerable doubt of impact or industry pressure to confirm impact, 9 = no pressure / doubt regarding impact/threat to fisheries

- ** **Potential importance to fisheries** (ranked 1-3) - 1- weak host specificity/all fisheries susceptible, 2 = low host range or specialist of common carp 3 = specialist of fish not common carp

Table 3.4. Prioritised list of Category 2 parasites in order of those warranting most urgent attention.

| Rank (most important first) | Parasite species |
| --- | --- |
| 1 | *Paraergasilus longidigitus* |
| 2 | *Ergasilus briani* |
| 3 | *Ergasilus sieboldi* |
| 4 | *Neoergasilus japonicus* |
| 5 | *Bothriocephalus acheilognathi* |
| 6 | *Atractolytocestus huronensis* |
| 7 | *Pomphorhynchus laevis* |
| 8 | *Monobothrium wageneri* |
| 9 | *Philometroides sanguinea* |

### 3.3.6. Identification of further studies

In order to improve understanding of the Category 2 parasites, further studies were identified (Fig 3.5). In the light of extensive published information and the ability to conduct relatively robust risk assessments, *E. sieboldi* and *B. acheilognathi* were considered to be of lower priority for further study than other species. For very poorly understood species, in particular *M. wageneri, N. japonicus, E. briani, A. huronensis* and *P. sanguinea*, studies focussed upon host level impacts. For many of these parasites, pathological changes had not previously been described. Consequently, this information required confirmation before progressing to population studies. As understanding of parasites improved, so the remaining gaps in knowledge tended to be in the area of population effects (Fig 3.5).

Fig. 3.5. Impact matrix populated with available information pertaining to each of the Category 2 parasites. Prioritised gaps in understanding are shown by the red circles

| Impact Level | Host Level Changes | | | | Population Level | | | Fishery Level | | | National Level | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Impact Factor / Parasite | Mort | Cond | Growth | Pathol | Distrib (P/I) | Reprod | Recruit | Catch | Product | Profit | Spread | Distrib | Output |
| A. huronensis | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| M. wageneri | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| P. sanguinea | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | Y | Y | FA |
| E. briani | ? | ? | ? | ? | Y | ? | ? | ? | ? | ? | Y | Y | FA |
| N. japonicus | N | ? | ? | ? | Y | ? | ? | ? | ? | ? | Y | Y | FA |
| P. longidigitus | N | N | N | ? | Y | N | ? | ? | ? | N | Y | Y | FA |
| P. laevis | ? | Y | Y | Y | Y | N | Y | ? | ? | ? | Y | Y | FA |
| B. acheilognathi | Y | Y | Y | Y | Y | ? | Y | ? | ? | ? | Y | Y | RA |
| E. sieboldi | Y | Y | Y | Y | Y | ? | ? | Y | Y | Y | Y | Y | RA |

Key: **Y** – reliable evidence to say parasite can cause this impact.
**N** – reliable evidence to say parasite does not cause this impact
**?** - Uncertainty of evidence requiring confirmation or further study

**Outputs:** **RA** – Enough information known to conduct risk assessment
**FA** - Further assessment needed before able to risk assess

## 3.3.7. Prioritisation of further studies

Based upon current levels of understanding and the aforementioned ranking process, four of the Category 2 parasites were chosen for further study. These were *P. longidigitus, E. briani, A. huronensis* and *P. sanguinea* . These studies included host, population and experimental investigations (Table 3.5). The justification for these studies is summarised as followed;

### *P. longidigitus*

Current studies indicate that *P. longidigitus* requires urgent attention to improve understanding. Published information suggests that the strict site preference of *P. longidigitus* for the nares limits disease potential at the individual level. However, it has long been perceived that damage to the nares could reduce the olfactory sensitivity of infected fish. This in turn could have important implications for fish populations by preventing the detection of reproductive pheromones used to synchronise spawning. For this reason the proposed avenue of study for *P. longidigitus* involved confirmation of pathology and potential for reduced olfactory sensitivity disruption to reproduction. Understanding in this area was urgently required due to the extensive distribution of the parasite, the perceived lack of impact that has long surrounded the parasite and to substantiate growing economic costs upon the fish movement trade as a result of fish movement restrictions.

### *E. briani*

After *P. longidigitus, E. briani* was the next parasite requiring studies to establish disease potential. Very little information exists on the impact of *E. briani* to wild fish populations. Epidemiological studies have revealed that the parasite has a predilection

for very small fish and that very heavy infections in farmed and experimental host can cause mortality (Bauer *et al.*, 1969; Alston, 1994). However, the effect of the parasite upon condition, survival and growth of wild fish remains very poorly understood. This represented an important area of research in view of the importance of fry growth and survival to fishery recruitment.

## *A. huronensis*

Although *A. huronensis* was not high on the list of prioritised parasites (Table 3.3), published literature and preliminary histopathological investigations provided little evidence to suggest the parasite was an important pathogen. Damage caused to the gut of infected carp was generally mild and localised. Due to the high economic value of common carp and the rising number of carp movements that take place throughout England and Wales, any restrictions upon trade require sound justification. At this stage in the study, two stillwater fisheries, both infected with *A. huronensis* were due to be de-watered for fishery management purposes. The entire fish populations from both waters were offered to the study. This provided a rare opportunity to progress understanding of the parasite and build upon preliminary studies with both host and population level observations.

## *P. sanguinea*

At a similar time in the study, another opportunity arose to conduct studies upon the nematode *P. sanguinea*. The very sparse distribution of the parasite within England had, up until this time, made it very difficult to obtain infected material for study. The epidemiology and pathogenicity of the parasite was very poorly understood and literature was generally fragmented. This represented an important area of investigation in light of declining crucian carp populations in England (Bolton *et al.*, 1998) and

documented reports of mortality in fry as a result of infections in Europe (Moravec, 1994). Although *P. sanguinea* was ranked as the lowest priority parasite, it was considered important to pursue this opportunity and improve understanding of the threats posed to crucian carp fisheries. Studies were therefore conducted alongside the work on *P. longidigitus, A. huronensis* and *E. briani.*

Table 3.5. Further studies identified to improve understanding of Category 2 parasites.

| Parasite | Reason for study | Area of study? |
|---|---|---|
| *P. longidigitus* | • Growing distribution, thus restrictions upon the fish movement trade.<br>• Perceived lack of impact.<br>• Poor understanding of population effects to substantiate controls. | Does *P. longidigitus* affect the olfactory sensitivity of fish and reduce potential for successful reproduction? |
| *E. briani* | • Records of host impacts under farmed and experimental conditions.<br>• Very poor understanding of impacts to wild fish populations.<br>• Unknown role of parasite upon fry health and fishery recruitment. | Does *E. briani* effect the growth, condition and survival of fry in wild fish populations? |
| *P. sanguinea* | • Recorded pathogen of 0+ crucian carp.<br>• Vulnerable status of crucian carp populations in the UK.<br>• Very poor understanding of general ecology, pathology and impact to fisheries. | Is *P. sanguinea* a threat to the health and survival of crucian carp populations in England and Wales? |
| *A. huronensis* | • High economic importance of common carp to fisheries.<br>• Economic costs of fish movement restrictions<br>• Very poor understanding of impact | Is *A. huronensis* an important pathogen of common carp fisheries? |

## 3.4. Discussion

During a review of non-native species introductions to the UK, Bullock *et al.,* (1996) stated that for every successful translocation, it should be a priority to carry out a full assessment of consequences in the light of best possible information. However, for many non-native parasites, little attention has been given to assessing effects on fisheries. For some of the Category 2 parasites, the current study is the first to assess impacts to wild fish populations. Kennedy (1994) summarised the characteristics of fifteen non-native helminths and crustaceans introduced into the British Isles. Although the author divided these parasites into pathogenic and non-pathogenic species, the basis for this division was not always clear. Kennedy (1994) described *Monobothrium wageneri* Nybelin, 1922 as non-pathogenic, despite a lack of any literature describing pathology or host effects. This assumption goes against literature that indicates a number of *Monobothrium* spp. are capable of severe lesion formation within the gut of infected fish (Mackiewicz *et al.,* 1972). Similarly, Kennedy (1994) also described *Tracheliastes polycolpus* von Nordmann, 1832 as non-pathogenic, despite very little attention given to the parasite within fisheries. Subsequent studies suggest the parasite may be the cause of disease in wild dace populations (Loot *et al.,* 2004). This highlights the importance of comprehensive assessments of impact incorporating host and population level changes. The assumption that a lack of literature about a parasite reflects a lack of pathogenicity is considered dangerous and unreliable. A primary objective of the current study was to collate information on the impacts of the Category 2 parasites and assess the threats posed by these species to fisheries within England and Wales. Despite a number of limitations, current efforts succeeded in progressing understanding of these parasites.

Chapter 3.

The omission of certain parasites from this study was necessary in order to make the project manageable. It was clear from the start of this work that a balance was needed between the depth of understanding gained and the number of parasite species studied. Whilst a number of species fell beyond the immediate scope of this work, the need to progress understanding of their impacts to wild fish populations remains.

Considerable variation was found in the quantity and quality of literature pertaining to each of the Category 2 parasites. In the case of *E. sieboldi* and *B. acheilognathi*, large quantities of information detailed most aspects of parasite biology, life-cycle development, morphology, pathology and control. This provided a relatively reliable foundation on which to assess disease risks to fisheries. In the case of *B. acheilognathi*, the sheer quantity of published literature was in itself difficult to handle, collate and review. Conversely, little published information was found on *P. sanguinea, P. longidigitus, A. huronensis, M. wageneri* and *N. japonicus*. For many of these species, an absence of any pathological or epidemiological data limited an assessment of impact. The production of literature reviews for each of the Category parasites, combined with observations of histopathology provided a valuable foundation of information on which to structure further studies.

The impact matrix provided a useful framework to summarise current understanding, compare impacts of different parasites, identify gaps in knowledge and direct future research efforts. This also supported the risk analysis process, which provided a mechanism for channelling large amounts of information into a single, easily interpreted output. This was illustrated with the parasites *E. sieboldi* and *B. acheilognathi*, both of which represent a high disease risk to fisheries. These parasites, in addition to *A. crassus, L. cyprinacea*, KHV and *Lactococcus garvieae* clearly

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represent undesirable additions to native aquatic environments. However, it is recognised that there are limitations and generalisations associated with this approach.

Although the proposed risk assessment provides a useful indication of disease threat, it does not confirm that a parasite is, or will be the cause of disease to fisheries. It is also recognised that in the absence of direct epidemiological observations, a risk assessment may be based largely upon published information. This can strongly influence the certainty and applicability of the output gained. Whilst it is unrealistic to expect to know everything about a parasite, or that all-available information is obtained from observations in fisheries, it is important to be aware of the quality and reliability of any information used to support decision making. Where possible, efforts are therefore needed to substantiate published literature with observations of impact within wild fish populations. Furthermore, the current impact assessment requires further development to improve understanding of the actual consequences of these parasite invasions (Peeler *et al.*, 2006c). This would involve the measurement or prediction of economic and ecological changes to fisheries on a national scale. This can only be attempted once host and populations level changes are understood.

This work represents the first to evaluate the economic costs of fish movement restrictions placed upon the Category 2 parasites in England and Wales. Although this assessment relied upon a number of generalisations, it revealed a clear relationship between parasite distribution and the financial impact upon the fisheries trade. It is clear that for a number of Category 2 parasites, the restriction of fish stocking activity causes considerable economic impacts. The wide distribution of a parasite, weak host specificity and high commercial value of susceptible fish species serve to increase the magnitude of these costs.

For many parasites, these restrictions were directly correlated with the period of time the species had been established within fisheries. This may be expected in view of the frequency and scale of fish movements to support angling interests. Irrespective of impact, the spread of non-native parasites represents a considerable problem for authorities involved with disease management and regulation. This highlights a number of key issues. The first is to promote the rapid detection of any newly introduced parasite. This is vital if spread is to be minimised. The second is to conduct a rapid risk assessment to highlight disease risks. If eradication is not a feasible option, then efforts must be focussed upon minimising spread. For high-risk introductions, impact assessment must be combined with strict control measures and nationally co-ordinated enforcement. Only then is there a realistic chance of preventing rapid dissemination with fish movement activity. In the current climate of de-regulation, free-trade and limiting resources this poses a serious challenge. However, it is hoped that the use of risk assessment will allow more effective control of the greatest risks. The application of more stringent controls placed upon a smaller number of high-risk parasites is likely to afford better protection to fisheries, minimise impact to trade and promote development of more sustainable policies. Conversely, if a parasite becomes widely dispersed or has an unknown impact, it remains possible that resource costs associated with control and impact upon trade could outweigh the risks of disease. It is this situation that made *P. longidigitus, E. briani, A. huronensis P. sanguinea* essential candidates for further investigation.

## Chapter 4. Importance of *Atractolytocestus huronensis* in common carp fisheries

### 4.1. Introduction

Common carp fisheries represent one of the fastest growing areas of freshwater fishery development in England (North, 2001; Environment Agency, 2004). It has been estimated that of the 30,000 stillwaters fished within England and Wales, over 60% contain carp. A recent review of fishery development conducted by the Environment Agency estimated that angling in England and Wales contributes over £3 billion to GDP. It is believed that as much as half of this may be attributed to common carp fisheries. Of the 3 million coarse anglers within the UK, over 40% regard carp as their primary target species (Environment Agency, 2004). This interest and economic value have lead to growing demands for carp to stock fisheries and an increase in the illegal movement of high-value specimen fish from abroad. The fish movement industry currently conducts over 1,700 legal common carp stockings annually to fisheries within England and Wales (LFMD, 2006). These movements comprise over 1 million fish with an estimated value of over £7 million. In order to support this industry and ensure the continued development of carp fisheries, protection from disease is an important consideration.

The movement of common carp to support aquaculture, the ornamental industry and fishery stocking have facilitated the spread of many parasites far beyond their natural geographical ranges (Bauer & Hoffman, 1976; Kennedy, 1975, 1993, 1994; Paperna, 1991; Dove & Fletcher, 2000; Hoole *et al.*, 2001). During a review of non-native parasite invasions into the British Isles, Kennedy (1994) revealed that the parasites with greatest colonisation potential are those that infect carp. The introduced parasite fauna of common carp in England now exceeds 18 species (Environment Agency, 1999; Kirk,

2000a; Kirk *et al.,* 2003a). Many of these introductions have been recorded within the last 30 years and include representatives from most major parasite groups. Recent examples include a number of intestinal cestodes, namely *Bothriocephalus acheilognathi* (Andrews *et al.,* 1981), *Khawia sinensis* (Chubb & Yeomans, 1995) and *Atractolytocestus huronensis* (Chubb *et al.,* 1996).

*A. huronensis* is an intestinal, caryophyllaeid tapeworm of common carp. The parasite was first recorded in 1993 from a stillwater fishery in Wales (Chubb *et al.,* 1996; Williams, 2005). Little is known about the pathogenicity of *A. huronensis* to carp populations and literature is generally sparse (Anthony, 1958; Jones & Mackiewicz, 1969; Mackiewicz, 1994; Scholz *et al.,* 2001; Kirk *et al.,* 2002, 2003b). It was not until the recent discovery of *A. huronensis* in carp farms of central Europe (Majoros *et al.,* 2003; Oros *et al.,* 2004) that studies have moved beyond those of taxonomic or morphological interest. Despite this attention, considerable uncertainty surrounds the disease potential of the parasite within both wild and farmed populations (Molnar *et al.,* 2003; Oros *et al.,* 2004).

Intestinal cestodes can have varied impacts upon their host, ranging from relatively benign effects to severe pathological changes. A number of species are capable of causing reduced growth, condition loss and in extreme cases, death (Bauer, 1961; Korting, 1974; Hoffman, 1975; Zitnan & Hanzelova, 1982; Shostak & Dick, 1986; Hoole, 1994; Williams & Jones, 1994; Dick & Choudhury, 1995a; Hoole *et al.,* 2001; Shields *et al.,* 2002). Heavy tapeworm burdens can also cause host debilitation, disruption of haematological parameters and disturbance to gut function (Lozinska-Gabska, 1981, Kadav & Agarwal, 1982; Arme *et al.,* 1983; Matskasi, 1978, 1984; Hoffman *et al.,* 1986; Hoole, 1994; Williams & Jones, 1994; Dick & Choudhury,

1995a). However, whilst considerable attention has been given to certain species of economic importance (Scott & Grizzle, 1979; Paperna, 1991; Hoole & Nissan, 1994) many species are very poorly understood. Very little attention has been given to the effects of caryophyllaeid cestodes on wild fish populations or fishery performance (Hoole, 1994; Williams & Jones, 1994). Consequently, there is clear need to improve understanding of the pathogenicity of A. *huronensis* in common carp and evaluate the importance of the parasite to the growing socio-economic development of carp fisheries.

This chapter describes studies undertaken to improve understanding of A. *huronensis* in common carp fisheries. A review of published literature provided an initial assessment of disease potential and highlighted gaps in current understanding. Both field and laboratory-based investigations were conducted to identify the effects of the parasite at both host and population level. Studies at the individual level included descriptions of pathological changes, characterisation of infections within the intestinal tract and effects of the parasite upon host condition. Epidemiological studies conducted on two stillwater fisheries in southern England provided an understanding of the prevalence and intensity of infections, distribution of parasites within infected populations, identification of host preferences and observations of gross disease across a range of parasite intensities. Observations of parasite prevalence from entire fishery populations allowed an assessment of the effectiveness of current sampling protocols used in the control of A. *huronensis*.

The specific aims of the study were:

To collate and review literature on *A. huronensis* in order to establish current understanding of parasite impact, biology, management and disease potential to fish populations.

To describe the pathology caused by *A. huronensis* to the intestinal tract of common carp.

To assess the distribution, prevalence and intensity of *A. huronensis* within common carp populations.

To establish the importance of host species, size and sex upon infections of *A. huronensis*.

To evaluate the potential for disease, condition loss, intestinal occlusion and physiological disruption from *A. huronensis* infections.

To assess the effectiveness of current sampling protocols employed for the control of *A. huronensis* within England and Wales.

To examine the distribution of *A. huronensis* within England and Wales as a measure of potential impact to fisheries.

## 4.2. Materials and methods

### 4.2.1. Literature reviews

Literature reviews were collated as described in Chapter 3.

### 4.2.2. Fish sampling and maintenance

Waters infected with *A. huronensis* were identified from a database of fish-health records held by the Environment Agency (Environment Agency, unpublished). For the purpose of pathology studies, infected fish were obtained from a number of fisheries located within the south-east England. Efforts were made to examine pathological changes at a range of parasite intensities and from a range of host sizes.

During the summer of 2004, it became known that two stillwater fisheries were to be fully drained and de-stocked for the purpose of fishery management. Frenches Pond, Redhill (NGR: TQ 2821 5144) and Mill Pond, Bracknell (NGR: SU 8590 6808), are located in the south-east of England and have historic records of *A. huronensis* (Environment Agency, unpublished). The entire cyprinid populations from both fisheries were donated to this project, providing a valuable opportunity to undertake population studies. All fish were captured by seine-netting or electro-fishing and transported alive to holding facilities at the Environment Agency, National Fisheries Laboratory, Brampton. Although common carp were the primary species targeted during the study, orfe *Leuciscus idus*, crucian carp *Carassius carassius*, goldfish *Carassius auratus* (L.), carp hybrids and ornamental carp variants were also retained for examination. Fish were maintained in either 25 l, 250 l or 30,000 l holding tanks supplied with bore-hole water.

## 4.2.3. Fish examinations

Fish were killed by a lethal overdose of benzocaine solution. Each fish was measured, weighed and examined for the presence of external abnormalities. The body cavity of each carp was opened, allowing fish to be sexed and the intestine to be removed in its entirety. The intestinal tract was opened under a dissecting microscope and examined for parasites. When necessary, phosphate buffered saline (PBS) was used to clear ingesta from the gut to aid parasite detection. The intensity of infection, distribution of worms within the gut and presence of other intestinal parasites were recorded. Parasite distribution within the gut was recorded according to Hair & Holmes (1975). This was measured to assess the potential for intestinal occlusion, and to provide an indication of impact within different regions of the intestinal tract. This involved recording the location of parasites within the gut as a percentage, with the oesophageal opening being 0% and the anus 100%. A number was therefore assigned to each fish based upon how far parasites extended down the intestinal tract.

Areas of infected gut with parasites attached were removed and fixed in 10% neutral buffered formalin (NBF) for histopathological examination. A small number of parasites were removed from each fishery sample to confirm identification. In addition, detailed parasitological examinations were conducted on a small number of fish from each fishery to ensure absence of disease or other important pathogens. The condition factor (K) of both infected and uninfected fish was recorded by the equation $K = 100 \times W/L^3$ (where W = fish weight in grams, L = fork length in centimetres) as stated by Jobling (1995).

## 4.2.4. Histopathology and scanning electron microscopy (SEM)

Fixed samples were trimmed, dehydrated in alcohol series, cleared and embedded in paraffin wax. Sections of 3-5μm thickness were stained using Mayer's Haematoxylin and Eosin (H&E). Stained, mounted sections were examined microscopically for pathological changes. Photomicrographs were taken using a Fuji Velvia film within a Nikon FX-35DX camera mounted on a Nikon Eclipse E400 microscope. Observations of scolex penetration and disruption to intestine surface were aided through scanning electron microscopy (SEM). This work was conducted at the Cefas laboratories, Weymouth. Specimens for SEM were fixed in 10% neutral buffered formalin (NBF), dehydrated in graded alcohol series, critically point dried in $CO_2$, sputter coated with gold and viewed with a Jeol Scanning Electron Microscope.

## 4.2.5. Blood sampling and analysis

Blood samples were taken from both infected and uninfected carp to assess the impact of *A. huronensis* upon physiological condition. Approximately 2 ml of blood was taken from the caudal vessel of each fish with a 5 ml syringe, left to clot and centrifuged at 12,000 rpm for 5 minutes to allow serum removal. Serum samples were immediately frozen and stored at $-20^{\circ}C$. The number of fish examined and range of blood parameters tested was partly dictated by cost and ease of analysis. Parameters chosen for the study were serum protein, glucose, sodium, potassium, alanine amino transaminase and alkaline phosphatase. These parameters were chosen as indicators of general fish health (Stoskopf, 1993; Hoole *et al.,* 2000), normal intestinal function in carp (Bucke, 1976; Bucke *pers. comm..*) and potential disruption as a result of parasite infection (Lozinska-Gabska, 1981; Foott & Hedrick, 1990; Williams & Jones, 1994;

Steinhagen *et al.,* 1997). Blood biochemistry analysis was conducted at Addenbrokes Hospital, Cambridge.

### 4.2.6. Assessment of sample sizes for parasite detection

The prevalence of *A. huronensis* and distribution within infected populations were recorded to evaluate effectiveness of current parasite screening protocols employed for disease controls by the Environment Agency. These observations were used to establish the number of fish needed to ensure detection of a single parasite within an infected fishery using different sample sizes and at different levels of confidence. This was achieved through use of the internet based epidemiological software Win Episcope 2.0 (http://www.clive.ed.ac.uk/winepiscope).

### 4.2.7 Distribution of *A .huronensis* in England and Wales.

Records of *A. huronensis* distribution were collated from a database of fish health records held by the Environment Agency. Records held by independent fish health professionals were not obtained, due to time constraints and issues concerning data protection. Distribution maps were constructed from National Grid Reference (NGR) details with use of the GIS package 'Arcview 3.1' (ESRI GIS and Mapping Software).

## 4.3. Results

### 4.3.1. Literature review of *A. huronensis*

- **Description and taxonomy**

*Atractolytocestus huronensis* (Anthony, 1958) is a caryophyllaeid cestode of common carp. The parasite is native to North America, and was first discovered during routine parasite surveys of an impoundment of the Huron River, Michigan, USA (Anthony, 1958). *A. huronensis* was first detected in the British Isles in 1993 and is considered to be an introduced species (Chubb *et al.*, 1996; Kirk *et al.*, 2002; Environment Agency, 1999). *A. huronensis* is a relatively small, white monozoic tapeworm, measuring 5-20mm in length (Anthony, 1958; Mackiewicz, 1994; Oros *et al.*, 2003). The worm has an unspecialised scolex, which is arrow-shaped when relaxed (*'Atract'* - arrow) (Scholz *et al.*, 2001). The neck of the parasite is distinct and narrower than both body and scolex. The body of the parasite is dorso-ventrally flattened and oval in cross section (Anthony, 1958; Schmidt, 1986; Scholz *et al.*, 2001). The only reliable way to detect *A. huronensis* is by dissection of freshly killed hosts.

It should be recognised that at the time of writing, uncertainty exists over the taxonomic identity of specimens within British Isles. Remarkable similarity exists between *A. huronensis* and the later described Asian parasite *Markevitschia sagittata* (Kulakovskaya & Akhmerov, 1965). This has lead to confusion over the possible synonimity of the two parasites and instigated numerous morphological and taxonomic studies (Hoffman, 1967; Jones & Mackiewicz, 1969; Williams, 1977; Protasova *et al.*, 1990; Mackiewicz, 1994; Kirk & Lewis, 1996; Kirk *et al.* 2002, 2003b; Hoole *et al.* 2001; Oros *et al.*, 2004).

*M. sagittata* differs from the congener species *A. huronensis* only by a greater number of testes (Scholz *et al.,* 2001). However, Kirk *et al.,* (2002) confirmed that specimens obtained from fisheries in southern England possessed a testes count between that used to distinguish the two parasites. Recently, the genera *Atractolytocestus* and *Markevitschia* have been synonimised (Mackiewicz, 1994) although strictly the two species have not (Scholz *et al.,* 2001; Oros *et al.,* 2004). This has fuelled considerable debate over the distinction of the two species, which according to many workers cannot be separated by testes number alone (Chubb *et al.,* 1996; Olsen, *pers. comm.*). Molecular studies to confirm the taxonomic identity of British specimens are underway (Kirk *pers. comm.*). For the purpose of this study, the two species are considered distinct and specimens will be referred to as *A. huronensis*. However, literature that pertains to *A. sagittatus* (syn *M. sagittata*) will be discussed where relevant.

- **Host susceptibility**

Host records indicate that *A. huronensis* is primarily a parasite of common carp (Anthony, 1958, Jones & Mackiewicz, 1969; Mackiewicz, 1972; Rubertone & Hall, 1975; Hensly & Nahhas, 1975; Chubb *et al.,* 1996, Environment Agency, 1999; Hoole *et al.,* 2001; Kirk *et al.,* 2002). A single exception to this was detailed by Amin (1986) who recovered parasites from the sucker *Catostomus commersonii* (Lacepéde) in Wisconsin. However, it is unclear if this finding represents a favoured host record for the parasite, a chance finding or even an erroneous identification.

During a study of *A. huronensis* in Hungary, Majoros *et al.,* (2003) detailed frequent infections of *A. huronensis* in common carp, but absence of parasites in gibel carp *Carassius auratus gibelio* (Bloch), grass carp *Ctenopharyngodon idella* (Valenciennes) and silver carp *Hypophthalmichthys molitrix* (Valenciennes), despite their co-habitation

in the same rearing ponds. The Amur strain of carp *C. carpio haematopterus* (Martens) has been described as a host for *A. sagittatus* (recorded as *M. sagittata*) in Asia (Kulakovskaya & Akhmerov, 1965; Jones & Mackiewicz, 1969). Specimens have also been recorded from ornamental koi carp in Japan (Scholz *et al.*, 2001). Within the British Isles, *C. carpio* is the only documented host of *A. huronensis* despite the examination of other fish species from infected fisheries (Environment Agency, unpublished). In view of this apparent host specificity, any impact caused by *A. huronensis* is likely to be restricted to fisheries containing established populations of common carp.

- **Life cycle development**

The life-cycle of *A. huronensis* has not been described. However, it is generally accepted that the life-cycle of the parasite is likely to be consistent with other caryophyllidean cestodes, where an oligochaete worm serves as an intermediate host (Mackiewicz, 1972). Support for this assumption was given by Mackiewicz (1972) who figured the oligochaete worm *Limnodrilus hoffmeisteri* Claparède infected with a larval procercoid of *A. huronensis*. However, no acknowledgement or reference to this plate was given within the document text. Developmental studies of *A. sagittata* (recorded as *M. sagittata*) confirm that the oligochaete worms *Limnodrilus udekemianus* Claparède and *L. hoffmeisteri* are suitable intermediate host species for the parasite (Demshin & Dvorjadkin, 1981). This further supports the likelihood that life-cycle development of *A. huronensis* is consistent with other members of the Caryophyllidea. Accepting this, it may be assumed that adult *A. huronensis* shed eggs into the lumen of the carp intestine, which are expelled along with faeces into the water. Eggs settling on, or into the benthic substrate are ingested by an oligochaete worm in

which larval development takes place. Carp presumably acquire infection of *A. huronensis* through the ingestion of infected worms (Chubb *et al.*, 1996).

- **Distribution and dissemination**

*A. huronensis* is considered common and relatively widespread in north America (Hoffman, 1967; Mackiewicz, 1972; Hensley & Nahhas, 1975; Rubertone & Hall, 1975; Williams, 1977; Amin & Minkley, 1996). However, records of distribution are generally sparse and fragmented. Within the UK, the parasite was first recorded from a stillwater fishery in Powys, Wales (Chubb *et al.*, 1996; Kirk *et al.*, 2002). Specimens were also recorded from a Hampshire lake later in the same year (Environment Agency, unpublished). *A. huronensis* is considered to be a non-native addition to the UK parasite fauna (Chubb *et al.*, 1996; Environment Agency 1999). However, the avenue of introduction of *A. huronensis* into the British Isles remains unclear.

Until very recently, the only record of *A. huronensis* in Europe was that of British specimens. However, the recent detection of parasites in a Hungarian fish farm has raised awareness of *A. huronensis* and led to subsequent discoveries in Czech Republic, Slovakia and Germany (Majoros *et al.*, 2003; Molnar *et al.*, 2003; Oros *et al.*, 2004; Kappe *et al.*, 2006). Kappe *et al.*, (2006) highlighted the potential for rapid dissemination throughout Europe due to the occurrence of the parasite in many European fish farms.

- **Pathology**

The pathology caused by *A. huronensis* to the intestine of common carp has been included in studies by Mackiewicz *et al.* (1972), Nolan (1994) and Molnar *et al.* (2003). However with the exception of the latter study, descriptions are sparse and limited to

brief observations. During a review of pathology caused by 15 caryophyllidean cestodes, Mackiewicz *et al.,* (1972) concluded that damage caused to the gut of fish is inversely proportional to the degree of scolex specialisation, with the most pronounced pathological changes resulting from worms that lack specialised attachment structures (e.g. hooks, bothria etc). As *A. huronensis* has an unspecialised scolex, some workers have accepted that the damage caused by the parasite may be pronounced (Williams & Jones, 1994; Molnar *et al.,* 2003). Whilst Mackiewicz *et al.,* (1972) provided little detail of pathological changes, descriptions given suggest that the damage caused by *A. huronensis* may actually be relatively mild.

Nolan (1994) compared the pathology of three intestinal tapeworms of common carp, which included histopathological observations of *A. huronensis.* This work was the first to examine infections within the British Isles. However, these observations were restricted to only two infected carp and were confused by concurrent bacterial and parasite infections within these hosts (Nolan, 1994). This was recognised by the author, who stated that further work was necessary before the pathogenicity of the parasite could be evaluated.

The detection of *A. huronensis* in a fish farm in Hungary (Majoros *et al.,* 2003; Oros *et al.,* 2004), prompted the first comprehensive study of pathology to the intestinal tract of infected common carp (Molnar *et al.,* 2003). This study provided descriptions of damage caused to the intestine through scolex penetration, which was considered by the authors to be pronounced. Although this work has contributed considerably to understanding of the parasite, descriptions were made solely from cultured carp fry. It is therefore unclear if these observations are consistent with parasite infections in fishery populations in the British Isles. This work is also limited by potential differences in the

identity of worms described from Europe and England (Kirk *et al.*, 2002). The pathology caused by *A. huronensis* to carp fishery populations in England and Wales requires elucidation before an assessment of disease potential can be made.

• **Impact to fish populations**

To date, no reports of clinical disease or mortality have been associated with infections of *A. huronensis* (Environment Agency, 1999; Molnar *et al.*, 2003; Kappe *et al.*, 2006). The occurrence of parasites in moribund fish from fisheries experiencing mortality events may represent incidental findings, making it difficult to assess the role of these infections on host health, debilitation and condition. Majoros *et al.* (2003) described 100% prevalence in cultured-carp ponds with infections reaching 32 parasites in carp fry. No reference to disease or host impact was given. However, this work does confirm that infected fry were capable of over-wintering with the observed infections (Majoros *et al.*, 2003). No comprehensive epidemiological studies have been conducted on *A. huronensis*. These are necessary to clarify characteristics of infection within host populations and the effects of the parasite at both host and fishery level. Until then, the threat posed by *A. huronensis* to carp fisheries remains unclear.

• **Management and control**

In view of the high economic value of carp, significant attention has been given to the pathogens and diseases that limit their production (Hoole *et al.*, 2001). The use of antihelminthics have been widely employed to control tapeworm infections in cultured or ornamental fish (Brandt *et al.*, 1981; Wildgoose & Lewbart, 2001; Hoole *et al.*, 2001; Mitchell, 2004). However, no specific studies have been conducted on the control, treatment or eradication of *A. huronensis*. The chemotherapeutic control of intestinal tapeworms in extensive fisheries is problematic. There are no licensed drugs

to treat intestinal cestodes of fish in the British Isles (Veterinary Medicines Directorate, 2006). The only recognised method of *A. huronensis* eradication from infected fisheries is through draining, drying and liming (Environment Agency, 1999; Hoole *et al.*, 2001). However, until the pathogenicity of the parasite is clarified, it is debatable whether such disruptive management procedures are necessary. Bauer & Hoffman (1976) emphasised that the easiest and most effective means of tapeworm control is by the prevention of introduction. This is currently supported by the Environment Agency fish movement policy, which promotes parasitological health checks on all fish prior to fishery stocking.

## 4.3.2. Pathological changes caused by *A. huronensis* to the intestinal tract of carp

- **General observations and attachment characteristics**

During the study, *A. huronensis* were always attached to the intestinal tract of common carp. Attachment involved penetration of the parasite's arrow-shaped scolex (Fig. 4.1) deep into the intestine wall. During attachment, the worm's white, un-segmented body extended into the gut lumen allowing relatively straightforward detection (Fig. 4.2). Attachment was often very firm, requiring the use of forceps and even dissection of the intestinal tract to remove individuals without damage. Parasites were primarily found within the anterior third of the intestine, although small numbers of worms were occasionally located in more posterior regions. *A. huronensis* had a dispersed distribution throughout the anterior gut, and did not show any obvious attachment characteristics like clustering (Fig. 4.2). Adult and juvenile worms were often recorded within the same regions of the intestine (Fig. 4.3).
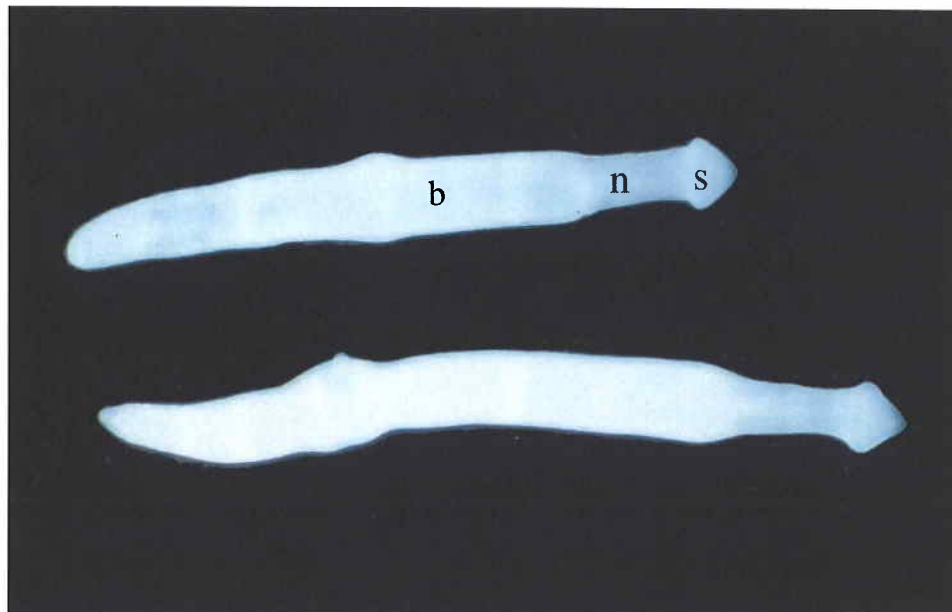


Fig. 4.1.Two relaxed *A. huronensis* detached from the intestine, showing the parasites' characteristic arrow-shaped scolex (s), narrow neck (n) and unsegmented body (b)

Fig. 4.2. Numerous parasites dispersed through the anterior region of intestine of common carp. The scoleces of the parasites are inserted deeply into the gut wall whilst the bodies extend into the gut lumen.
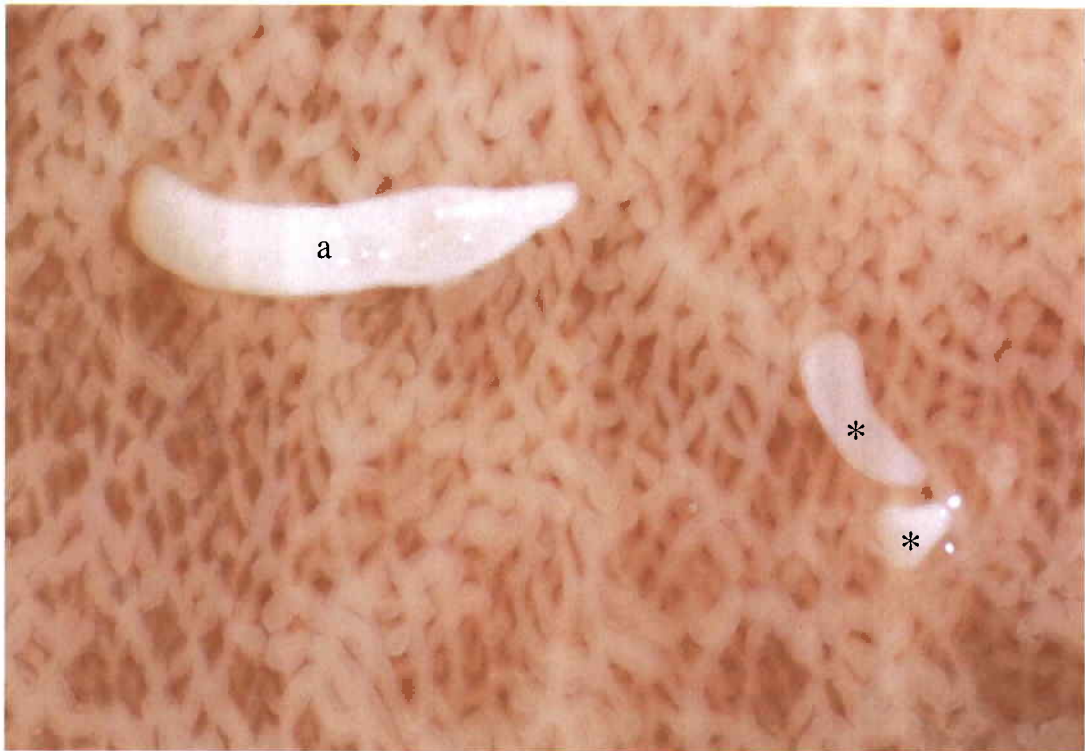


Fig. 4.3. Both adult (a) and juvenile (*) worms attached within intestine. The translucency and smaller body size of juvenile worms made detection more difficult, especially in large hosts where parasites were occasionally nestled deeply between the intestinal folds.

- **Gross pathological changes**

Fish examined for pathology studies showed no gross signs of disease. Prior to dissection of the intestinal tract, infected fish were generally indistinguishable from uninfected hosts. Penetration of the scolex into the intestine did not result in any gross abnormalities to the gut wall.

- **Histopathological changes**

The pathological changes caused by *A. huronensis* to the intestinal tract of common carp could be divided into those caused by scolex attachment and those associated with host responses to infection. For this reason, the following descriptions will be divided accordingly.

During attachment, the scolex and neck of *A. huronensis* penetrated deeply between the intestinal folds into the gut wall (Fig. 4.4, 4.5). Penetration of the scolex extended through the mucosal epithelium deep into the lamina propria (Fig. 4.5). In smaller hosts the scolex occasionally approached the muscularis. Parasite attachment caused distortion of normal gut folding (Fig. 4.5), plus compression of the lamina propria and epithelium (Fig. 4.6) which was particularly pronounced along the sides of the scolex (Fig. 4.6). Whilst the epithelium usually remained intact, localised erosion and necrosis occurred around the sides and tip of the scolex. During infections in smaller hosts, loss and desquamation of epithelium was observed adjacent to the parasite's body (Fig. 4.7).

Around the scolex, attachment also caused damage to the epithelium which occasionally coincided with focal ulceration. Within these areas the scolex was separated from the lamina propria only by the basement membrane (Fig. 4.6). Contact between the scolex of *A. huronensis* and the host tissue was often intimate. This was

characterised by the presence of a thin eosinophilic interface layer between the parasite cuticle and host tissue (Fig. 4.8). On the internal surface of the intestine, erosion and pyknosis of epithelial cells was an occasional finding in smaller hosts. This occurred at the site of parasite entry into the gut (Fig. 4.10 and Fig. 4.11). Localised congestion was often observed in tissues surrounding the tip of the scolex. In a single infection, plaques of bacteria were recorded along the shoulders of the parasite at the point of entry into the gut lumen (Fig. 4.9). In areas removed from the direct site of attachment, the intestine was normal (Fig. 4.12 & Fig. 4.13).

The inflammatory responses caused by *A. huronensis* were generally varied and localised. These reactions did not necessarily reflect intensity of infection and often only mild cellular changes accompanied heavy parasite infections. *A. huronensis* provoked a localised cellular inflammatory response that comprised increased numbers of macrophages, lymphocytes, neutrophils and eosinophilic granular cells (EGCs). Occasionally a mild lymphoid response was recorded on the peritoneal surface of the intestine opposite attached parasites. Two parasite infections resulted in more pronounced inflammatory reactions which included marked lymphocytic infiltrations (Fig. 4.14). During a single low level infection, large numbers of EGC's were recorded migrating through the intestine toward the cuticle of the parasite (Fig. 4.15). These cells surrounded the worm in a distinct halo of inflammatory cells and represented a pronounced inflammatory response (Fig. 4.16).

Fig. 4.4. A single *A. huronensis* attached to intestine of carp, showing insertion of scolex and neck regions into gut wall and extension of body into gut lumen. Scale bar = 1mm

Fig. 4.5. Penetration of arrow-shaped scolex (S) into intestinal wall caused mechanical compression and distortion of intestinal folds (arrows). Scale bar = 200μm
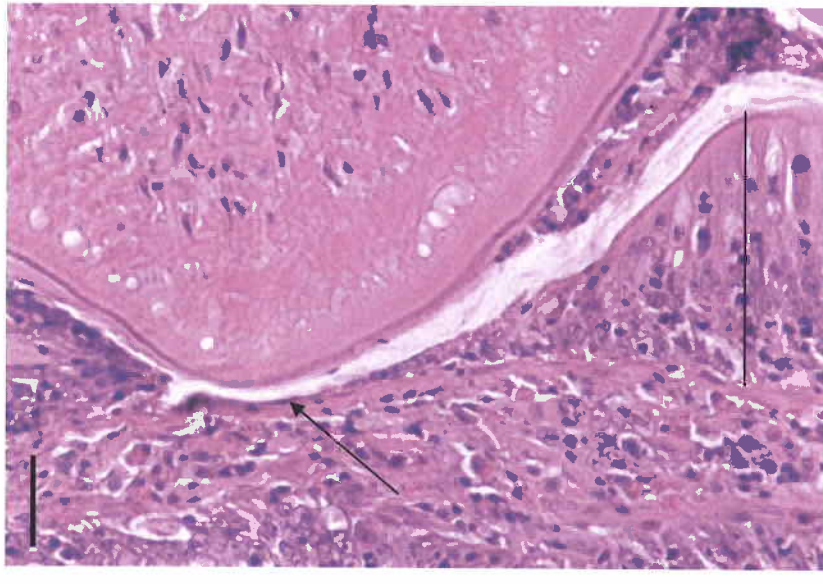
Fig.4.6. Scolex penetration caused compression and loss of normal mucosal epithelium as far as the basement membrane (arrow). Normal depth of the epithelial layer beyond the parasite is shown (bar). Scale bar = 80μm.
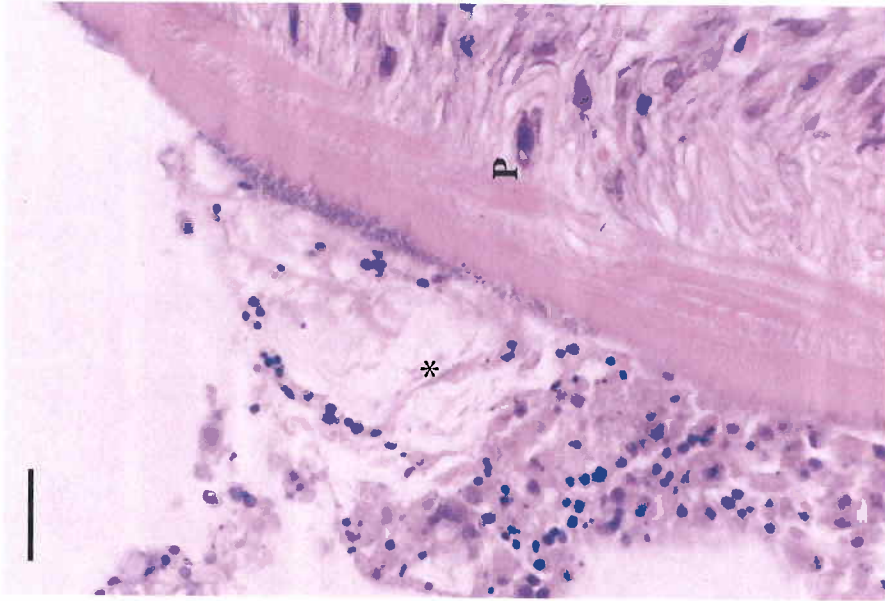
Fig. 4.7. During some infections, bacteria, combined with localised desquamation of epithelium (*) occured at the surface of the intestine where the parasite (P) entered the intestinal crypt. Scale bar = 40μm.
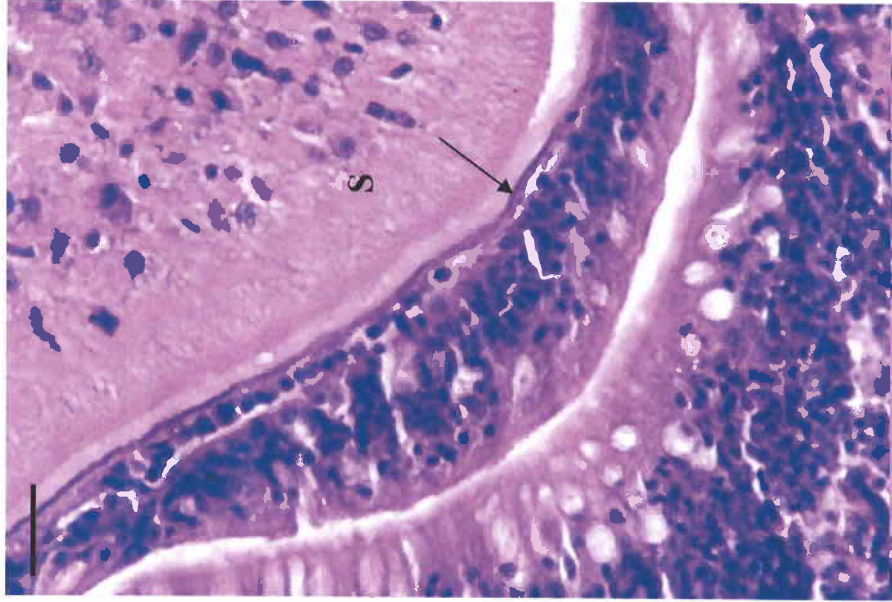
Fig. 4.8. At the point of host-parasite contact, the presence of a thin eosinophilic interface layer (arrow) between the scolex (S) and intestine (I) indicated intimate connection. Scale bar = 40μm.
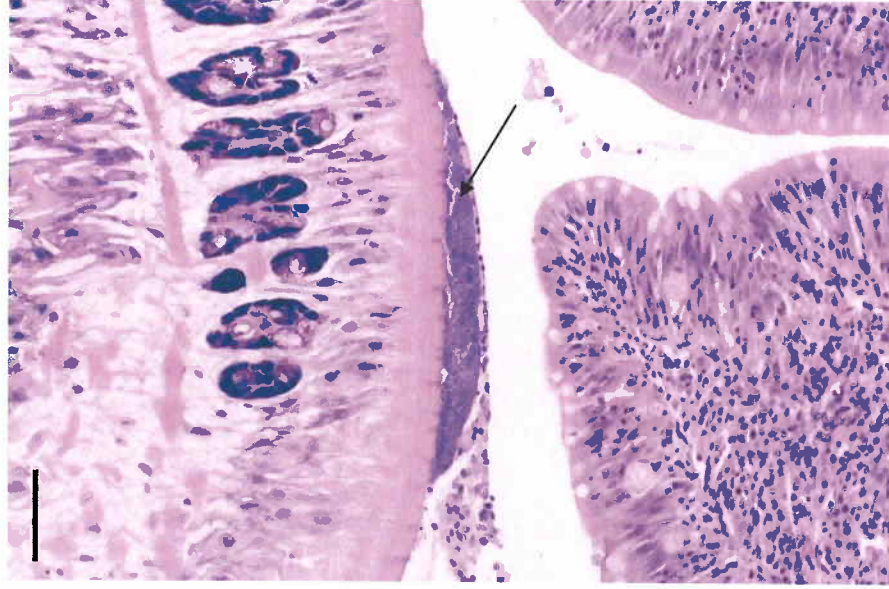
Fig. 4.9. Plaques of bacteria attached to the body of A. *huronensis* within the lumen of the intestine (arrow). Note intact epithelial surface adjacent to the body of the worm. Scale bar = 40μm.
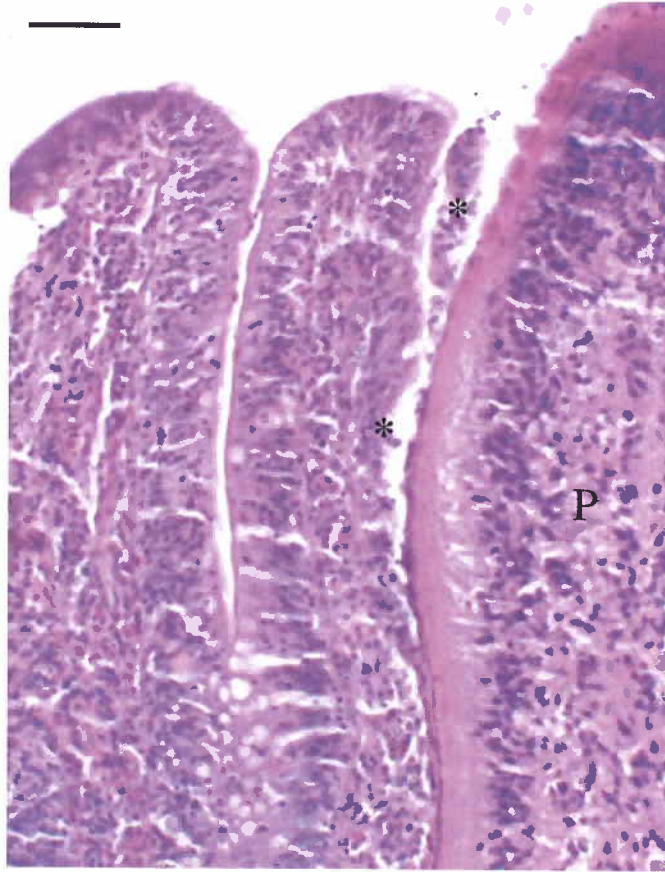
Fig. 4.10. Disruption and loss of epithelium (*) adjacent to the body of the parasite (P) infecting a small carp. Scale bar = 40μm.
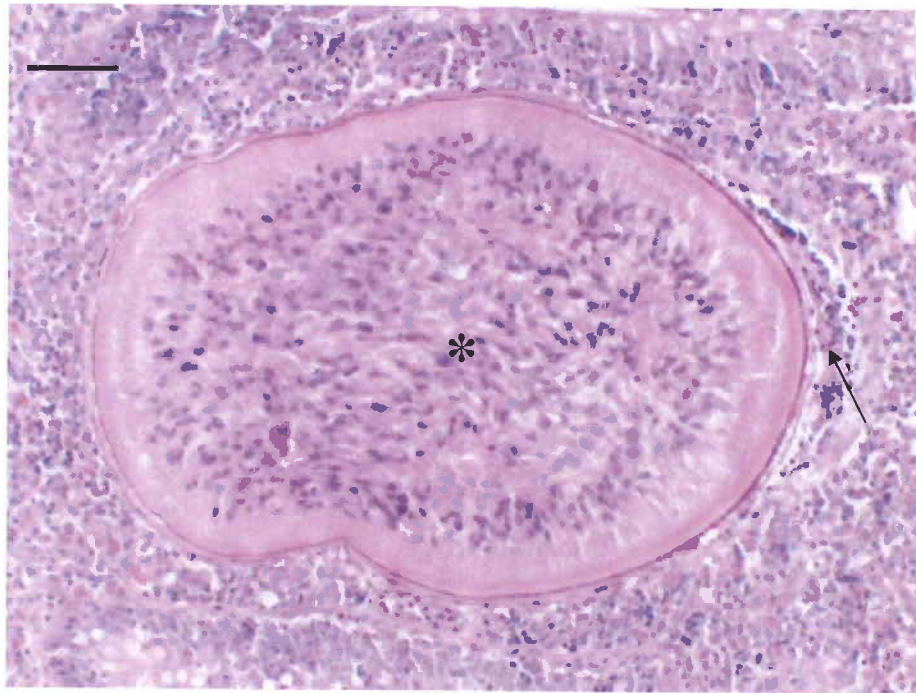


Fig. 4.11. Cross section of *A. huronensis* (*) showing disruption of the cells adjacent to the parasite's cuticle (arrow). Scale bar = 80 μm.
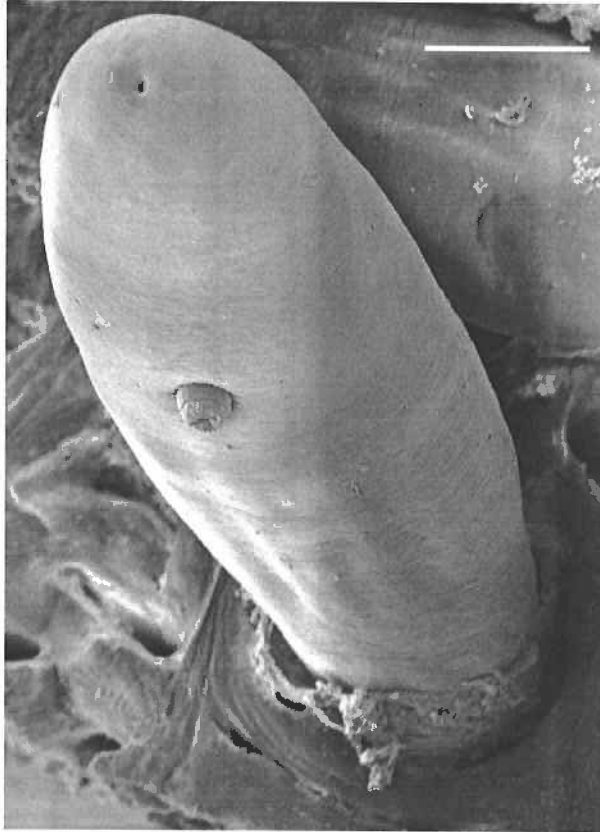
Fig.4.12. SEM of a single *A. huronensis* penetrating the gut wall of a common carp. Scale bar = 0.5mm
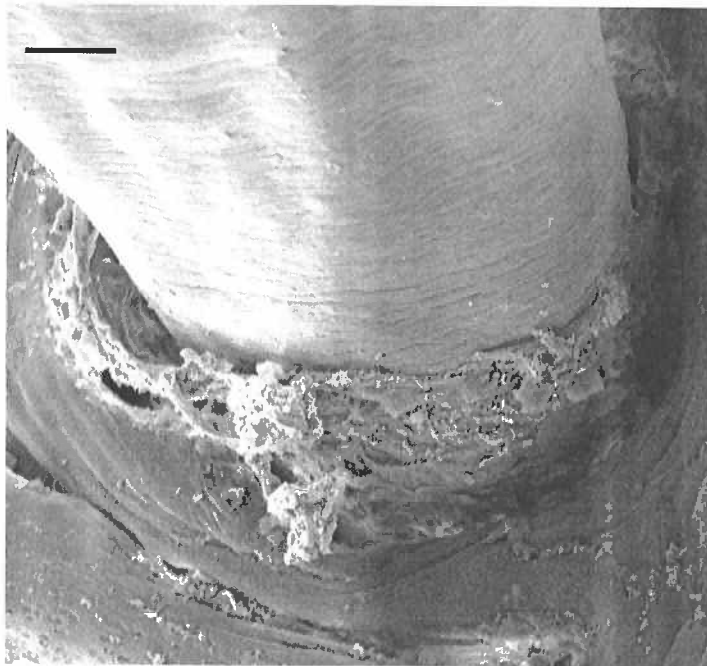


Fig. 4.13. Insertion of the scolex into the gut wall caused localised disruption of the intestine at the site of parasite entry. Elsewhere the gut remained normal. Scale bar = 100μm.
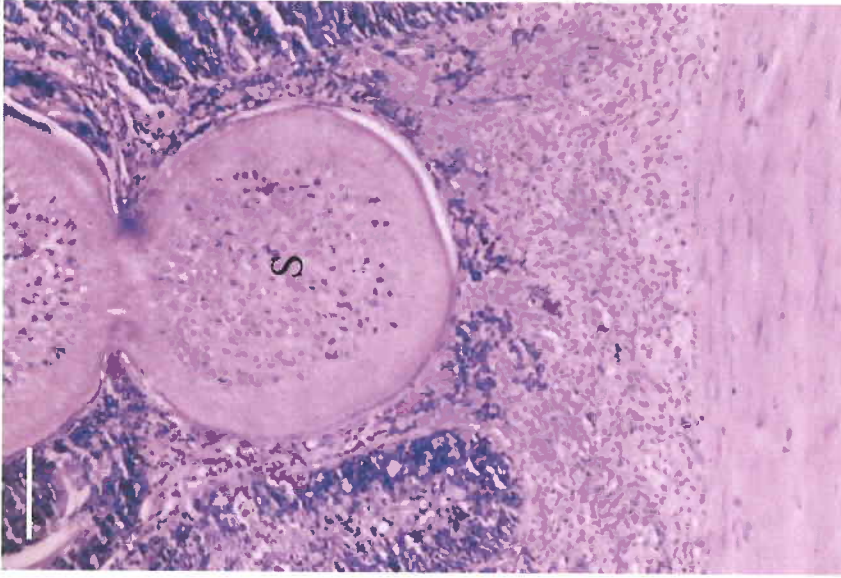
Fig. 4.14. Penetration of the scolex (S) may extend to the lamina propria and provoke a marked inflammatory response with increased lymphocyte activity around the parasite. Scale bar = 80µm.
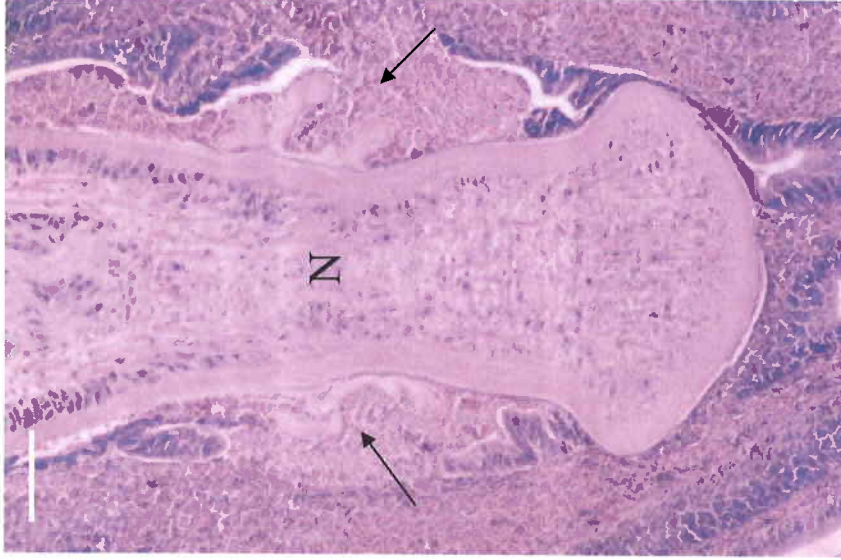
Fig. 4.15. Insertion of the scolex has provoked the migration of numerous EGC's (arrows) through the lamina propria toward the neck of the parasite (N). This represents a pronounced inflammatory response. Scale bar = 200µm.
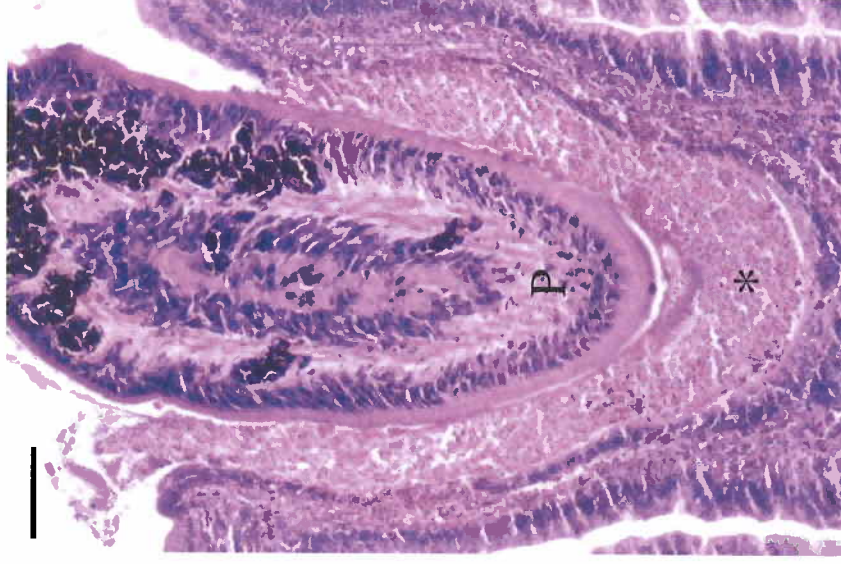
Fig. 4.16. A mass of EGC's surrounding the parasite (P) in a pronounced eosinophilic hollow (*). This represents a pronounced inflammatory reaction to infection. Scale bar = 200µm.

## 4.3.3. Population studies of *A. huronensis* from two carp fisheries

- **Prevalence and intensities of infection**

A total of 371 fish were examined from the two stillwater fisheries studied (Table 4.1).
*A. huronensis* was recorded from both carp populations, although prevalence and
intensities of infection varied considerably (Table 4.2). Parasite prevalence was greatest
at Frenches Pond with 83.5% of the common carp population infected. Intensities of
infection were also greatest within this water, reaching a maximum of 214 parasites
(mean, 23.5 parasites). At Mill Pond, prevalence of infection was 17.3% with a
maximum intensity of 68 worms (mean, 5.3 parasites). Within this fishery only 9 out of
231 fish (3.8%) had infections exceeding 5 worms.

Table 4.1. Infection data of *A. huronensis* from Mill Pond and Frenches Pond.

| Water details (Date) | Fish Species | Number examined | Length range | Prevalence (%) | Intensity range (mean / sd) |
|---|---|---|---|---|---|
| **Mill Pond Bracknell, Berkshire SU85906808** **(23 Oct 04)** | Common carp | 231 | 11.3-70.6 | 40/231 (17.3) | 1-68 (5.3 / 10.8) |
| | Goldfish | 19 | 9.3 – 31.9 | 0 | 0 |
| | Carp/ goldfish hybrid | 1 | 33.1 | 0 | 0 |
| | Crucian carp | 1 | 13.1 | 0 | 0 |
| **Frenches Pond Redhill, Surrey TQ28215144** **(3 August 04)** | Common carp | 109 | 13.1-54.1 | 92/109 (84.4) | 1-214 (23.5 / 34.4) |
| | Orfe | 1 | 17.1 | 0 | 0 |
| | Goldfish | 5 | 13.2-18.1 | 0 | 0 |
| | Carp/ goldfish hybrid | 4 | 16.9-33.5 | 2/4 (50) | 1-21 (5.2 / 10.3) |

- **Host preferences and distribution of parasites within the carp populations**

During the study, four species of cyprinid fish and a hybrid of goldfish and common carp were examined for *A. huronensis*. Common carp were the predominant species sampled during the study, and represented the host in which the majority of worms were recorded (98.5%). Both waters contained very small numbers of orfe, crucian carp and goldfish, preventing a detailed assessment of susceptibility in these species. None of these species was infected with *A. huronensis*. However, two common carp x goldfish hybrids, sampled from Frenches Pond contained both juvenile and adult worms with infections of 1 and 21 parasites respectively. In both waters, *A. huronensis* was shown to be over-dispersed (variance/mean ratio greater than 1) within the common carp populations (Fig. 4.17).
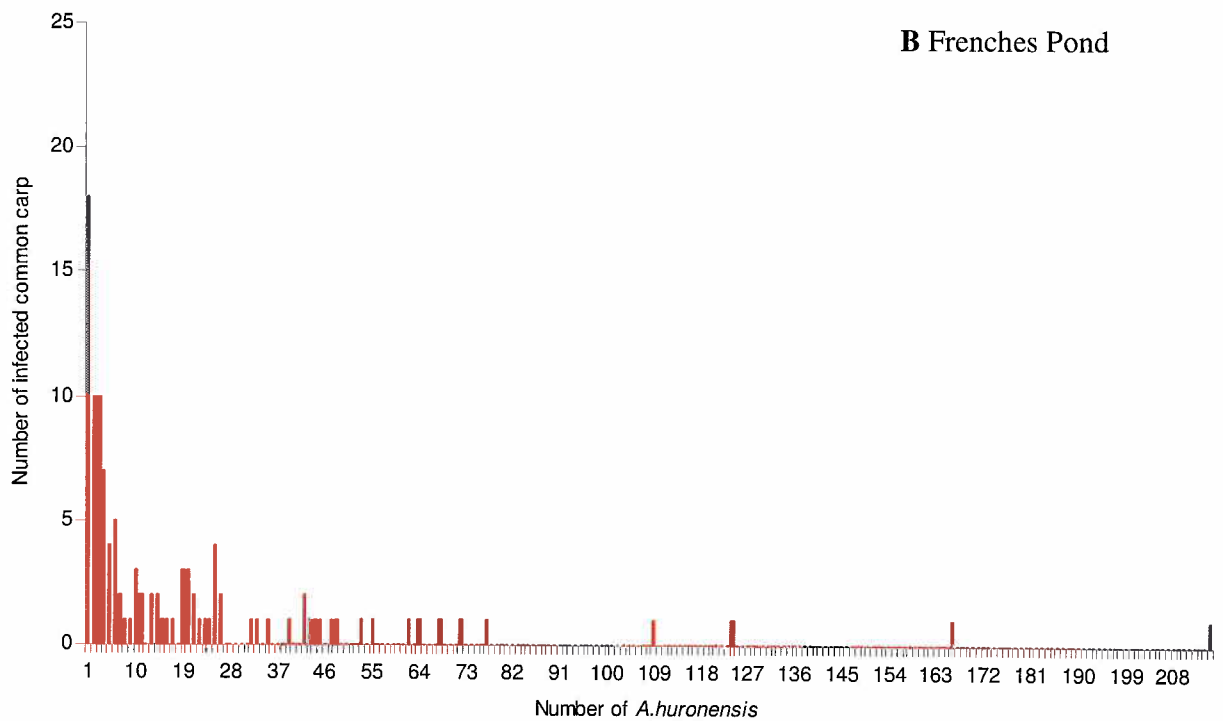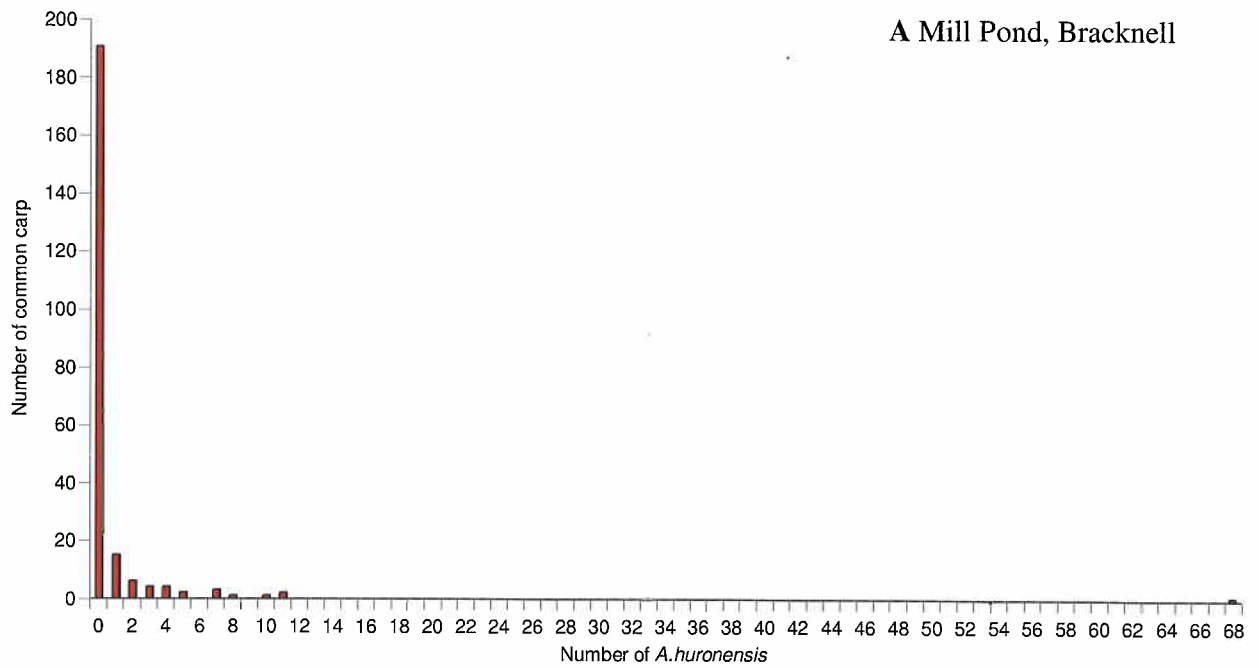
Fig. 4.17. Frequency distribution of *A. huronensis* in common carp from **A.** Mill Pond and **B.** Frenches Pond, both showing a negative binomial distribution. This distribution confirms that *A. huronensis* is over-dispersed within these host populations.

- **Role of host sex upon *A. huronensis* infection**

In both carp populations, host sex did not affect the prevalence or intensity of *A. huronensis*. Parasites were also found in both adult and juvenile hosts. Of the 40 infected carp examined from Mill Pond, 15 (37.5%) were female fish, 13 male (32.5%) and 12 (30%) juvenile hosts. Mean intensity of infection within female carp was 6.8 parasites (intensity range 0-68 parasites), compared with 3.6 in male fish (intensity range 0-11 parasites). Of the 92 carp examined from Frenches Pond, *A. huronensis* were found in 40 (43.4%) male carp and 43 (46.7%) female fish. 9 fish were unsexed or immature. Mean intensity of infection within female carp was 26.28 worms (intensity range 0-214 parasites), compared with 23.7 in male fish (intensity range 0-165 parasites). Host sex did not have a significant effect upon the intensity or prevalence of infection at either of the fisheries studied (Mann Whitney $P<0.05$).

- **Role of host size upon *A. huronensis* infection.**

During the study, *A. huronensis* were recorded in common carp ranging from 13.1 to 70.6cm in length. The heaviest parasite infection recorded from Frenches Pond and Mill Pond were in hosts measuring 34.3cm and 55cm respectively. Due to the low prevalence of infection at Mill Pond and the predominance of large fish within this water, efforts to establish a relationship between host size and parasite infection were focussed upon the sample gained from Frenches Pond. This relationship, using quartiles as a division of host size, is summarised in Fig. 4.18.

Observations from Frenches Pond indicate a general trend of increasing parasite prevalence and mean intensity with host size, peaking at the host range of 32.3 – 36.8cm. Intensity of *A. huronensis* infection was significantly greater within this size

group than the two smaller size ranges (Mann Whitney, P<0.005). However, there was

no significant difference in *A. huronensis* infections between the two largest size ranges

of fish examined. It is recognised that both waters contained very limited populations of

small carp or fry, preventing an assessment of infections within these hosts.
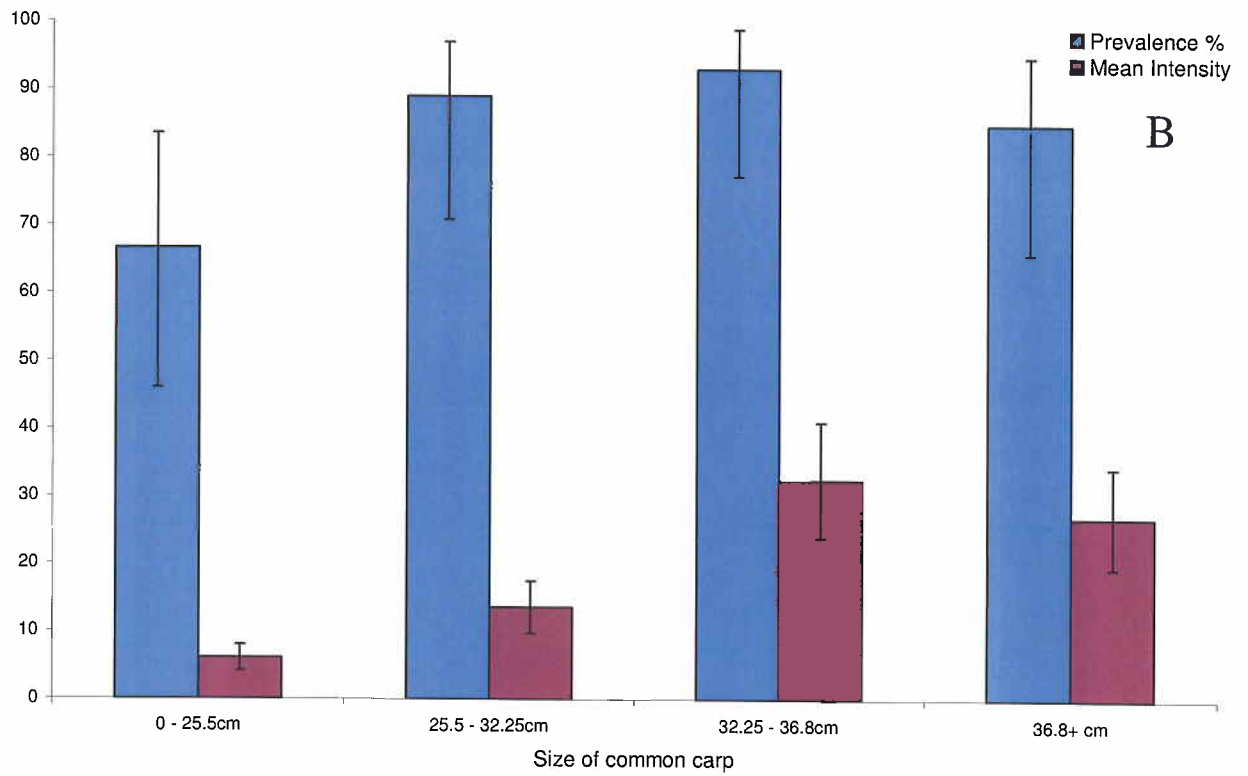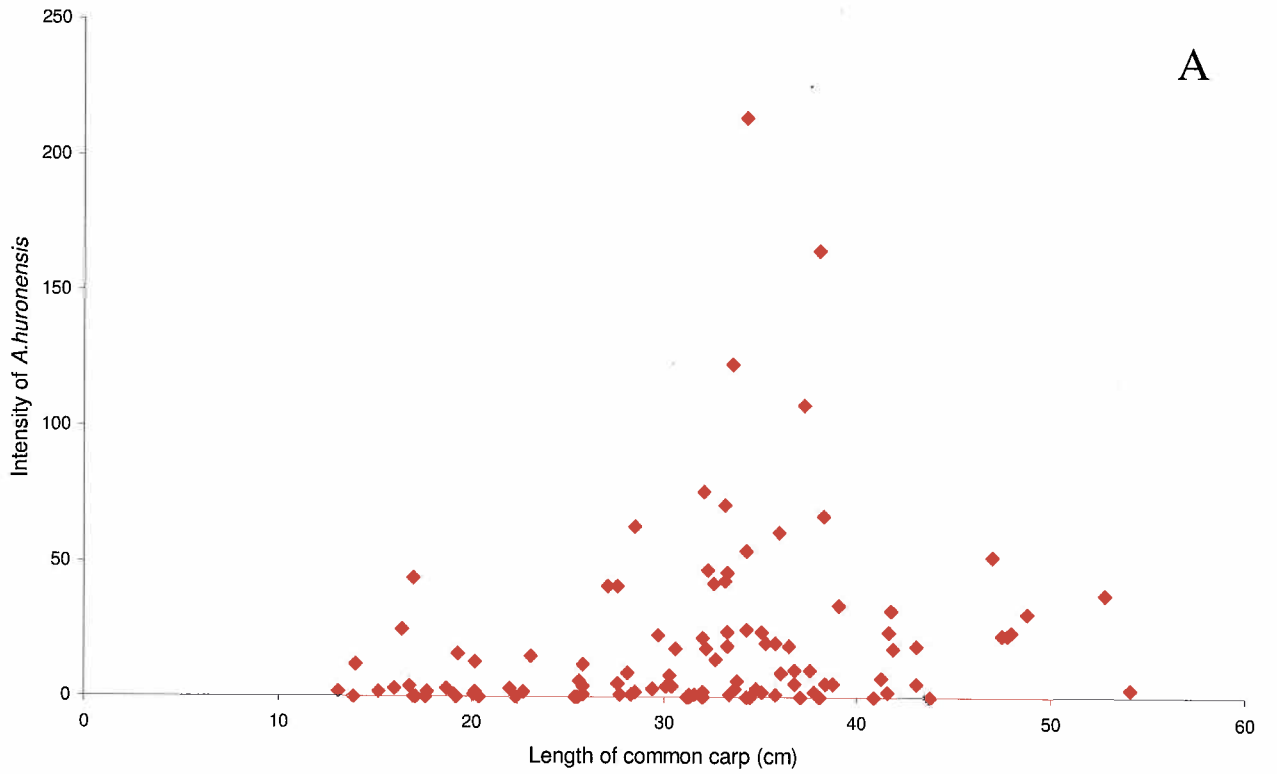
Fig. 4.18. A) Relationship between host length and intensity of *A. huronensis* within Frenches Pond. B) Differences in prevalence (with 98% confidence) and intensity of *A. huronensis* with length of common carp in Frenches Pond (grouped according to quartile of length data).

- **Attachment characteristics of *A. huronensis* and evidence of intestinal occlusion.**

*A. huronensis* attached primarily within the anterior third of the intestinal tract (Fig 4.19). Only 17 (12.8%) out of the 132 infected carp examined had parasites attached posterior to the first bend of the intestine. In only two of these fish were parasites found within the lower half of the intestinal tract. Even within these hosts, the majority of parasites were localised within the anterior region of the gut, with only occasional individuals extending to more posterior regions.

Despite congregation of parasites within the anterior region of the gut, no evidence was gained to suggest that *A. huronensis* causes gut blockage or distension of the intestinal tract. Furthermore, parasites remained relatively scattered within infected areas of the gut and did not show signs of clustering or localised lesion formation. In most of the fish examined, *A. huronensis* occurred as a single infection within the gut of common carp, although light infections of the tapeworm *Khawia sinensis* Hsü, 1935 were recorded as concurrent infections in nine common carp from Mill Pond.

**A   Mill Pond**

Position of *A. huronensis* within the intestinal tract % (0 = oesophagus, 100% = anus)

**B   Frenches Pond**

Position of *A.huronensis* within the intestinal tract % (0 = oesophagus, 100 = anus)
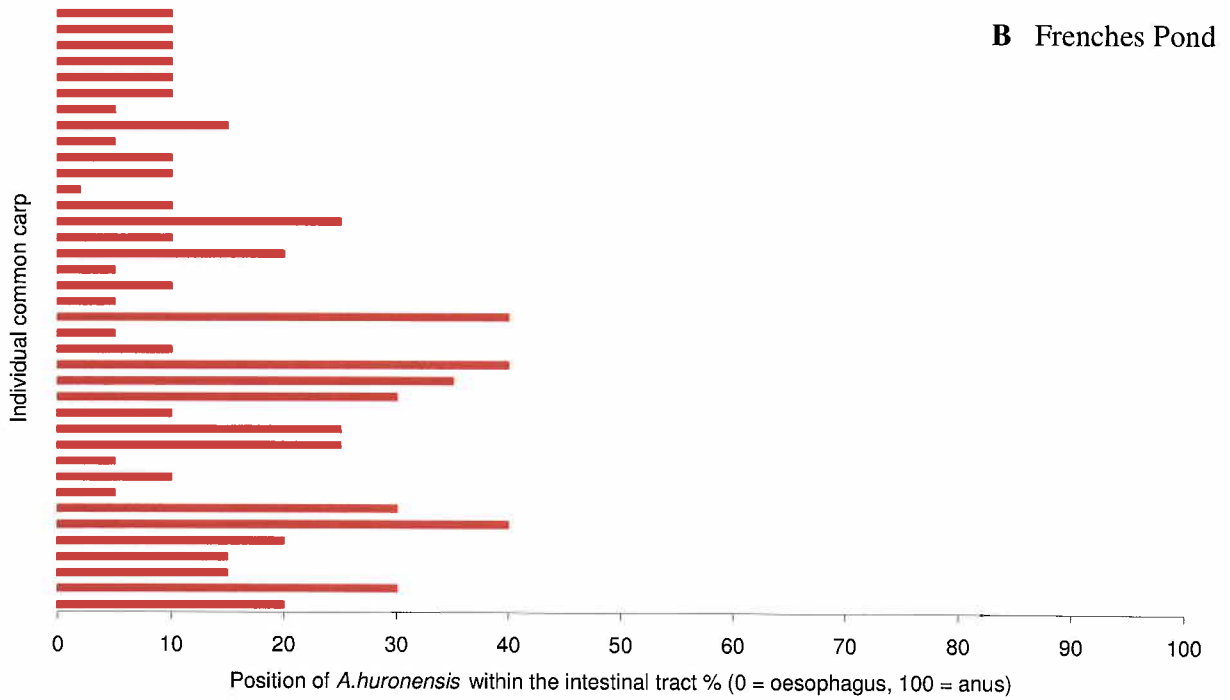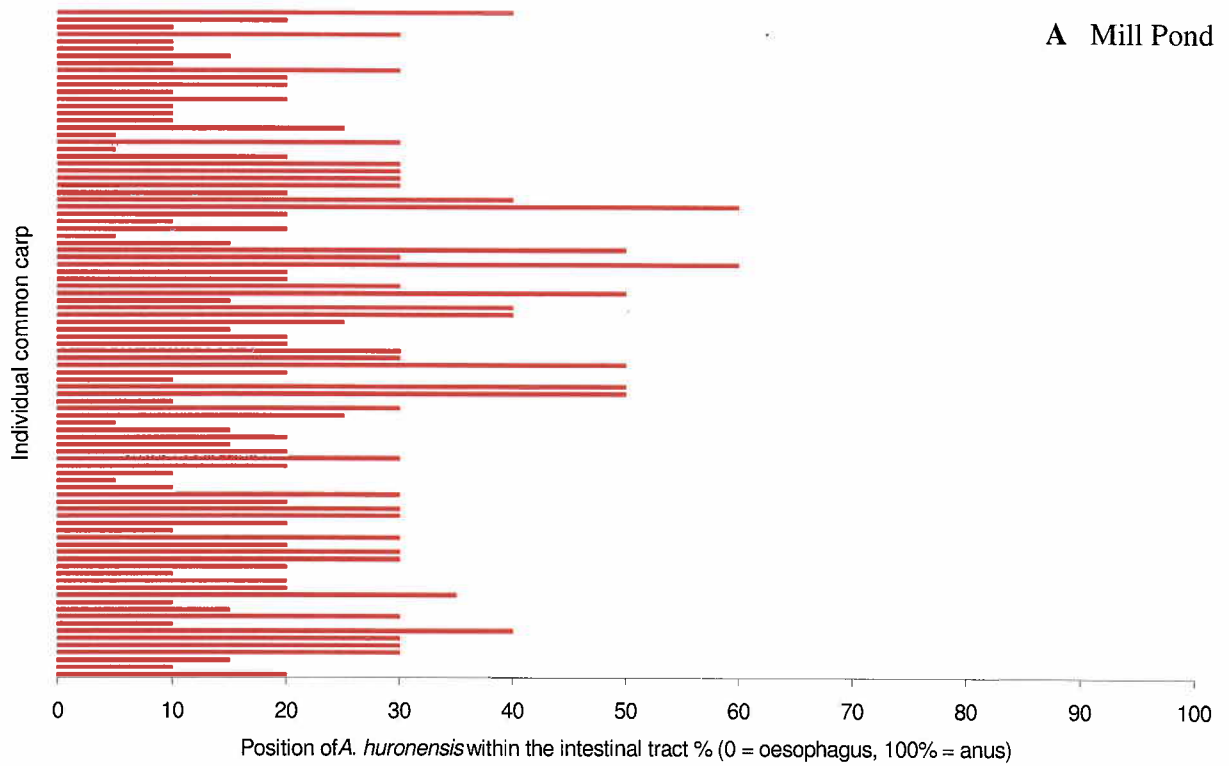
Fig 4.19. Regions of the intestinal tract of common carp infected with *A. huronensis*. Red bars show how far down the intestine parasites were attached (0-100% on the x axis represents oesophagus to anus).

- **Effect of *A. huronensis* upon host condition.**

Infection by *A. huronensis* had no significant adverse effect upon the condition of common carp examined from either Mill Pond or Frenches Pond (Table 4.2). This included a comparison between infected and infected fish, as well as a breakdown of light (1-10 parasites), moderate (11-50 parasites) and heavy parasite infections (>50 parasites). In both fisheries, even the most heavily infected hosts maintained condition factors within the range of uninfected individuals.

Table 4.2. Condition of common carp from Mill Pond and Frenches Pond infected with varying intensities of *A. huronensis*.

| | Mill Pond (fish length range 11.3 - 63.3cm) | | Frenches Pond (fish length range 13.1-54.1) | |
|---|---|---|---|---|
| **Parasite Intensity** | **Mean condition** | **Range of condition** | **Mean condition** | **Range of condition** |
| Uninfected | 2.34 (n= 191) | 1.37 - 3.28 | 2.24 (n= 18) | 1.86 – 3.17 |
| Infected | 2.45 (n= 40) | 1.96 – 3.38 | 2.14 (n= 90) | 1.55 – 3.38 |
| Light infection (<10) | 2.28 (n= 36) | 2.02 – 2.59 | 2.16 (n= 43) | 1.55 – 3.38 |
| Moderate inf. (11-50) | 2.46 (n= 3) | 1.96 – 3.38 | 2.10 (n= 36) | 1.60 – 2.69 |
| Heavy infection (>51) | 2.61 (n=1) | 2.61 | 2.23 (n= 11) | 1.81 – 2.80 |

- **Effects of *A. huronensis* upon haematological parameters of common carp.**

A total of 60 blood samples were analysed during the study to establish any obvious physiological effects of *A. huronensis* upon common carp (Table 4.3). Unfortunately, samples from Mill Pond were not tested for Alanine amino transaminase, preventing an assessment of this parameter. Considerable variation was observed in many of the blood parameters examined within the two fisheries studied. When comparing differences between infected and uninfected carp, there were no significant differences in any of the blood parameters measured (Mann Whitney, $P<0.05$).

Table 4.3. Measurements of blood parameters in common carp infected and uninfected with *A. huronensis*.

| A = Frenches Pond (n = 36) | Infected carp | | Uninfected carp | |
|---|---|---|---|---|
| **Blood parameter (unit)** | mean | sd | mean | sd |
| Sodium (mmol/L) | 139.24 | 5.28 | 134.14 | 8.93 |
| Potassium (mmol/L) | 2.95 | 3.61 | 5.96 | 7.74 |
| Glucose (mmol/L) | 6.18 | 2.61 | 3.7 | 2.63 |
| Protein (g/L) | 24.10 | 3.79 | 23.14 | 2.41 |
| Alkaline phosphatase (U/L) | 13.41 | 11.68 | 13.83 | 14.13 |
| Alanine amino transaminase (U/L) | 15.67 | 3.96 | 15.29 | 4.68 |

| B = Mill Pond (n = 24) | Infected carp | | Uninfected carp | |
|---|---|---|---|---|
| **Blood parameter (unit)** | mean | sd | mean | sd |
| Sodium (mmol/L) | 142.29 | 3.55 | 143.76 | 2.73 |
| Potassium (mmol/L) | 1.81 | 0.79 | 1.15 | 0.58 |
| Glucose (mmol/L) | 3.16 | 1.09 | 3.41 | 0.79 |
| Protein (g/L) | 22.86 | 4.30 | 28.76 | 4.21 |
| Alkaline phosphatase (U/L) | 9.43 | 6.83 | 12.71 | 6.70 |
| Alanine amino transaminase (U/L) | N/A | N/A | N/A | N/A |

- **Sampling requirements for parasite detection**

The prevalence of *A. huronensis* recorded from both fisheries indicate that a sample of 30 fish would be adequate for parasite detection at a 95% level of confidence. Assuming a 95% confidence level, detection of *A. huronensis* would be achieved with sample sizes of 16 carp from Mill Pond and 3 fish from Frenches pond. This assumes successful detection and identification of all tapeworms within the intestine and random sampling of the fishery population. The relationship between parasite prevalence and confidence of detecting a single infected carp in a population of up to 1,000 fish is shown in Fig. 4.20.
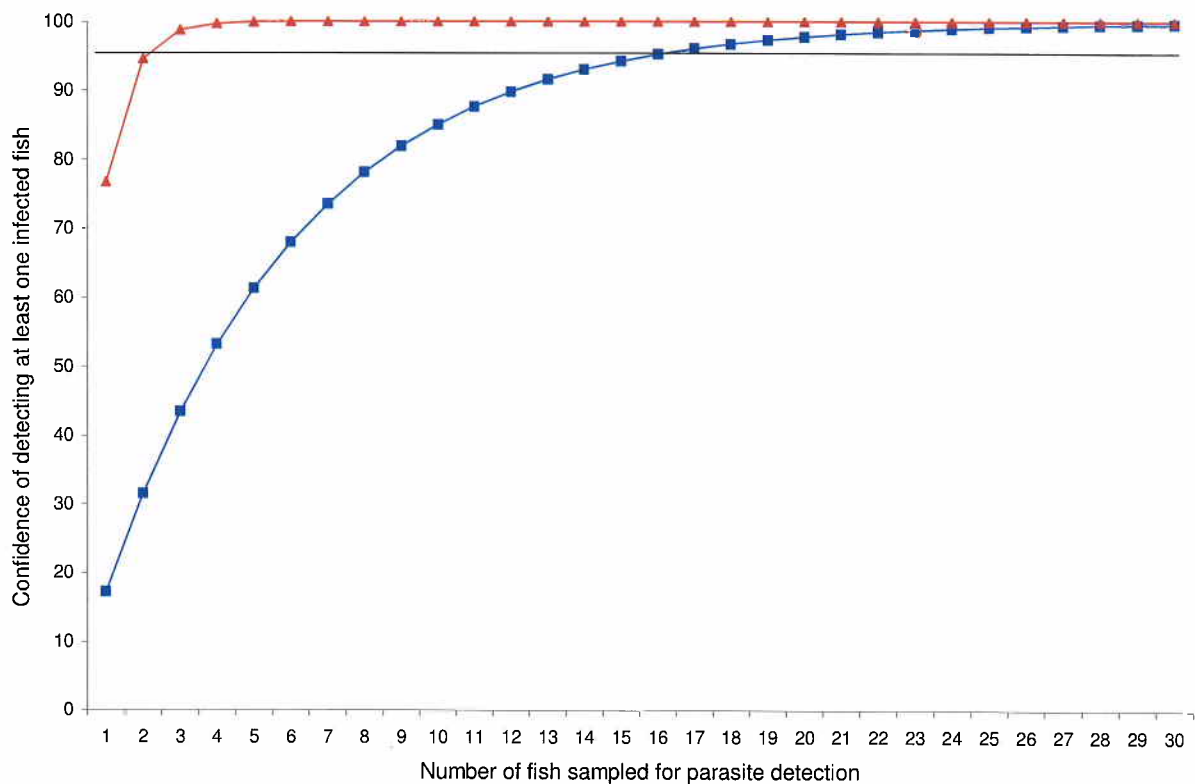


Fig. 4.20. Relationship between fish sample size and confidence of detecting at least one infected fish within populations from Frenches pond and Mill pond. The 95% confidence level currently used for Section 30 health checks is shown (adapted from Win Episcope v2.0). (red line – Frenches Pond, blue line – Mill Pond)

## Distribution of *A. huronensis* within England and Wales

Examination of Environment Agency fish health records from 1993-2003 reveal that *A. huronensis* has been recorded from a total of 35 freshwater fisheries in England and Wales (Fig. 4.21). These records primarily involve stillwaters, but include records of infection in a river and a single fish farm. The detection of *A. huronensis* in the River Medway followed the escape of fish from an infected fish farm during flooding. Consequently, it remains unclear whether the parasite has successfully established within this catchment.

*A. huronensis* has a relatively widespread distribution within south-east England (Fig. 4.21). The parasite has not been recorded from the north of England and appears to have a restricted distribution within the south west of the country. Although the parasite was first detected in a fishery in Powys, Wales in 1993, this remains the only record within this country.
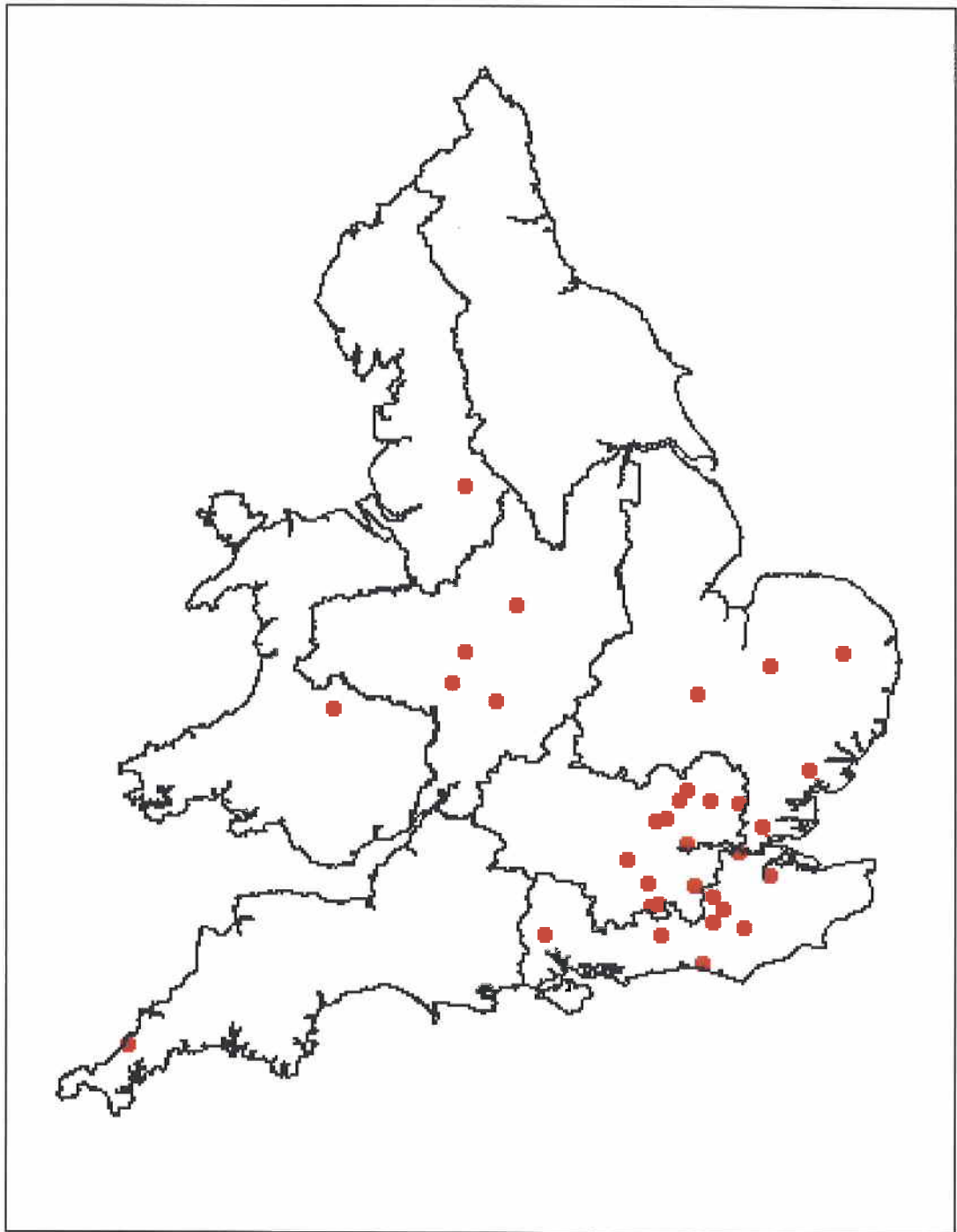
Fig. 4.21. Distribution of *A. huronensis* in freshwater fisheries within England and Wales collated from Environment Agency records between 1993 and 2003.

## 4.4. Discussion

**Pathological changes caused by *A. huronensis***

Cestodes can cause pronounced pathological changes to the intestine of teleost fish (Mackiewicz & McCrae, 1962; Mackiewicz *et al.,* 1972; Hayunga, 1979a; Shostak & Dick, 1986; Schäperclaus, 1991; Williams & Jones, 1994). These include loss of intestinal architecture (Scott & Grizzle, 1979; Hayunga, 1979a), nodule formation (Janisezewska, 1954; Mackiewicz & McCrae, 1962; Korting, 1977), severe inflammatory responses (Hayunga, 1979a,b; Mackiewicz *et al.* 1972), development of lesions (Hayunga, 1979a; Shostak & Dick, 1986), ulceration (Mackiewicz *et al.,* 1972) and intestinal occlusion (Korting, 1984; Shostak & Dick, 1986; Chakravarty & Tandon, 1989; Paperna, 1991; Hoole, 1994). According to some workers, the caryophyllideans include a number of pathogenic species (Mackiewicz, 1972; Chakravarty & Tandon, 1989; Williams & Jones, 1994). However, despite studies spanning the last century, the pathogenicity of many caryophyllidean cestodes are poorly understood (Williams & Sutherland, 1981; Korting, 1984; Schäperclaus, 1991; Williams & Jones, 1994; Chubb & Yeomans, 1995). In particular, there is very little literature detailing impacts of these parasites to fisheries (Dick & Choudhury, 1995a). The current study represents the first to comprehensively describe the lesions caused by *A. huronensis* within wild common carp populations in the British Isles.

The most pronounced and consistent pathological changes caused by *A. huronensis* involved mechanical damage as a result of scolex attachment. This was characterised by distortion of the intestinal folds, compression and erosion of epithelium and inflammatory responses in tissues adjacent to the scolex and neck of the worm. When

removed from the gut, live *A. huronensis* continually elongates and then expands the sides of its arrow-shaped scolex. This creates a burrowing movement that forces the anterior of the parasite deep between the intestinal folds. Once in place, lateral expansion of the scolex plugs the worm firmly to the gut wall. Epithelial compression, localised necrosis and congestion adjacent to the scolex, indicates that parasite penetration is forceful and capable of exerting considerable pressure. Based upon this attachment characteristic, mechanical damage caused by *A. huronensis* is likely to be greatest in small hosts where limited space between the intestinal folds increases the pressure necessary to accommodate the scolex. This is consistent with observations of infection in very large carp, where worms are easily accommodated with relatively minor epithelial damage. Conversely, pressure changes in small carp appear to be more pronounced, especially from adult worms (Molnar *et al.*, 2003).

According to Molnar *et al.*, (2003) the epithelial lesions caused by *A. huronensis* are considered severe, based upon comparisons with the tapeworms *K. sinensis* (Vikhman & Kapustina, 1975) and *B. acheilognathi* (Scott & Grizzle, 1979; Hoole & Nissan, 1994). However this work does not recognise that the main pathological changes caused by *B. acheilognathi* are associated with the parasite's body rather than scolex attachment, a characteristic of other pseudophyllidean infections (Smyth, 1969). Furthermore, the pathogenicity of *K. sinensis* remains unclear, preventing comparisons of disease potential (Chubb & Yeomans, 1995).

Studies by Mackiewicz *et al.*, (1972) indicate that cestodes lacking scolex specialisation are capable of causing considerable pathology as a result of attachment. Current studies suggest this is not the case for *A. huronensis*. Despite localised epithelial damage

adjacent to the parasite's scolex, the observed lesions were not considered severe and were less pronounced that those recorded in a number of other caryophyllideans (Hayunga, 1979a,b). Whilst epithelial compression appears to be a common consequence of attachment by caryophyllaeid cestodes (Mackiewicz *et al.,* 1972; Karanis & Taraschewski, 1993; Nolan, 1994; Morley & Hoole, 1995), there is little evidence to suggest that *A. huronensis* causes damage capable of major functional disruption within the gut. According to Wanstall *et al.,* (1988), epithelial cells can remain functional despite compression. Molnar *et al.,* (2003) confirmed that loss of epithelium was an unusual consequence of *A. huronensis* infection in fry. Consequently, the localised attachment of *A. huronensis* and observation that areas beyond immediate scolex penetration remain normal indicate limited impact. This may explain the absence of any records detailing clinical disease, host debilitation, degenerative changes or condition loss as a result of *A. huronensis* infection.

The observed preference of *A. huronensis* for the anterior third of the intestinal tract is a characteristic of many intestinal cestodes (Kennedy, 1983; Zaman & Seng, 1988; Mackiewicz, 1972, 1994; Hoole, 1994). It is believed that this site specificity is determined by gut morphology, activation of worms by bile secretions during establishment and areas favourable for absorption of nutrients across the cuticle of the parasite (Kennedy, 1983). Unlike some caryophyllaeid cestodes, in particular members of the genus *Monobothrium, A. huronensis* does not attach in tight clusters (Janisezewska, 1954; Mackiewicz *et al.,* 1972; Hayunga, 1979a). Consequently, the damage caused to the gut remained relatively constant for each worm and did not result in cumulative lesion formation during heavy infections. The congregation of parasites within the anterior third of the gut raises potential for intestinal occlusion (Korting,

1974, Hoole, 1994). This is supported by the fact that tapeworm growth and thus size are likely to be maximised within this region (Kennedy, 1983). However, at no time during the current study was there any evidence of gut blockage, stretching of the gut wall or loss of normal gut architecture. This may be explained by the scattered distribution of A. *huronensis* and the relatively small size of the worm compared with many other intestinal cestodes (Chubb *et al.*, 1987; Scholz, 1989; Mackiewicz, 1994; Scholz *et al.*, 2001). Unlike certain pseudophyllidean cestodes, where parasite size can be inversely proportional to intensity of infection (Read, 1951), A. *huronensis* remains relatively small regardless of parasite burden. Consequently, it appears that gut blockage is an unlikely consequence of A. *huronensis* infection (Molnar *et al.*, 2003).

The occurrence of a homogenous eosinophilic interface layer around the scolex and neck of the parasite indicates intimate host-parasite contact. This phenomenon has been described for a number of caryophyllaeid cestodes (Hayunga, 1979a) and is believed to arise through either lytic or adhesive secretions (Mackiewicz *et al.*, 1972; Hayunga, 1979a; Karanis & Taraschewski, 1993). Large number of scolex glands have been described in the neck and scolex of A. *huronensis* which are thought to be associated with secretary mechanisms (Mackiewicz, 1972). However, it remains unclear to what extent such secretions provoke host responses and thus pathological changes (Mackiewicz *et al.*, 1972; Hayunga, 1979a,b). In some tapeworms, scolex secretions are believed to be strong irritants that lead to pronounced inflammatory responses (Hayunga, 1979 a,b). Current indications of secretory material combined with pronounced infiltration of lymphocytes and EGC's support this possibility. However, these reactions were recorded in only a very small number of infections and did not lead to major inflammatory changes like nodule formation. The lack of marked host

responses or tissue damage in the majority of infections suggests that these may not be important or consistent pathological changes associated with *A. huronensis* infection. It is possible that such secretions have an adhesive function, or are used only during initial establishment of worms within the gut. The varied inflammatory responses observed in the current study may therefore be related to the longevity of infection, being more pronounced during initial parasite establishment and becoming milder with longer lived parasite burdens (Dick & Choudhury, 1995a). The occurrence of mild and localised inflammation may be considered a normal host reaction to intestinal parasites (Rowley *et al.*, 1988; Castro, 1992).

According to Mackiewicz (1972), intestinal cestodes affect their host in three ways, namely mechanical obstruction of the intestinal tract, production of lesions or disruption to the physiological function of the gut, causing debilitation and increasing susceptibility to secondary infections. Current studies indicate that *A. huronensis* does not cause formation of severe lesions within the intestine or changes consistent with gut occlusion. Even during extreme worm burdens, pathological changes remained relatively mild and did not extend beyond the immediate site of scolex attachment. At no time during the study were any secondary infections recorded from infected common carp. These observations provide little evidence to suggest that *A. huronensis* is an important pathogen of common carp. In a number of cases, observations of infected tissue suggested that the mucosal layers of the intestine had adapted to accept the cestode, with only minimal and localised pathological changes.

**Prevalence, intensity and distribution of *A. huronensis* in carp fisheries**

The observed prevalence of 83.5% within Frenches Pond indicates that *A. huronensis* has the potential to infect a large proportion of a carp population. The marked difference in parasite prevalence and intensity between the two fisheries may reflect the importance of the intermediate host in the diets of the two populations. It is well recognised that host feeding is an important factor in determining cestode infrapopulations (Anderson, 1974; Esch, 1983). The marked differences in the two fisheries may therefore be explained by differences in the feeding behaviours of the two populations. Frenches Pond is very rarely fished and thus the nutrition of resident carp is gained primarily from natural food items. In contrast, Mill Pond is a heavily fished water where carp have at least partial reliance upon anglers bait (Sutton *pers. comm.*). Whilst there are many variables that may influence tapeworm infections in fish, including intermediate host population dynamics (Esch, 1983; Courtney & Christensen, 1987, 1988), environmental conditions (Anderson, 1976; Chubb, 1980; Esch, 1983; Kennedy, 1994) host physiology (Bauer, 1961; Arme *et al.*, 1983), seasonality (Kennedy, 1969; Anderson, 1974; Chubb, 1980) and immunity (Rickard, 1983; Hart *et al.*, 1988; Dick & Choudhury, 1995a), fishery management may be an additional influence in the structure of parasite populations in fisheries.

The occurrence of up to 214 parasites in a single host represents the heaviest documented infection of *A. huronensis*. Similarly, the recorded prevalence of 85% is the highest recorded for any fishery population and the highest recorded since the detection of the parasite in 1993 (Environment Agency, unpublished). It may therefore be assumed that Frenches Pond constitutes a heavily infected fishery. Despite these

infections, at no time during the study were any diseased, debilitated or moribund fish observed.

*A. huronensis* showed a highly over-dispersed distribution within the populations examined. This distribution is well documented in most host parasite systems (Crofton, 1971; Anderson, 1974; Alston, 1994) and is likely to be related to the heterogeneous distribution of the oligochaete intermediate host, combined with heterogeneity in carp feeding behaviour. The effect of over-dispersion in the parasite population means that most fish harbour no parasites or only low level infections. This may reduce the impact at the population level, if only small numbers of individuals are heavily parasitised at any one time.

**Host preferences of *A. huronensis***

Literature suggests that *A. huronensis* is a specialist of common carp (Jones & Mackiewicz, 1969; Mackiewicz, 1972; Rubertone & Hall, 1975; Chubb *et al.*, 1996; Majoros *et al.*, 2003; Environment Agency, unpublished, Natural History Museum Host Parasite Database). The detection of parasite infections in two carp hybrids therefore represents a new host record for the parasite. The presence of adult and juvenile worms within these hosts as well as intensities of up to 21 parasites, suggests that hybrids may be suitable hosts for the parasite allowing reproduction and potential transmission. This raises implications for the dissemination of the parasite with carp hybrids as well as with common carp. This is of particular importance as demand for carp hybrids for fishery stocking has risen significantly in the last 5 years (LFMD, 2006). This finding also suggests that any impact from the parasite may not be limited to common carp fisheries, but may also affect those comprising hybrids.

## Role of host size and sex upon *A. huronensis* infection

Host sex did not affect the prevalence or intensity of *A. huronensis* infections in the carp fisheries studied. It is known that for some tapeworm species, infection is exclusively associated with either male or female hosts, influenced by hormonal differences in the two sexes (Esch, 1983). However, this appears not to be the case with *A. huronensis,* where both male and female carp are equally susceptible to infection.

The observation that greater infections of *A. huronensis* were recorded in larger hosts may simply be a function of carp feeding behaviour, with larger fish ingesting larger numbers of the intermediate oligochaete host. It is well recognised that the nutritional behaviour of fish can strongly influence susceptibility to intestinal cestodes. This is well documented for certain cyprinids, where development coincides with nutritional shifts involving different proportions of aquatic invertebrates (Lammens & Hoogenboezem, 1991; Couchman, 1997). *Bothriocephalus acheilognathi* primarily infects small carp due to the importance of copepods within the diet of juvenile fish (Brouder, 1999). However, the occurrence of *A. huronensis* in carp fry (Molnar *et al.,* 2003) as well as adult fish exceeding 65cm, confirms that all size ranges of common carp are susceptible to infection. This suggests that the detection of *A. huronensis* from infected fisheries may be successfully achieved with the examination of both adult and juvenile fish.

## Effects of *A. huronensis* upon host condition and physiological disruption

Pathogenic cestodes can have detrimental effects upon the physiology, condition and blood composition of infected fish (Bauer, 1961; Bauer *et al.,* 1969; Kurovskaya & Kititsyna, 1986; Williams & Jones, 1994). However, the effects of tapeworms upon physiological disruption can be difficult to establish and often requires detailed

examinations, involving large sample sizes monitored under controlled conditions (Arme *et al.,* 1983; Powell, 2006; Arme *pers. comm.*). Such changes can be particularly difficult to identify in extensive water bodies where many variables influence fish health and host-parasite relationships. Blood parameters in particular can fluctuate considerably within 'normal' ranges, and can even be influenced by factors like netting, transportation and handling. Despite these problems, current studies were undertaken in efforts to determine whether *A. huronensis* was responsible for obvious or severe physiological disruption to common carp. Despite small sample sizes, there was no evidence of adverse changes in any of the parameters examined. Even the heaviest infected fish had blood parameters within ranges on uninfected individuals. The absence of gross pathology, condition loss or clinical signs of disease in any of the fish examined, suggests that carp were capable of tolerating the observed infections and were not nutritionally compromised. Whilst it is important to understand the impact a parasite may have upon infected hosts, it was beyond the scope of these studies to attempt to tackle the potential effects of *A. huronensis* upon nutrient malabsorption. It remains possible that, to some degree, *A. huronensis* disrupts the nutritional efficiency or intestinal function of common carp (Arme, *pers. comm.*). However, it is questionable whether this represents a serious impact to the performance and socio-economic development carp fisheries.

**Detection of *A. huronensis* to minimise risks of parasite dissemination**

The examination of entire fishery populations is unusual in parasitological investigations. Whilst extensive sampling is unnecessary to reveal general patterns of infection (Thrusfield, 1995; Cameron, 2002), such effort provides a robust understanding of infections throughout the whole fishery, including extreme worm

burdens that may be missed through sub-sampling. The examination of a total of 371 fish during the current study represents the largest study conducted on *A. huronensis* in carp fisheries. Whilst this did not accommodate observations of seasonal influences, the current studies provided a sound foundation on which to base a preliminary assessment of pathogenicity. However, the absence of infections in very small carp prevented understanding of impact within these hosts.

Sampling protocols employed for the detection of fish diseases rely strongly upon an understanding of prevalence within the host population (Ossiander & Wedemeyer, 1973; Simon & Shill, 1984; Thrusfield, 1995). The examination of two fishery populations allowed a robust assessment of the protocols used by the Environment Agency for the control of *A. huronensis*. These protocols are based upon a sample size of 30 fish, with a 95% confidence of detecting a 10% infection within host populations. The observed prevalence of infection at both fisheries would allow the successful detection of infected individuals from a sample comprising 30 carp. However, whilst health check samples may comprise a total of 30 fish, this may include just 10 fish of each species (Environment Agency, unpublished). In such cases, the risk of not detecting *A. huronensis* may increase significantly. This remains an important consideration for the future control of non-native parasite introductions. Although the obvious answer to such a problem is to increase the size of the fish sample examined, it is recognised that disease controls involve financial and practical considerations as well as robust sampling strategies. In view of the economic value of common carp, increasing sample size would increase the cost of health checks and thus raise potential for more illegal fish movements. This is where understanding the threats posed by any

non-native parasite would allow the Environment Agency to prioritise resources and implement controls proportionate to disease risk.

**Distribution of *A. huronensis* within England and Wales**

Distribution records indicate that *A. huronensis* has a relatively widespread distribution within south-east England. However, the parasite appears to have maintained a restricted distribution within Wales and the north and south-west of England. This distribution is likely to reflect the regions where most carp fisheries are located, largest numbers of carp movements take place and thus greatest detection effort from health check examinations. As the current studies focused solely upon Environment Agency data, the observed distribution of *A. huronensis* is likely to be an under-estimate of the parasites true distribution. This assumption is supported by the fact that health check examinations are conducted only when fish movements are destined for an open water body or on-line fishery.

The detection of *A. huronensis* in 1993 involved simultaneous recordings in Powys, Wales and Hampshire, England. The wide geographical separation in these records suggest that the parasite was either present within the British Isles prior to this date and spread to one or both fisheries through carp movements, or that both sites had gained infection from separate introductions of infected common carp. Due to the number and regularity of parasitological examinations conducted on common carp prior to 1993, it is likely that *A. huronensis* is a non-native introduction to the parasite fauna of England and Wales (Chubb *et al.*, 1996). The relative ease of parasite detection and morphological distinction of the scolex of *A. huronensis* compared with other British cestodes supports this assumption and minimises the risk of mis-identification (Chubb

*et al.,* 1987). An exception to this involves juvenile stages of *Khawia sinensis,* which may possess a narrow, pointed scolex until relaxed (*pers. ob.*).

Until the identity of British parasites has been confirmed, it is difficult to assess the likely routes of *A. huronensis* introduction. However, it is widely recognised that the translocation of many helminths result from movements of infected carp (Bauer & Hoffman, 1976; Kennedy, 1975, 1993, 1994; Andrews *et al.,* 1981; Molnar, 1982; Bauer, 1991; Chubb & Yeomans, 1995; Majoros, *et al.,* 2003). The cosmopolitan distribution of common carp throughout most parts of the world (Bauer, 1991; Dove & Fletcher, 2000; Hoole *et al.,* 2001), vast global trade of this species and growing numbers of fisheries being stocked with carp raise potential for rapid dissemination (Bauer & Hoffman, 1976; Bauer, 1991; Kennedy, 1976, 1994). However, the transfer of intermediate life stages with water, mud or vegetation represent additional routes for parasite introduction (Chubb & Yeomans, 1995). The importation of infected oligochaete worms from Europe with live fishing bait, or on aquatic plants intended for the ornamental industry represent potential avenues for parasite invasion (Garner, *pers. comm.*). Despite this, the direct movement of infected carp represents the largest and most likely route of *A. huronensis* introduction.

It has been speculated that *A. huronensis* was introduced in to the British Isles either with ornamental koi from Asia, or with infected carp from Europe for the purpose of stocking fisheries (Kirk *et al.,* 2002). Support for an Asian origin includes the detection of parasites within Japanese koi carp and records of infection within ornamental koi ponds within the UK (Environment Agency, unpublished). Fish imported for the ornamental industry are not routinely examined for parasites, providing the potential for

future parasite invasions (Andrews 1984; Crawshaw & Sweeting, 1986; Kim *et al.,* 2002). The occurrence of *A. huronensis* in fisheries, ornamental ponds and a fish farm, combined with the frequency of common carp movements for the purpose of fishery stocking, provides high potential for the future dissemination of *A. huronensis* within the British Isles. However, current evidence suggests that this may not pose a major threat to fishery populations.

## 4.5 Summary and risk assessment

Current studies of pathology and epidemiology provide little evidence to suggest that *A. huronensis* is an important pathogen of common carp. The lesions caused by the parasite were generally mild and considered unlikely to pose a serious threat to infected hosts (Ferguson *pers. comm.*). The localised damage caused to the gut, over-dispersed distribution of parasites within carp populations, absence of condition loss or clinical disease, in even the most heavily infected individuals and apparent lack of obvious physiological disruption support this assumption. In all of the hosts examined, effects at both host and population level suggest that common carp are able to tolerate infections of *A. huronensis* comprising up to 214 worms. No records of parasite-induced debilitation or mortality exist in current literature, including fry (Oros *et al.,* 2004; Kappe *et al.,* 2006) and no disease events have been recorded within the British Isles as a result of *A. huronensis* infection (Environment Agency, unpublished).

Although current studies were primarily based upon only a small number of infected fisheries, the sample sizes provided a robust assessment of infection characteristics. However, it is recognised that the current work did not confirm infections within juvenile carp and were based on spot sampling. It remains possible that heavy infections

of *A. huronensis* may have some adverse effect upon the intestinal function or nutritional efficiency of common carp. Similarly, the influence of fishery management practice, seasonality and host nutritional status cannot be ignored and may increase the susceptibility of fish to such infections. Further studies are necessary before a complete picture of impact is obtained. However, it is recognised that any studies undertaken, or changes identified, must be placed into context with the rapid socio-economic development of carp fisheries. Whilst these factors may be important, the lack of serious pathological changes to the gut of common carp and maintenance of host condition make it very unlikely that the parasite causes serious population changes. A risk assessment based upon published literature and current findings is given in Fig. 4.22. and summarised in Fig. 4.23.

Fig. 4.22. Risk analysis process for evaluating the probability of *A. huronensis* causing undesirable economic and ecological impacts to fisheries. This is based upon available published literature and information gained during the current study.

| Parasite species being assessed – *Atractolytocestus huronensis* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact** <br><br> 1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise ecologically important fisheries? | 0.1 | | |
| **B Economic impact** <br><br> 1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise economically important fisheries? | | | 0.5 |
| **A Ecological impact risk analysis** <br> What is the risk of the parasite having an adverse ecological effect on fisheries? | = 0.1 x 0.1 x 0.1 = 0.001 | | |
| **B Economic impact risk analysis** <br> What is the risk of the parasite having an adverse economic effect on fisheries? | = 0.1 x 0.1 x 0.5 = 0.005 | | |

Fig. 4.23. Risk analysis matrix to prioritise the potential impact of *A. huronensis* upon the ecological and economic development of fisheries. ·

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low**<br>(0.001 – 0.005) | **Medium**<br>(0.009 – 0.027) | **High**<br>(0.045 – 0.125) |
| **Economic Risk** | **Low**<br>(0.001 – 0.005) | **Low**<br><br>*A. huronensis* | Medium | High |
| | **Medium**<br>(0.009 – 0.027) | Medium | Medium | High |
| | **High**<br>(0.045 – 0.125) | High | High | High |

## Chapter 5. Effects of *Ergasilus briani* on juvenile cyprinid populations

### 5.1. Introduction

*Ergasilus briani* is a crustacean gill parasite of freshwater fish. The parasite has a well-documented preference for small hosts and is most commonly found infecting fish less than 100mm in length (Alston, 1994; Alston & Lewis, 1994). Literature confirms that ergasilid parasites can cause considerable damage to the gills of infected fish, leading to respiratory dysfunction, osmoregulatory failure, haematological disruption, condition loss and death (Kabata, 1970; Einszporn-Orecka, 1970, 1973a, 1973b; Hoffman, 1977;, Hogans, 1989; Abdelhalim, 1990; Pathiratne, 1992; Alston & Lewis, 1994; Dezfuli *et al.*, 2003). A number of species are economically important pathogens and have therefore received considerable attention throughout the world (Dogiel *et al.*, 1958; Lahav & Sarig, 1967; Kabata, 1970; Abdelhalim, 1990; Paperna, 1991; Lester & Roubal, 1995). Six species of ergasilid parasite have been recorded from wild freshwater fish within the British Isles (Fryer, 1982; Hawkins, 2001). Five of these are considered to be non-native additions to the parasite fauna of England (Fryer, 1969, 1982; Mugridge *et al.*, 1982; Fryer & Andrews, 1983; Hawkins, 1999, 2001; Kerne, 2005). However, with the exception of *E. sieboldi*, the role of many of these parasites in causing disease to fishery populations remains poorly understood.

*E. briani* was first recorded in Britain in 1982, from a stillwater fishery in Yorkshire (Fryer & Andrews, 1983). At the time of detection, the only published record of impact from *E. briani* was that of Bauer *et al.*, (1969), who described losses of 0+ tench in a Russian fish farm associated with intensities of 200-1139 parasites. Alston (1994) was the first to conduct comprehensive studies on *E. briani* in the British Isles. This work

vastly improved understanding of the parasite's life-cycle, morphology and host preferences. During observations of life-cycle development, Alston (1994) confirmed that experimentally infected tench lost condition and died as a result of heavy *E. briani* infections. The same author revealed that in the wild, prevalences of infection exceed 80% in cyprinid fry (Alston, 1994). These findings, combined with the pathogenicity of other ergasilid species (Lahav & Sarig, 1967; Kabata, 1970, 1985; Abdelhalim, 1990; Lester & Roubal, 1995), susceptibility of fry to ergasilosis (Kelly & Allison, 1962) and invasive potential of many crustacean parasites (Bauer, 1991; Kennedy, 1993, 1994) raised concern regarding the threat of the parasite to native fish populations. This underpinned a precautionary approach to the management of *E. briani* in England and Wales (Kirk & Lewis, 1992; Alston & Lewis, 1994; Environment Agency, 1999). Despite this concern, the pathogenicity of the parasite in the wild remains largely unsubstantiated due to some fundamental gaps in understanding. To date, the histopathology caused by *E. briani* to the gills of infected hosts has not been described. Furthermore, no attempts have been made to measure the effects of *E. briani* upon the growth, condition or survival of hosts from wild fish populations. This has so far prevented a reliable assessment of impact on fisheries in England and Wales (Kirk & Lewis, 1992; Alston & Lewis, 1994; Alston, 1994).

The growth, condition and survival of fry are important for the recruitment of wild fish populations and thus performance of fisheries (Eggilshaw & Shackley, 1985; Sammons & Bettoli, 1998; Paxton & Winfield, 2000; Cowx, 2001; Frear & Cowx, 2003; Cowx & Frear, 2004). However, fry mortality is a natural phenomenon that is influenced by a wide range of biotic and abiotic factors (Frear & Cowx, 2003). These include environmental conditions, nutrition, predation, climatic changes and disease.

Chapter 5.

Considerable attention has been given to the diseases of fry and juvenile freshwater fish in aquaculture. Although literature includes records of parasitic infections (Matskasi, 1978, 1984; Buchmann *et al.*, 1993; Mohan & Shankar, 1995; Uldal & Buchmann, 1996; Serrini & Sarti, 1999; Cruz-Lacierda *et al.*, 2004), bacterial diseases (Madsen *et al.*, 2005), pathogenic fungi and viruses (Muench *et al.*, 1996; Woo & Bruno, 1998), these records almost exclusively involve cultured fish populations. These can be subjected to close scrutiny, are easily sampled and are usually maintained in relatively stable environments. Understanding the effects of parasites among wild fish populations can be far more difficult, especially when the target host is very small, delicate to handle, difficult to capture on demand and is influenced by a wide range of environmental stressors (Kennedy, 1993; Hedrick, 1998). Even sub-lethal parasite impacts like poor growth and condition loss are important to year class strength and can be key indicators in determining the future success of adult fish stocks (Sammons & Bettoli, 1998; Paxton & Winfield, 2000; Cowx, 2001; Frear & Cowx, 2003). Consequently, the pathogenicity of a parasite that has a predilection for fry requires careful assessment, if impacts to fisheries are to be appropriately managed.

This chapter details studies undertaken to improve understanding of *E. briani* infections in fisheries in England and Wales. A review of available literature provided a foundation of current understanding of the parasite. This also highlighted gaps in current knowledge regarding the potential of the parasite to cause disease. Histopathological descriptions of *E. briani* in the gills of juvenile cyprinids were made to establish the extent and severity of changes at the host level. Epidemiological studies were conducted at two fisheries to assess the distribution and disease potential of the

parasite within infected populations. The effect of *E. briani* upon the growth, condition and survival of juvenile cyprinids were studied.

This chapter is structured around the following aims:

1. To collate and review current literature on *E. briani* in order to improve understanding of impact, distribution, detection and management of the parasite in fisheries.

2. To describe the pathological changes associated with infections of *E. briani* in the gills of juvenile cyprinid fish from fisheries in England.

3. To identify the prevalence, intensity and distribution of parasites within infected fisheries.

4. To identify the effects of *E. briani* upon the growth, condition and survival of wild cyprinid fry populations.

5. To update understanding of the distribution of *E. briani* within fisheries in England and Wales.

## 5.2. Materials & methods

### 5.2.1. Literature reviews

Literature reviews were collated as described in Chapter 3.

### 5.2.2. Fish sampling and maintenance

Waters infected with *E. briani* were identified from a database of fish-health records held by the Environment Agency. The Basingstoke Canal between Fleet and Aldershot was chosen as a site for study due to the known presence of established *E. briani* populations, abundance of cyprinid fry and availability of historic data of parasite infections (Alston, 1994). Four sites along this stretch between Eelmoor Flash (NGR: SU84345280) and Ash Lock (NGR: SU89265150) were chosen based upon their suitability as fry habitat and clear access for vehicles and equipment. This water represented a wild fishery, which relied solely upon natural recruitment to support fish populations. This removed many of the variables associated with more highly managed waters (e.g. fish stocking).

All fish were captured with a 25m fry seine net. Each site was netted approximately 2-3 times along its length, with all small roach and bream retained. A maximum size of approximately 100mm was selected in order to maximise capture of infected individuals and ensure retention of all 0+ fish (Britton *pers. comm.*). Captured fish were transported to holding facilities at the National Fisheries Laboratory, Brampton and maintained in 30l tanks fed with bore hole water.

Initial fry samples were obtained from the Basingstoke Canal during October 2003. This time of year allowed fish a full growing season and was favourable for heavy

parasite infections due to the reproduction of parasite throughout the summer. Efforts were also made to obtain samples during August 2004, to identify impacts during warmer conditions where gill function would be more critical and fry smaller in size. Unfortunately, this latter sampling was hindered by extreme weather conditions, an initial failure to catch sufficient numbers of small fish and during the last attempt, the netting of a bomb from the canal, which forced a sudden halt to fishing.

To improve understanding of *E. briani* in different types of fishery, fish samples were also obtained from Birkett Hall Pond, Essex (NGR: TQ8005 9994). This 1 acre, fully-enclosed, highly stocked fishery was typical of a highly managed fishery. This sampling opportunity arose during April 2004 and coincided with removal of rudd from the fishery for the purpose of stock manipulation. This not only allowed observations to be made of *E. briani* populations within a more intensively stocked water body, but also enabled the effect of the parasite to be assessed in an over-wintered population. Fish capture and maintenance were consistent with that for the Basingstoke Canal sampling.

### 5.2.3. Fish examination

Fish were sacrificed by lethal anaesthesia (immersion in 5%w/v benzocaine solution). All fish were weighed and the fork length measured. Scales from the Basingstoke Canal fish were taken for age determination and growth analysis. All gill arches were removed in their entirety for parasite detection. Each gill was transferred to a petri dish and examined under a dissection microscope with base illumination. Parasite number, position within the gills and reproductive status were recorded (determined by body colour and presence of egg sacs). Infected gills were fixed in either 10% neutral buffered formalin or Bouin's fixative for histopathological examination.

## 5.2.4. Histopathology and scanning electron microscopy (SEM)

Methods used for histopathological investigations were as described in Chapter 4.

## 5.2.5. Fish ageing and condition

Scales were aged with use of a projectina held within the ageing department of the National Fisheries Laboratory, Brampton. Length frequency histograms were constructed for each species to determine the age composition of the sampled population. This allowed 0+ fry to be distinguished and analysed independently of older fish. Scales of both infected and uninfected fish were compared to establish evidence of disturbed or poor growth. The condition factor (K) of both infected and uninfected fish from both fisheries was calculated according to Jobling (1995), as detailed in Chapter 4.

## 5.2.6. Distribution records of *E. briani* within England and Wales

Distribution records of *E. briani* were collated as descried in Chapter 4.

## 5.3. Results

### 5.3.1. Literature review

Published literature pertaining to *E. briani* is generally sparse and fragmented. An exception to this lies in the work of Alston (1994). This author requires particular recognition due to his significant contribution to understanding of the parasite. The near sole reliance on this work for assessment of many aspects of the parasite's biology is recognised and will be evident in the following literature review.

- **Description and taxonomy**

*Ergasilus briani* is a crustacean parasite of the family Ergasilidae. It is one of six ergasilid species recorded from fisheries within the British Isles (Fryer, 1982; Alston & Lewis, 1994; Environment Agency, 1999; Hawkins, 2001). The taxonomic history of *E. briani* is complex and has long been disputed (Fryer, 1982; Fryer & Andrews, 1983; Alston *et al.,* 1993). This confusion stems from two independent descriptions of the same parasite, made during the early 1930s (Markewitsch, 1933; Halisch, 1934). *E. briani* was first described from specimens found in the Ukraine (Markewitsch, 1933), but described the following year as a different species from Germany (Halisch, 1934). Since then, considerable debate has been given to the morphology and synonymity of these species (Halisch, 1935, 1939; Romanovski, 1955; Yin, 1956; Bauer *et al.,* 1959; Gusev & Smirnova, 1962; Yamaguti, 1963; Fryer & Andrews, 1983; Alston, 1994). A comprehensive morphological study conducted by Alston (1994) detailed a number of features previously overlooked by other workers and for the first time clarified the taxonomic identity of the parasite by means of both light and scanning electron microscopy (Alston *et al.,* 1993; Alston, 1994).

Descriptions of adult parasites have been given by Halisch (1935, 1939), Romanovski (1955), Markewitsch (1956), Yin (1956), Fryer, (1982); Fryer & Andrews (1983) and Alston *et al.,* (1993). Adult parasites exhibit primitive cyclopoid morphology and measure approximately 0.8mm in length, excluding egg sacs. Adult female *E. briani* have an elongated and segmented body, which has been described as 'violin-shaped' in appearance (Kirk & Lewis, 1992). A pair of prominent antennae extend from the anterior of the body, which form the large inwardly curved 'claws' used for attachment (Fryer, 1982). Adult parasites possess four pairs of biramous swimming legs, the fifth being reduced to a small papilla bearing a single seta. The mouth of the parasite lies on the underside of the body, and possess serrated mandibles concealed beneath the labrum (Alston *et al.,* 1993). A detailed review of morphology and taxonomy is given by Alston (1994).

- **Host susceptibility**

*E. briani* is a generalist and shows low host specificity (Bauer *et al.,* 1959; Kennedy, 1994; Alston & Lewis, 1994; Environment Agency, unpublished). In the British Isles, host records for *E. briani* currently exceed 20 fish species, most of which are members of the Cyprinidae (Environment Agency, unpublished). Despite this wide host range, common bream, tench, and crucian carp appear to be particularly susceptible hosts to infection (Fryer & Andrews, 1983; Tuuha *et al.,* 1992; Alston, 1994; Environment Agency, unpublished). Conversely, parasites are rarely found in carp, roach, perch and pike (Lucky, 1977; Fryer & Andrews, 1983; Tuuha *et al.,* 1992; Alston, 1994). To date the parasite has not been recorded in salmonids.

A number of workers have shown that *E. briani* has a strong predilection for small fish (Bauer, 1962; Tuuha *et al.,* 1992, Alston, 1994; Alston & Lewis, 1994). Although individuals have been recorded on large hosts, infections of *E. briani* are generally rare on fish exceeding 150mm (Environment Agency, unpublished; Alston & Lewis, 1994). Both prevalence and intensity of infection are greatest in fish measuring less than 80-100mm (Alston & Lewis, 1994). Reasons given for this characteristic include differences in host behaviour, mainly mobility and mode of nutrition (Schäperclaus, 1991; Alston, 1994) host gill size (Nakai, 1991; Alston, 1994), host immunity (Noble *et al.,* 1963; Alston, 1994) and chemoreception of larval parasites prior to attachment (Bocquet & Stock, 1963). The discovery of sensillae and sensory pores on freshwater ergasilids, suggests that chemoreception may play an important role in the acceptance or rejection of suitable hosts (Abdelhalim, 1990). With respect to fisheries, these host records suggest that fisheries containing juvenile cyprinid populations are most likely to harbour *E. briani* populations.

- **Life-cycle development**

Confusion has long surrounded the life-cycle development of many ergasilid parasites, including *E. briani*. This has primarily stemmed from difficulties with the morphological distinction of the free-living stages (Zmerzlaya, 1972; Urawa *et al.,* 1980a,b; Kabata, 1981; Kirk & Lewis, 1992; Alston *et al.,* 1993, 1995; Alston, 1994). Alston (1994) was the first to overcome these problems, by completing the life-cycle of the parasite under controlled conditions and describing each life stage through use of scanning electron microscopy. He also described the seasonal development of *E. briani* from observations of natural infections in the south-east of England.

The life-cycle of *E. briani* is direct, requiring only the fish host for its completion. Life-cycle development comprises six free-living naupliar stages, five free-living copepodid stages and a single adult (Alston, 1994). Only post-mated adult females are parasitic and males die following copulation. On locating a fish host, parasites typically attach between the two hemibranchs of the gill on the inner surface of the filaments (Bauer *et al.*, 1959; Fryer, 1982). Ponyi & Molnar (1969) described *E. briani* on the fins of its host. However, due to morphological similarities this may represent a misidentification with *Neoergasilus japonicus*, a parasite specific to the fins of fish (Mugridge *et al.*, 1982; Abdelhalim, 1990; Hayden & Rogers, 1998). Attachment of *E. briani* is achieved by the clawed antennae, which are believed to firmly grip the gill tissue (Alston, 1994; Alston & Lewis, 1994). Once attached, parasites become permanent residents of the gills, losing the ability to detach and swim. Upon successful establishment on the gills, reproduction commences, resulting in the development of egg sacs, which trail from the posterior of the parasite.

Ecological studies have revealed that the maturation, development and reproduction of *E. briani* is seasonal within temperate climates and influenced strongly by temperature (Tuuha *et al.*, 1992; Alston, 1994). Reproduction is triggered during spring when water temperatures reach approximately $10^{\circ}C$. Epidemiological studies suggest that *E. briani* produces between two and three parasite generations annually (Tuuha *et al.*, 1992; Alston, 1994). Tuuha *et al.*, (1992) detailed how the first generation is produced from over-wintered parasites, whilst the second comes from parasites that develop and mature in the same year. This is reflected by two seasonal peaks of parasite infection per year, the first in July and the second from August onwards. Parasite populations in England increase throughout the year reaching a peak during autumn (Alston, 1994).

Development and reproductive activity ceases during the winter, although parasites retain the ability to over-winter on their host.

- **Distribution and dissemination**

*E. briani* is native to Eurasia, but has increased its geographical range with the anthropochore movement of infected fish (Alston *et al.,* 1993; Kennedy, 1994). Distribution records are generally patchy, but include countries throughout Europe and Asia (Markewitsch, 1956; Yin, 1956, 1962; Romanovski, 1955; Ponyi & Molnar, 1969; Bauer, 1987; Alston, 1994). These records fail to identify any obvious patterns of spread or dissemination. However, the discovery of *E. briani* almost concurrently in Russia (Markewitsch, 1933) and Germany (Halisch, 1934) suggests that the parasite had a wide geographical distribution in Eurasia before that time. The relatively small size of *E. briani,* and characteristic attachment to the inner surface of the hemibranchs makes it easy for the parasite to escape detection unless parasitological examinations are performed (Fryer & Andrews, 1983). According to Bauer *et al.,* (1959), this hidden location may be responsible for fragmented distribution records and lack of early parasite descriptions. The potential for confused identification with young *E. sieboldi* may also contribute to inaccuracies in early recordings (Bory, 1933; Bauer *et al.,* 1959).

*E. briani* is considered to be a non-native addition to the parasite fauna of the British Isles (Fryer & Andrews, 1983; Alston, 1994; Environment Agency, 1999; Kerne, 2005). The parasite was first recorded from common bream *Abramis brama* in a stillwater fishery in Yorkshire (Fryer & Andrews, 1983; Fryer, 1993). Although the exact source and route of introduction are unknown, evidence suggests that the parasite entered the British Isles with infected fish from Continental Europe. This is supported by the

discovery of heavy *E. briani* infections in fingerling tench imported directly from Holland and Croatia to a Yorkshire fish farm, for the purpose of stocking fisheries (Alston, 1994; Environment Agency, unpublished). Most metazoan parasites are disseminated by the movements of infected hosts (Kennedy, 1976; Majoros *et al.,* 2003). The spread of freshwater ergasilids has largely resulted from the translocation of infected fish without adequate health checks and restrictions on movement (Fryer, 1993). Large scale and frequent imports of cyprinids from many parts of the world into England support this avenue of introduction (LFMD, 2006). The movement of infected fish therefore represents the greatest risk of *E. briani* dissemination. However, the dissemination of free-living stages with aquatic vegetation, wet fishing tackle or water used for fish transportation are additional, yet less common sources of infection.

*E. briani* has become successfully established within the British Isles and extended its geographical range (Environment Agency, 1999). The parasite has many attributes of a successful coloniser, including a direct life-cycle, high reproductive capacity and a wide host range (Kennedy, 1994). This invasive potential is consistent with other non-native ergasilid species introduced into England (Abdelhalim, 1990; Hawkins, 2001; Environment Agency, unpublished). *E. briani* has been documented throughout England and Wales and has colonised most fishery types including lakes, canal systems, river catchments and fish farms (Alston, 1994; Environment Agency, unpublished). To date *E. briani* has not been recorded in Scotland or Ireland. However, the distribution of *E. briani* in countries with more northern and southern latitudes than Scotland suggests that climate is unlikely to pose a boundary to the colonisation of the parasite.

- **Pathology**

The histopathology of *E. briani* has not been described. The only author to have described damage caused by the parasite is Alston (1994). However, this work is limited to observations from scanning electron microscopy only. Observations included structural damage to the gills, caused mainly by attachment. Lesions included compression with penetration of gill filaments and destruction of secondary lamellae below the mouth and legs of the parasite. Based upon observations in other ergasilid species, workers have suggested that blood flow restriction might also occur as a result of *E. briani* attachment (Fryer, 1982). Comprehensive pathological descriptions are necessary to improve understanding of this area.

- **Impact to fish populations**

Records of disease caused by *E. briani* are scarce. Bauer *et al.* (1969) documented losses of 0+ tench in a Russian fish farm as a result of heavy *E. briani* burdens, which reached intensities of 1139 parasites per host. However, little detail is provided regarding disease characteristics or the magnitude of losses associated with these infections. Alston (1994) observed emaciation and condition loss in experimentally infected tench as a result of heavy *E. briani* infection. Mortalities were recorded in two-year-old tench harbouring 108-509 parasites. Low level infections in the same fish species caused no obvious gross abnormalities and only a slight reduction in host condition. The effect of *E. briani* on the growth and condition of wild fish populations have not been described. Within the British Isles, no records exist of disease or mortality as a result of *E. briani* infections. However, unless losses are acute, impacts on wild fry populations are likely to go undetected or be masked by natural predation (Blanc, 1997).

Epidemiology studies of *E. briani* have been reported by Tuuha *et al.* (1992) and Alston (1994), from Finland and south-east England respectively. The latter study focused on infections within wild fish populations and represents the most comprehensive epidemiological study of the parasite to date. Other records are restricted to brief records of parasite intensities and factors influencing life-cycle development (Halisch, 1939; Grabda-Kazubska *et al.*, 1987; Bricker *et al.*, 1978). Alston (1994) revealed that prevalence of infection within the Basingstoke Canal reached 66% in bream fry <4cm, with intensities of up to 9 parasites per host. This increased to 91% of bream measuring 4-6cm in length. Alston (1994) suggested that deleterious impacts from *E. briani* were likely to be restricted to fish fry, supporting experimental observations and earlier speculation by Kirk & Lewis (1992). This represents an important area of further investigation in order to support this hypothesis.

- **Management and control**

No specific studies have been undertaken on the control or treatment of fish with *E. briani*. However, due to economic losses, significant attention has been given to the chemical treatment of other ergasilid parasites, in particular *E.sieboldi* (Kabata, 1970; Hoffman, 1977; Lahav & Sarig, 1967; Kirk & Lewis, 1992). A review of chemotherapeutics used for the control of ergasilid parasites has been published by Kabata (1970) and Kirk & Lewis (1992). This focuses heavily upon the use of organophosphorous chemotherapeutics for problems in aquaculture. These drugs, including the recently banned compound Naled[R], are neurotoxic insecticides with low therapeutic index and high toxicity to fish, environment and handler (Kirk & Lewis, 1992). None of these compounds are currently licensed for use in fisheries within the UK, thus they will not be discussed further.

Chapter 5.

It has become accepted by a number of workers that the presence of weed growth in a fishery may limit populations of ergasilid parasites (Bauer, 1962). This is believed to be the result of reduced water currents, limiting parasite dispersal (Kabata, 1985; Schäperclaus, 1991; Kirk & Lewis, 1992; Hoole et al., 2001). The most effective means of ergasilid control is through the prevention of introduction (Hoffman, 1977; Cressey, 1983). Within the British Isles, draining liming and restocking infected waters is the only recognised means of parasite eradication from fisheries (Environment Agency, unpublished).

## 5.3.2. Pathological changes associated with *E. briani* infections

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- **General observations and attachment characteristics**

During the study period, *E. briani* were recorded in roach *Rutilus rutilus*, tench *Tinca tinca*, rudd *Scardinius erythropthalmus* and common bream *Abramis brama*, with infections ranging between 1 and 85 parasites. The maximum number of parasites recorded on a single holobranch was 22.

In all cases, *E. briani* were confined to the gills of infected fish (Fig. 5.1). Parasites were usually obscured by the gill filaments, requiring removal and microscopic examination to confirm identity (Fig. 5.2). However, in very small fish, parasites were often visible within the first gill arch following removal of the operculum (Fig. 5.1). The favoured site of attachment of *E. briani* was between the hemibranchs, tight to the interbranchial septum (Fig. 5.3 & 5.4). This strict site preference resulted in parasites forming a line of infection parallel to the gill arch (Fig. 5.3). With illumination from the underside of each gill, parasites could be seen silhouetted within this region (Fig. 5.3). The proportion of the filament length taken up by individual parasites varied in relation to host size (Fig. 5.4). This was also influenced by the gill morphology of different host species, in particular the position and prominence of the gill septum. In very small fish, parasites were generally more conspicuous as they extended beyond the distal edge of the gill (Fig. 5.4). Both established and newly attached parasites, identified by their orange pigmentation (Fig. 5.2) were found attached within this position.

At no time during the study were more than a single parasite recorded on the same gill filament. Dissection of the connective tissue running along the gill septum (with use of

fine forceps) allowed parasites to be removed without directly handling them (Fig. 5.5).

In very small hosts the filaments of the gill were occasionally displaced by the presence

of the parasite between the hemibranchs (Fig. 5.6). The occasional arching of the

parasite's body when attached to the gills became a characteristic movement that aided

detection.

Fig. 5.1. A small number of *E. briani* (arrows) attached to the gills of a juvenile common bream. Due to the small size of the gills, the light coloured parasites were easily detected within these hosts, especially during the reproductive period when parasites possessed white egg-strings.



Fig. 5.2. *E. briani* following removal from the gills. The parasite's antennae (arrows) and black eye spot are conspicuous characteristics. Newly established parasites also possess areas of orange pigmentation within the cephalothorax (*)

Fig. 5.3. A heavy infection of *E. briani* in gill of crucian carp, showing parasites silhouetted between hemibranchs. Parasites typically attached to the interbranchial septum (arrow) giving the appearance of being 'lined-up' parallel with the gill arch.
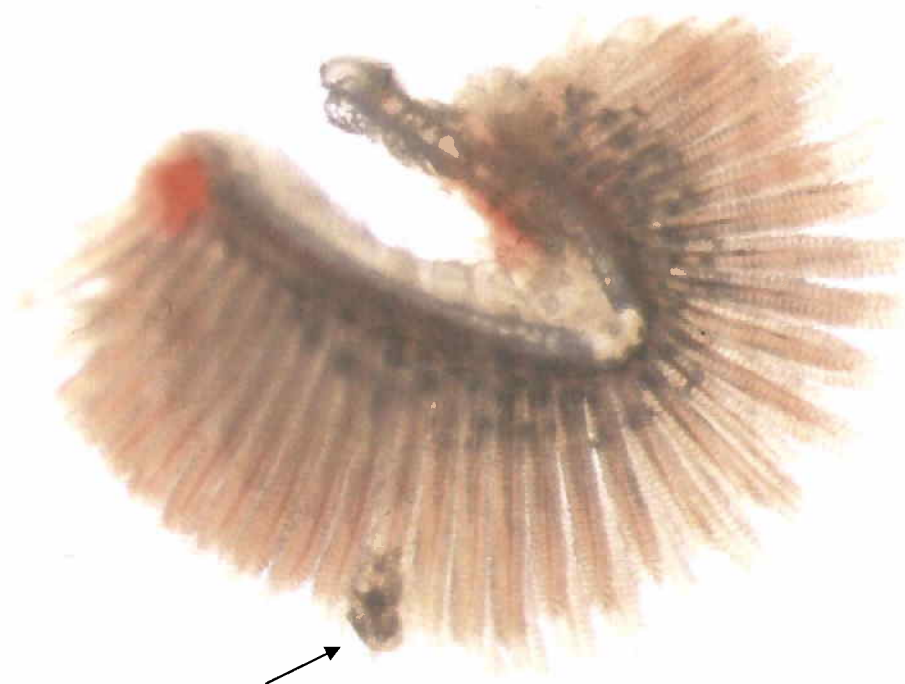


Fig. 5.4. A single *E. briani* (arrow) attached to gill of common bream fry. In very small hosts, parasites often extended beyond the distal edge of the gills.
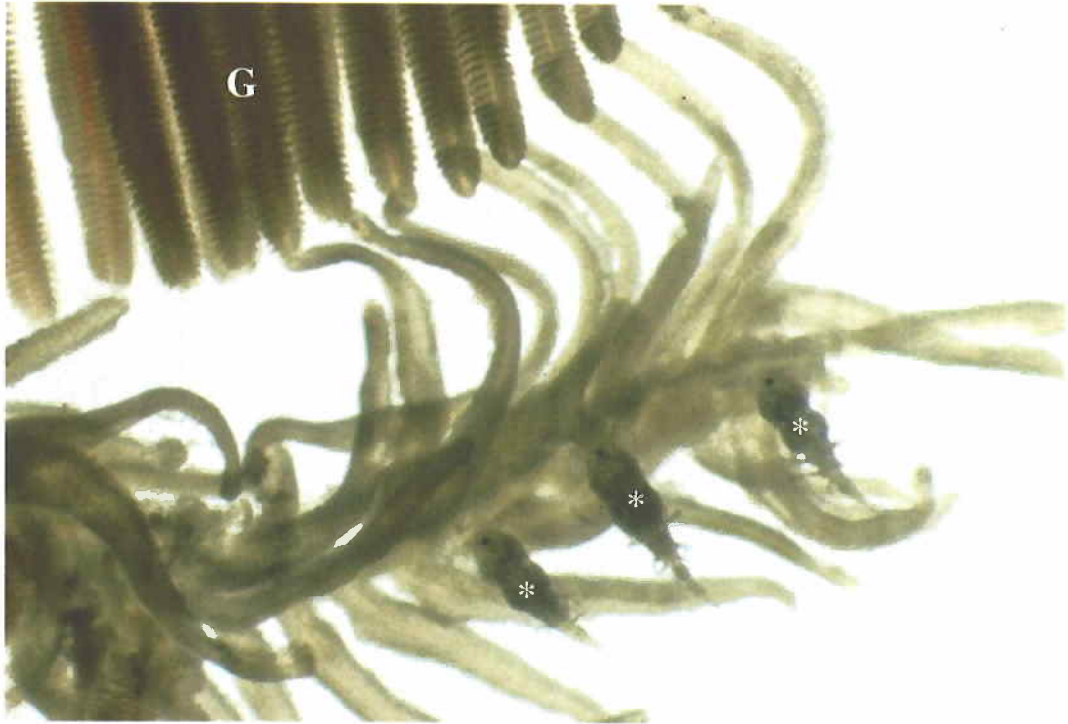
Fig. 5.5. Three *E. briani* (*) attached to the connective tissue of tench gill (G). Removal of this tissue from the gill with fine forceps enabled parasites to be examined without direct contact. The eggstrings are absent from these specimens.
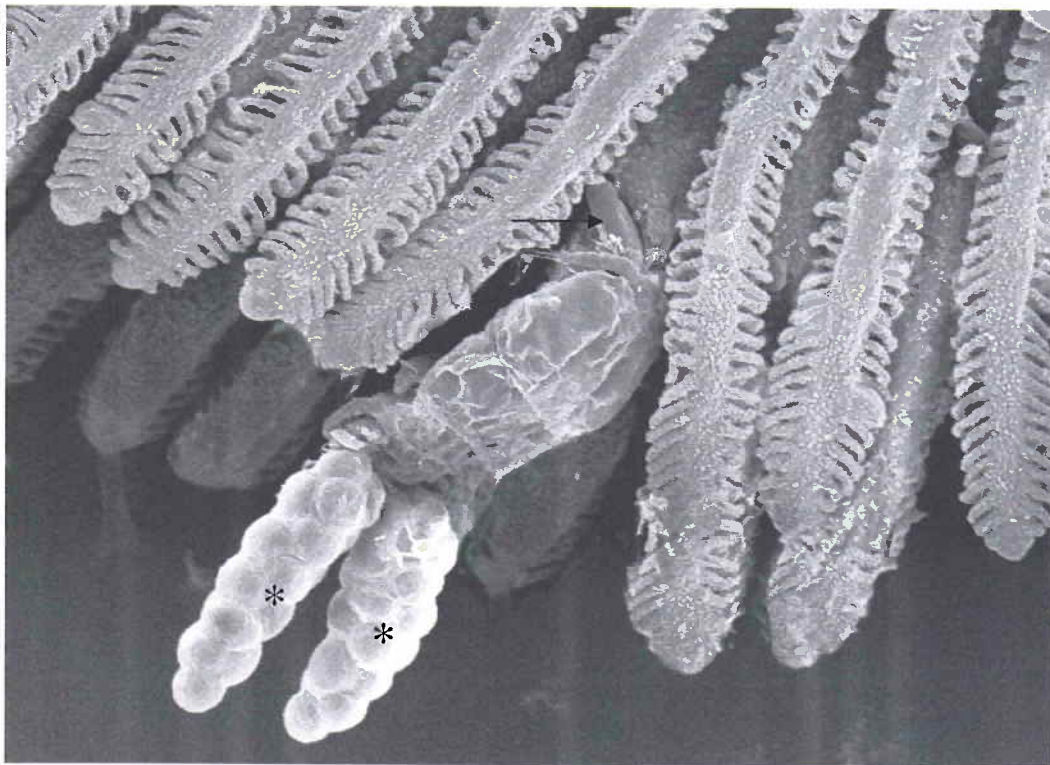


Fig. 5.6. SEM of *E. briani* attached to gills of a juvenile common bream. Attachment involved insertion of the parasite's antennae into the gill (arrow). Presence of the parasite, with eggstrings (*) often resulted in displacement of adjacent gill filaments.

- **Gross pathological changes**

During the study period, infected fish showed no obvious signs of gross pathological change. Although a small number of heavily infected hosts appeared to be in poor condition, these observations were not consistent and could not be attributed directly to infection. The gills of infected fish, including those of juvenile hosts did not reveal any gross abnormalities or clinical signs of disease. Prior to the removal of the operculum, infected fish were generally indistinguishable from uninfected individuals.

- **Histopathological changes associated with infections of *E. briani*.**

*E. briani* were primarily found attached to the ventral surface of the gill filaments, tight to the interbranchial septum (Fig. 5.7). Attachment involved use of the parasite's antennae to grasp the gill filaments. This brought the mouth-parts located on the underside of the body into close contact with the gill tissue (Fig. 5.8). Attachment was characterised by the antennae directed in a forward position toward the gill arch (Fig. 5.9). This allowed the parasite's body and eggstrings (when present) to lie parallel to the filaments. Attachment usually involved the antennae embracing two gill filaments (Fig. 5.10). This resulted in the ventral surface of the parasite hanging within the space between adjacent filaments rather than directly along the gill surface (Fig. 5.10). This behaviour appeared to be dependent upon host size, with the antennae grasping a single filament in larger hosts and spanning up to four filaments in very small fish. Insertion of the terminal segment of the antennae into the filament (Fig. 5.11) ensured firm attachment to the gills.

Epithelial hyperplasia was a frequently recorded consequence of *E. briani* infection. However, this host response was usually quite mild and rarely extended beyond the

immediate site of parasite attachment. Exceptions to this were observed when parasites were found on the medial and lateral surfaces of the filaments. This lead to more pronounced hyperplasia with loss of normal gill structure (Fig. 5.12). These changes were occasionally accompanied by capillary congestion and localised necrosis of the epithelium (Fig. 5.13). During most infections, pathological changes were localised, leaving the majority of the gill filament relatively normal (Fig. 5.14).

Due to the strict site specificity shown by *E. briani,* heavy parasite infections resulted in loss of space adjacent to the interbranchial septum (Fig. 5.15). The presence of the parasite's body within this region resulted in mechanical compression and distortion of the ventral filament surface. However, this varied considerably depending upon space availability and the position of parasites within this region. This was most pronounced in very small fish, where the filaments accommodated the anterior region of the parasite's body (Fig. 5.16). The pressure exerted by the body of the parasite, combined with the gripping action of the antennae caused compression of epithelium and thinning of tissue surrounding the efferent arteriole (Fig. 5.16). Arteriole narrowing was recorded during a number of infections, although this did not appear to cause complete vessel constriction.

Many of the pathological changes associated with *E. briani* resulted from attachment, namely insertion of the antennae into the gill and the resultant pressure exerted by the body of the parasite. The severity of pressure changes varied according to host size, space available between the hemibranchs and parasite morphology. Space was generally most limited in the region closest to the interbranchial septum. However, this region was primarily utilised by the narrow, forwardly positioned antennae (Fig. 5.17A) which were relatively easily accommodated. The greatest pressure changes were

associated with the thickest part of the parasites body, namely the anterior region of the cephalothorax. However, with increasing space availability away from the gill septum, damage remained relatively mild within this region (Fig. 5.17B). Despite their relatively large size, the eggstrings of *E. briani* were associated with few pathological changes. Space within this region minimised host parasite contact, leading to minimal gill damage (Fig. 5.17C). This was consistent in very small hosts, where the eggstrings extended beyond the gill in to the space of the opercular chamber.

Many of the pathological changes associated with *E. briani* were more evident in very small hosts. The presence of parasites in very small fish caused both lateral (Fig. 5.6) as well as dorso-ventral displacement of gill filaments (Fig. 5.18). In most infections, the anterior regions of *E. briani* maintained close contact with host tissues, whilst the posterior regions of the body, including the swimming legs and furcal-rami caused little damage (Fig. 5.19). Exceptions to this included minor flattening of the secondary lamellae and mild hyperplasia.

Due to the obscured position of parasites on the ventral surface of the gills, it was difficult to make direct observations of the feeding activity of *E. briani*. However, damage to the filaments adjacent to the mouth parts included erosion, haemorrhage and compression of the epithelium (Fig. 5.20 & 5.21). This was often most noticeable on the lateral surfaces of the filaments, suggesting that *E. briani* may adjust position during feeding. The combination of attachment and potential feeding appeared to place pressure upon the underlying efferent arteriole (Fig. 5.21). In a small number of hosts, accumulations of bacteria were observed around the body and attachment appendages of the parasites (Fig. 5.22). Inflammatory responses to *E. briani* infection were varied but usually mild.

Fig. 5.7. Section through whole gill of common bream. The typical position of *E. briani* (*) at the junction of the filaments (F) and interbranchial septum (S) can be seen. The gill rakers (R), gill arch (A) and filament musculature (M) are labelled. Scale bar = 0.5mm.
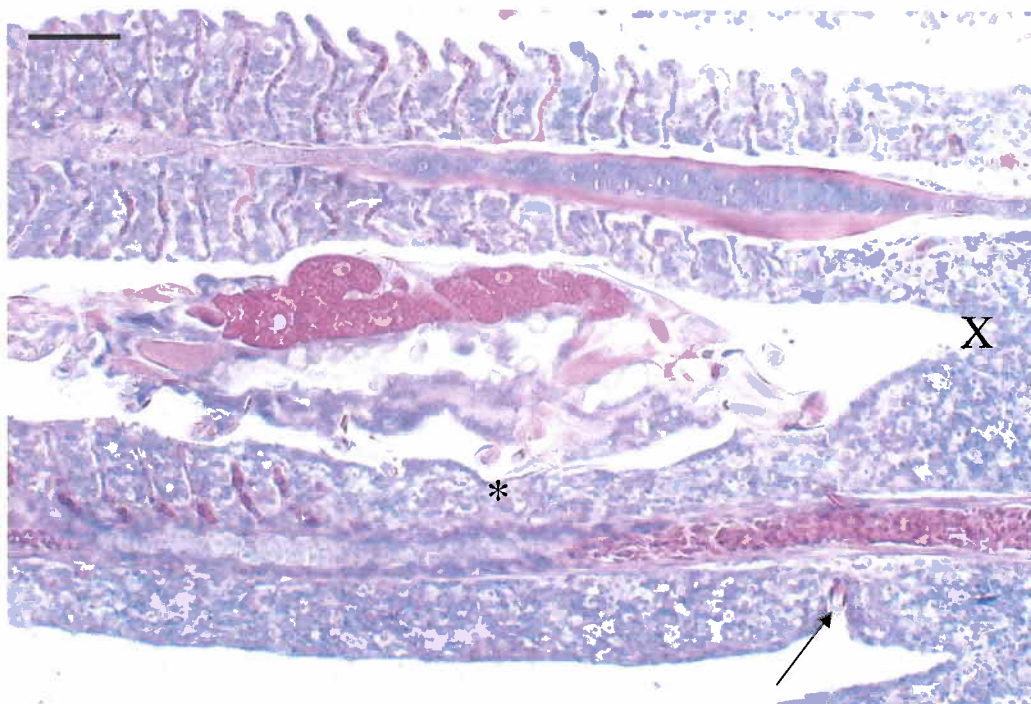


Fig. 5.8. Attachment of *E. briani* showing use of antennae to grip gill filaments (arrow) tight to the gill septum (X). Orientation of parasites was always in a forward-facing position with the body of the parasite running along the filaments. This allowed the mouth-parts (*) to come into close contact with the gill surface. Scale bar = 60 μm.
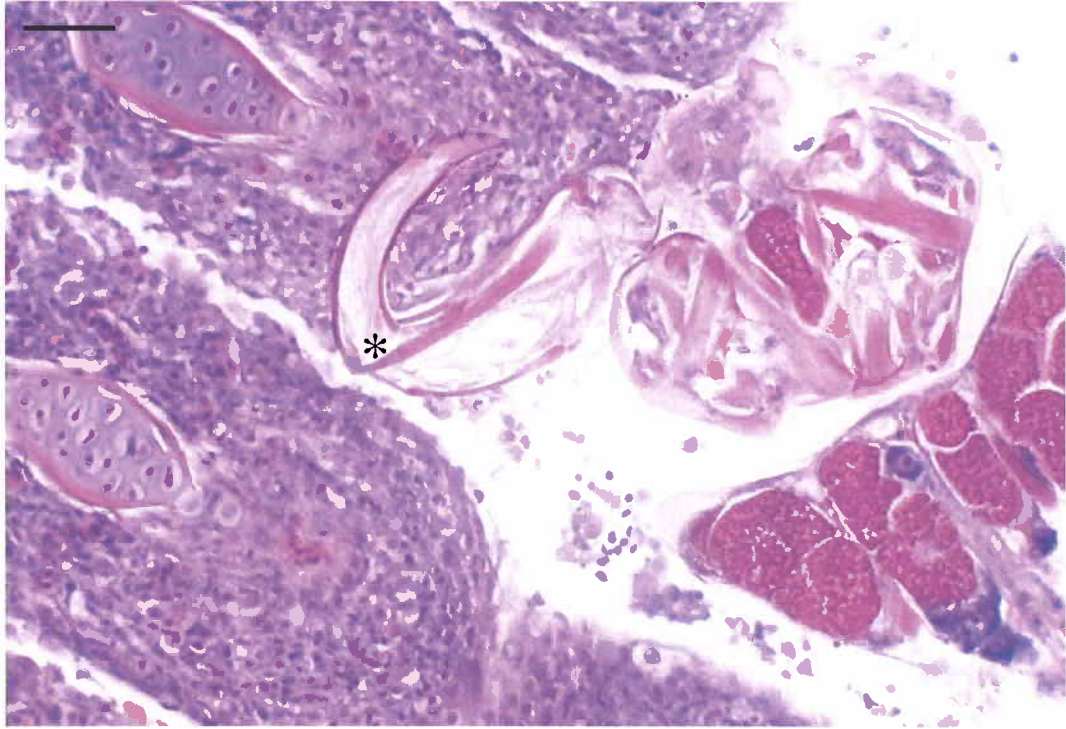
Fig. 5.9. Section showing attachment of *E. briani* to gill of tench. During all infections the antennae (*) of *E. briani* were orientated in a forward position toward the junction of the hemibranchs. Scale bar = 40μm.



Fig. 5.10. A single *E. briani* showing typical attachment tight to gill septum with antennae (*) engulfing two filaments. The body of the parasite lies between filaments rather than along the ventral surface. Pressure exerted by attachment caused noticeable indentation and thinning of the filament (arrow). Scale bar = 80μm.
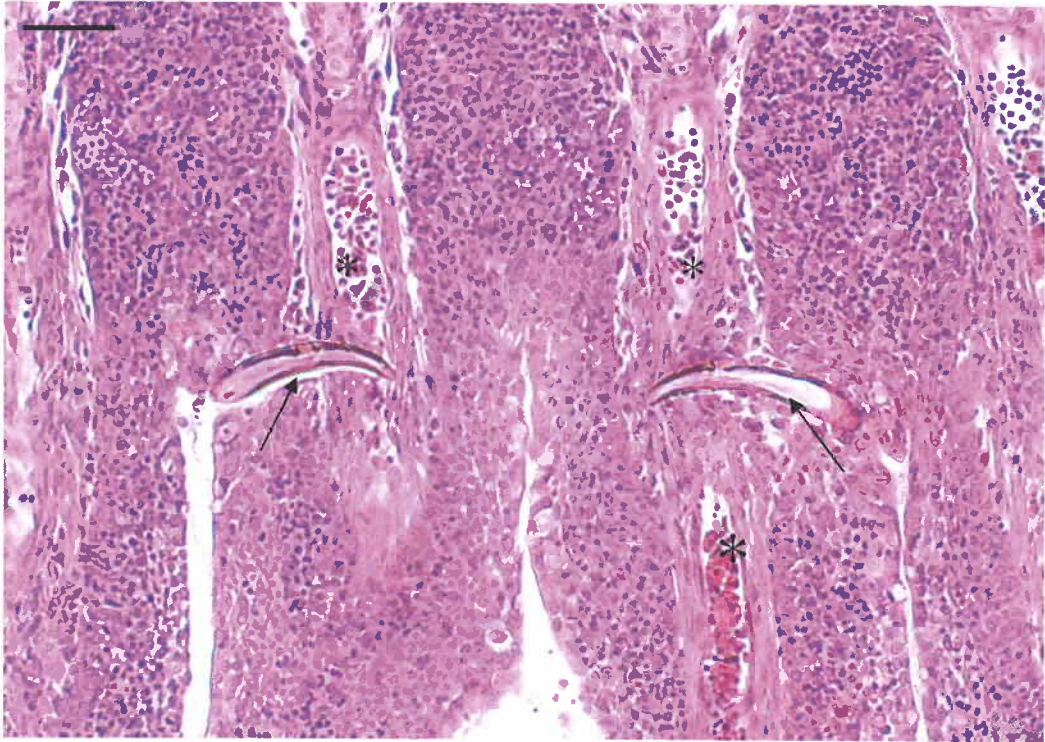
Fig. 5.11. Insertion of antennae terminal segments (arrows) during attachment of *E. briani* leading to puncture of adjacent filaments. Pressure exerted by the antennae and body of the parasite led to mechanical distortion of the filament, hyperplasia and slight compression of the efferent arteriole (*). Scale bar = 40μm.
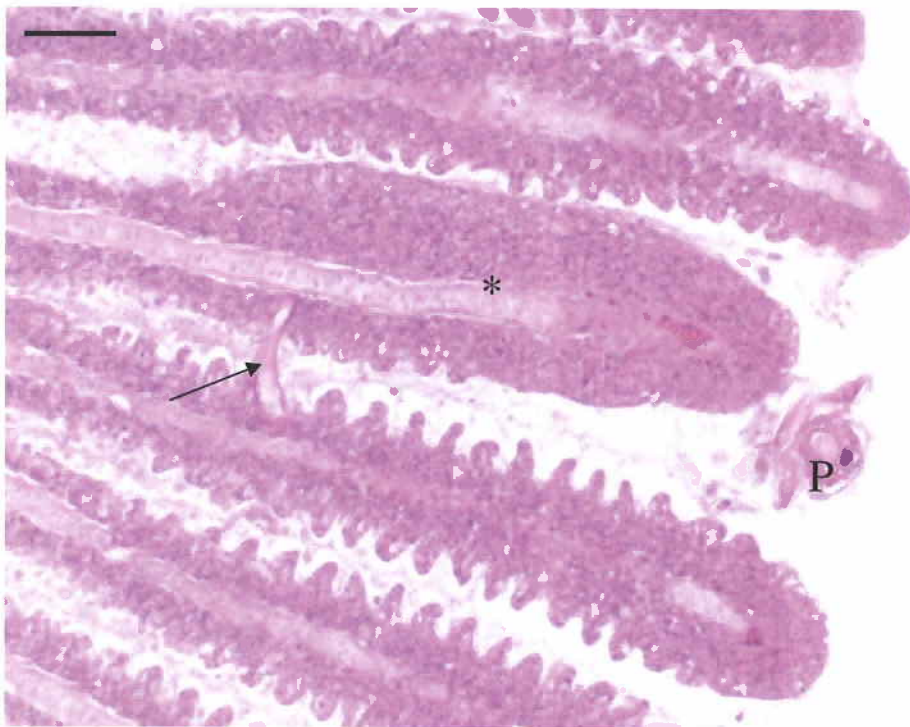


Fig. 5.12. Attachment of *E. briani* to distal regions of bream gill filaments. This resulted in more pronounced damage, including hyperplasia (*) and loss of normal gill structure in regions between the antennae (arrow) and body of the parasite (P). Scale bar = 80μm.
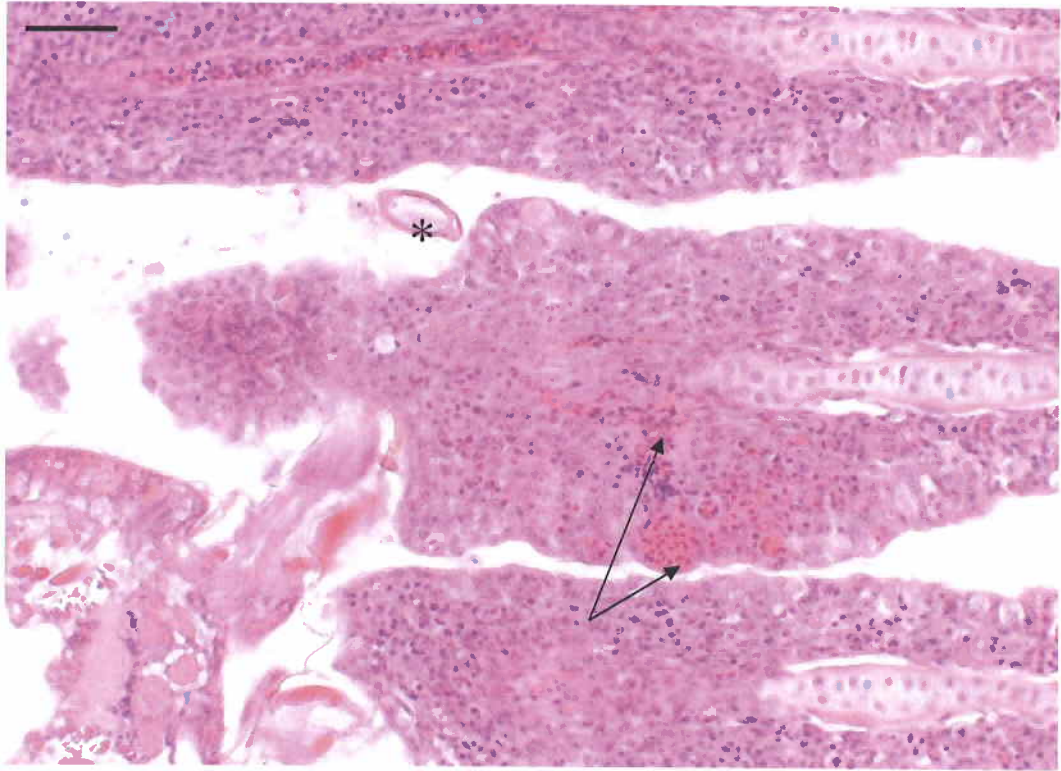
Fig. 5.13. Section showing attachment of *E. briani* (*) to distal region of rudd gill. Localised capillary congestion (arrows) and necrotic changes to the epithelium occurred within these regions. Scale bar = 40μm.
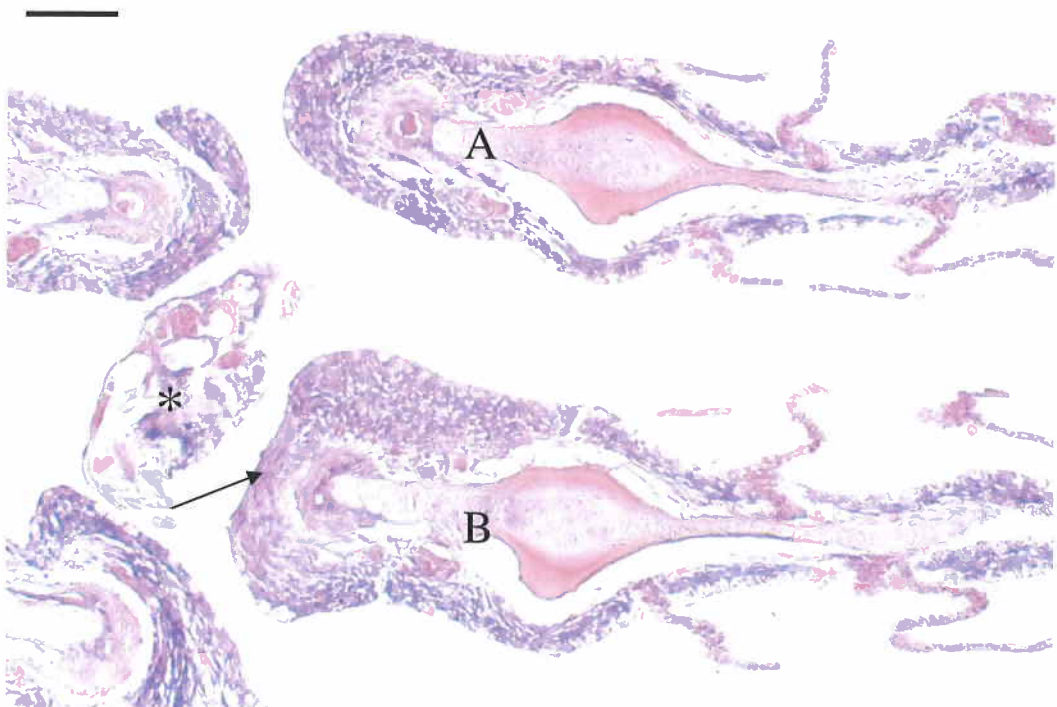


Fig 5.14. Section showing comparison of uninfected (A) and infected (B) filaments of bream gill. A single *E. briani* (*) is attached to the ventral gill surface. With the exception of mild distortion and epithelial compression (arrow) the filaments, including secondary lamellae remained relatively normal. Scale bar = 60μm.
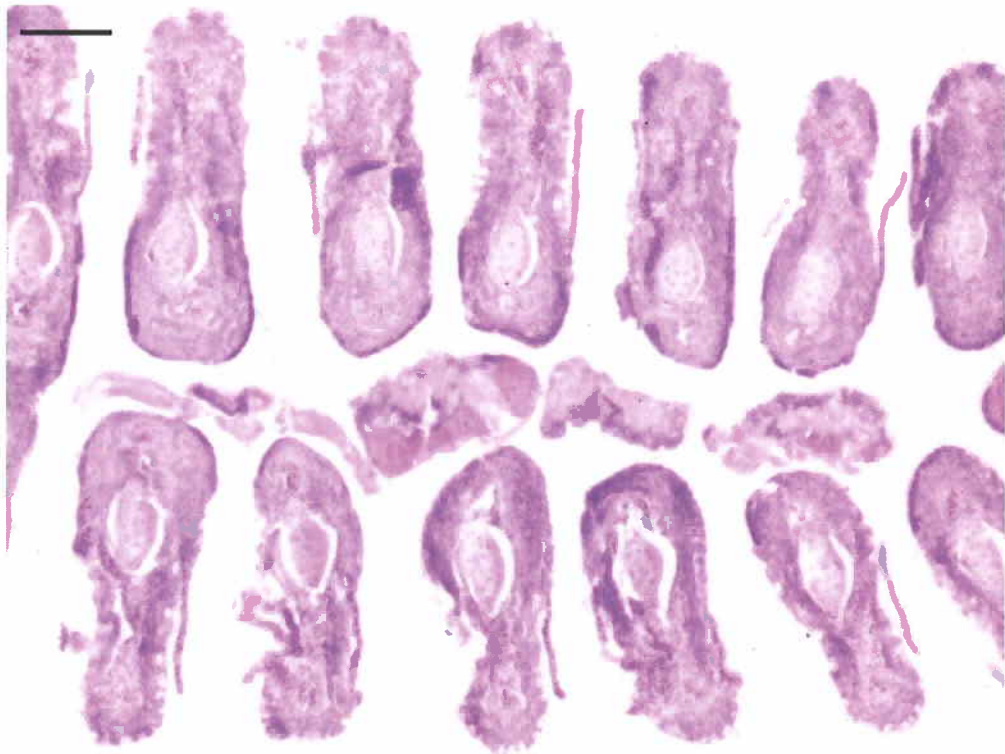
Fig. 5.15. A number of *E. briani* located between hemibranchs of roach gill. Competition for this preferred site of attachment led to loss of the inter-filament spaces. Scale bar = 100μm.
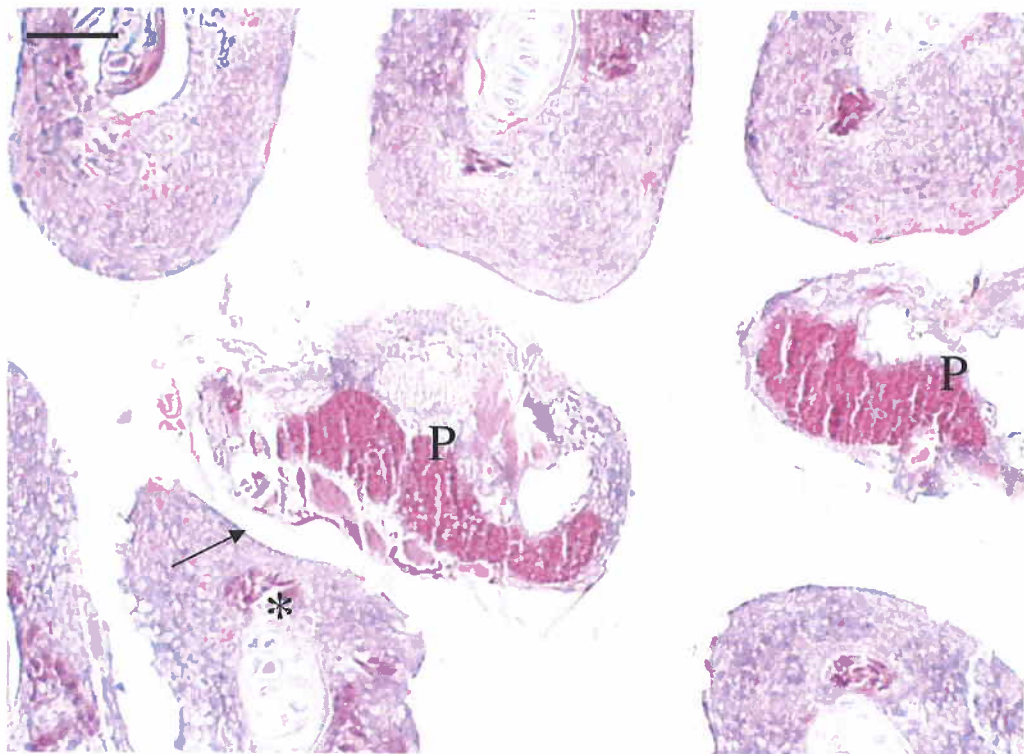


Fig. 5.16. Presence of parasites (P) between hemibranchs of tench gill. Pathological changes within these regions included localised distortion of the ventral surface (arrow) and compression of epithelium adjacent to the efferent arteriole (*). Scale bar = 40μm.
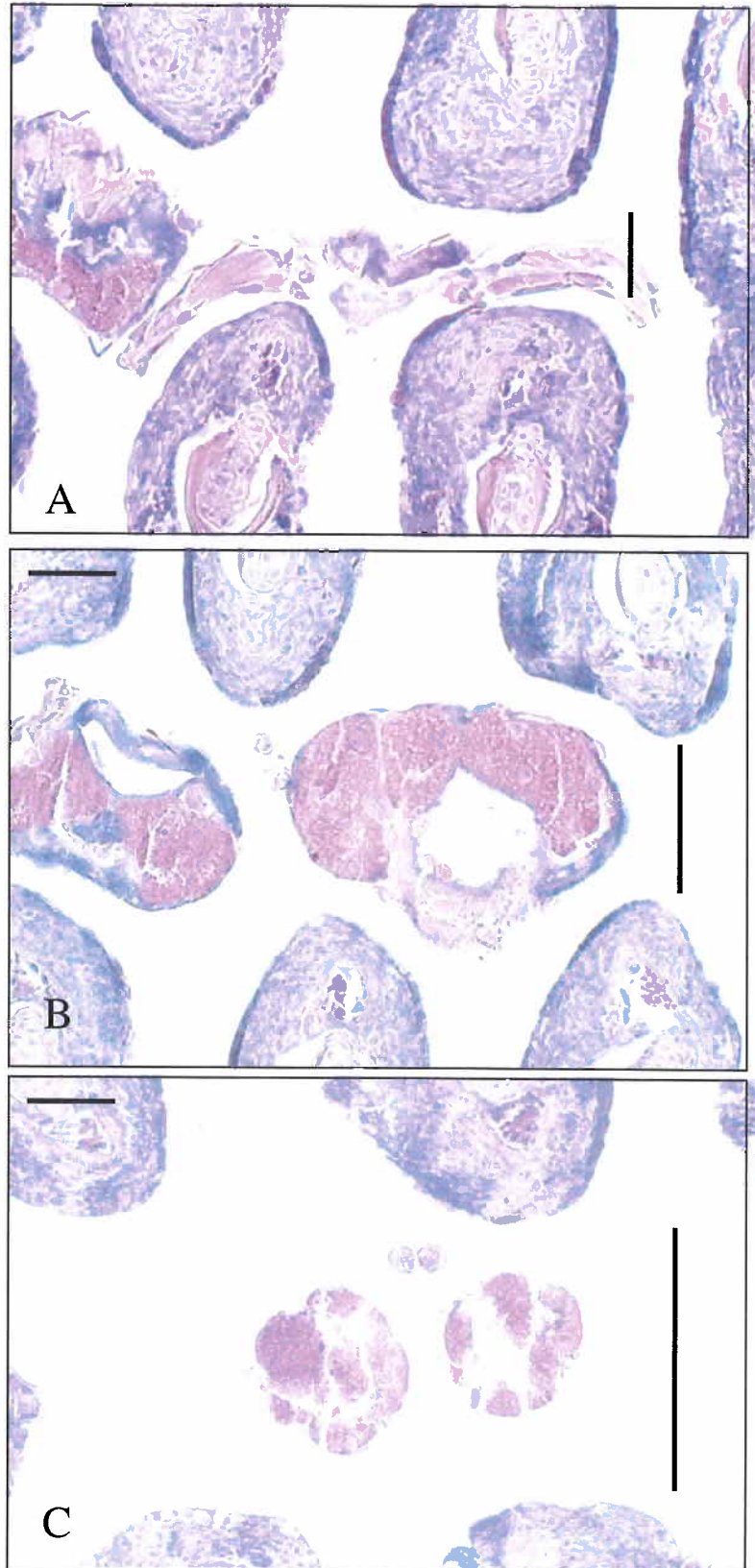
Fig. 5.17. Step transverse sections through bream gill from anterior junction of hemibranch (A) to distal regions of filaments (C). The space between the filaments can be seen to increase (vertical black lines), accommodating the parasites antennae (A), main body (B) and finally eggstrings (C) with minimal gill damage. Scale bar = 40µm.
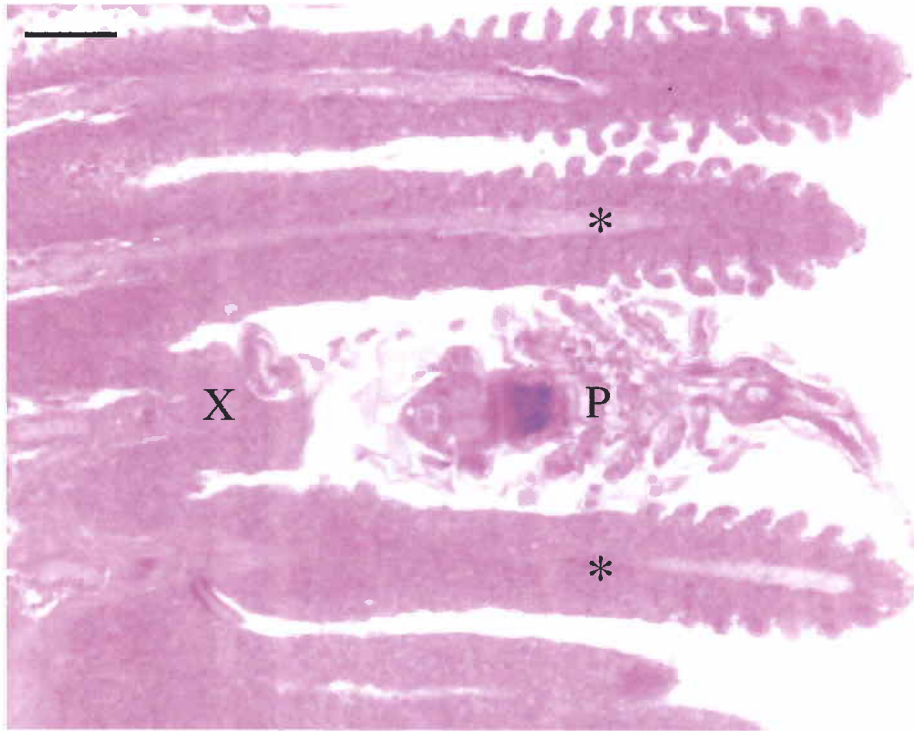
Fig. 5.18. Section showing attachment of *E. briani* to bream gill. Displacement of the gill filament (X) as a result of the parasite can be seen. Normal position of filaments (*) either side of the parasite (P) are shown. Scale bar = 80μm.
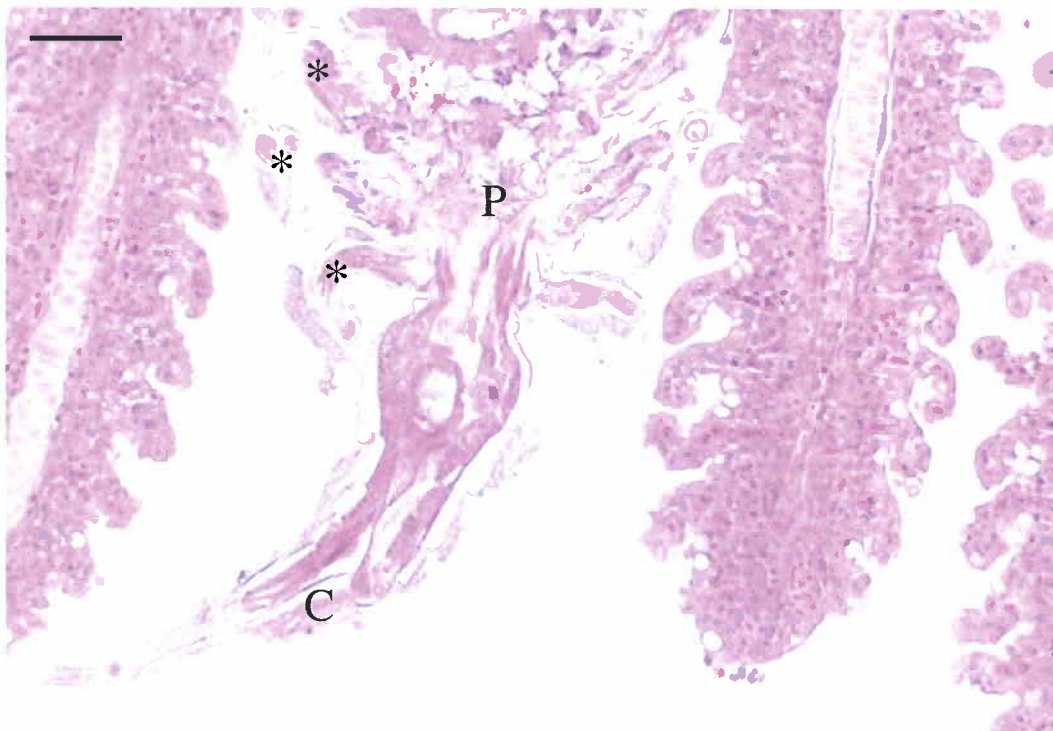


Fig. 5.19. Section showing *E. briani* between adjacent gill filaments of roach. Minimal damage was recorded as a result of contact from the parasite's body (P), swimming legs (*) and caudal rami (C). Scale bar = 40 μm.
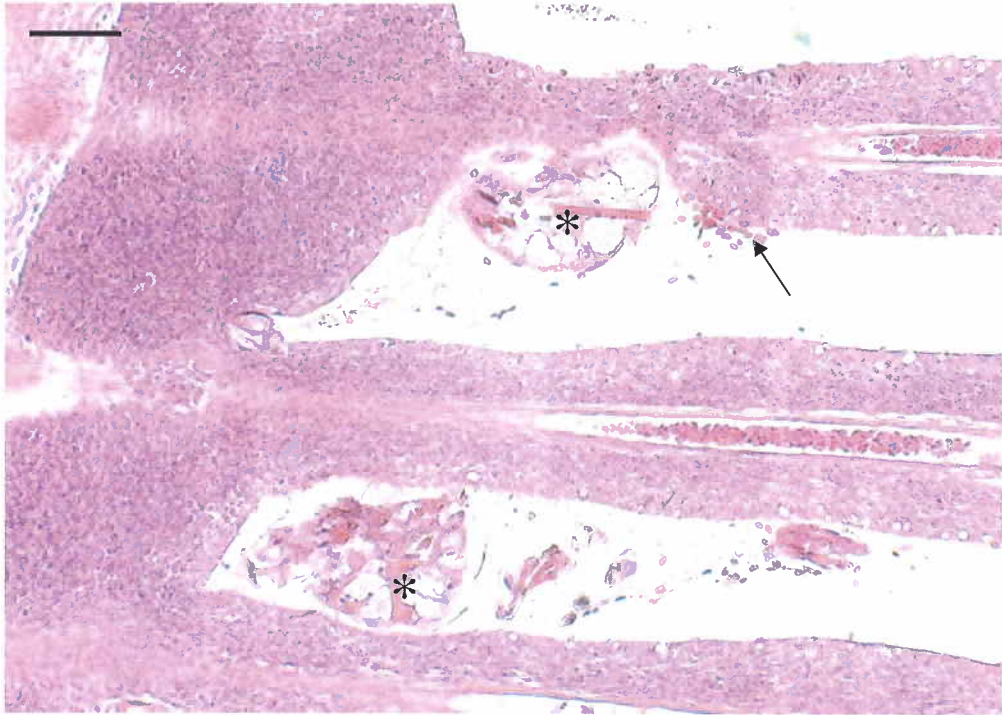
Fig. 5.20. Two *E. briani* (*) attached between adjacent filaments of bream gill. Pathological changes at the base of the filaments, included necrosis, epithelial erosion, compression and haemorrhage (arrow). These were considered the combined result of parasite attachment and feeding behaviour. Scale bar = 80μm.
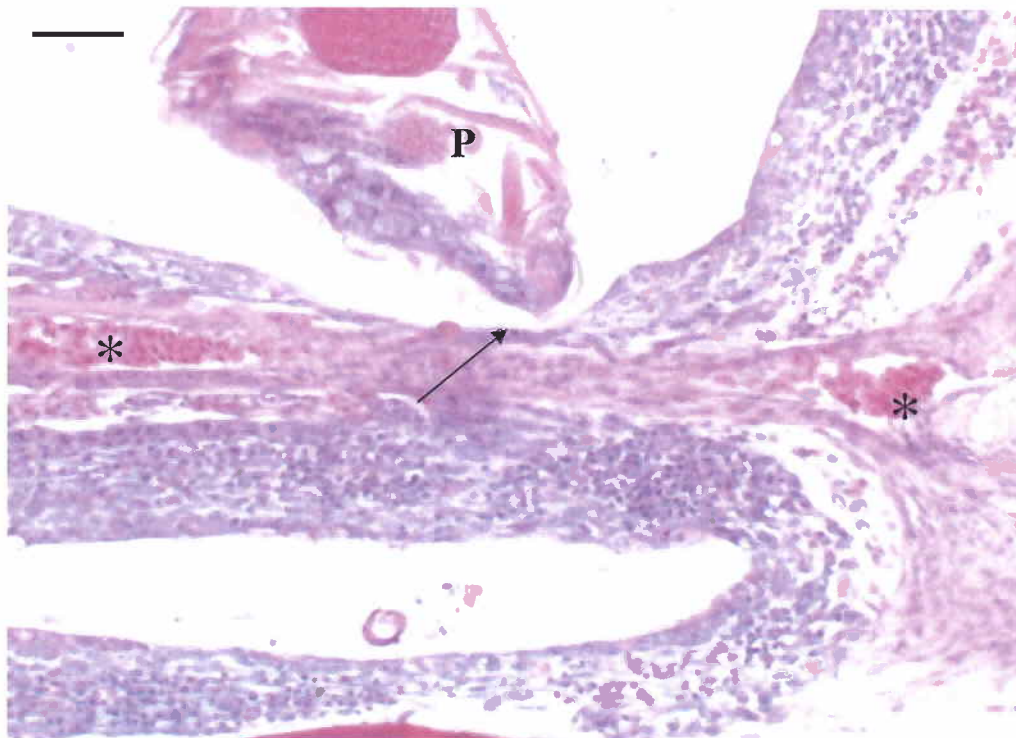


Fig. 5.21. Compression and erosion of epithelium at base of gill filament of bream (arrow). This represents a possible feeding site of *E. briani* (P). The blood sinus (*) has become constricted at this point as a result of pressure upon the gill filament. Scale bar = 40μm.
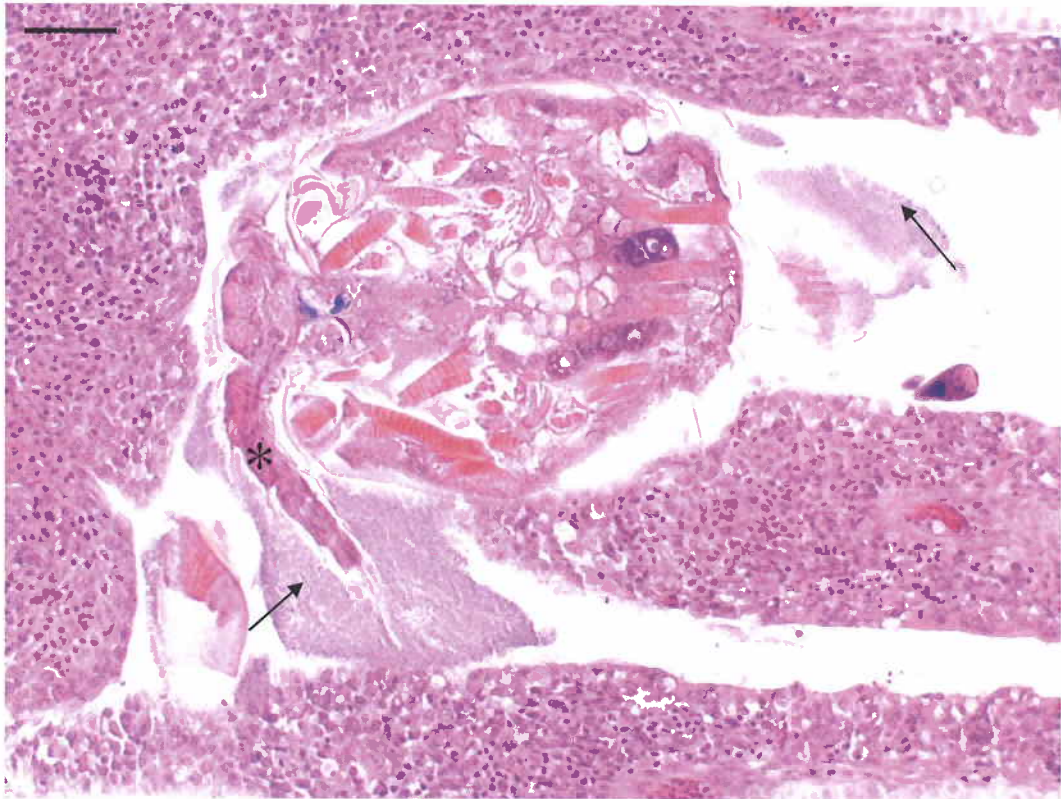
Fig 5.22. Accumulation of bacteria (arrows) surrounding the body and antennule (*) of *E. briani* whilst attached to the gill filaments of a common bream. Scale bar 40μm

### 5.3.3. Epidemiology studies of *E. briani* in fisheries

- **Prevalence and intensity of *E. briani* within the Basingstoke Canal**

During the study period a total of 439 fish were captured from the Basingstoke Canal and examined for the presence of *E. briani* (Table 5.1). This comprised 413 fish from the October 2003 sampling and a further 26 fish from August 2004. The 2003 sample comprised 155 bream, 247 roach and 11 roach/bream hybrids. The 2004 sample comprised exclusively of bream. Roach and bream sampled from the canal were aged between 1 and 4 years old. The length frequency histograms for roach and bream sampled during 2003 are shown in Figs. 5.23 A and B. Roach bream hybrids and fish captured during the 2004 sampling were not aged. Due to the small sample size obtained during 2004, most attention was given to analysis of the 2003 data. Similarly, very small numbers of 1-year-old bream captured from three of the four sites sampled limited spatial comparisons of parasite infections.

During the study period, roach, bream and roach/bream hybrids were all found to harbour infections of *E. briani*. However, both prevalence and intensity of infection were significantly higher in bream than in the roach and hybrid populations (Table 5.1 and 5.2). Prevalence of infections within the 2003 bream fry population reached 82.4%, compared with 17.1% in roach. Despite small sample sizes, prevalence of infection in 2004 exceeded 92% within the bream population. Intensities of infections ranged from 0-62 parasites in bream, whilst in roach the maximum infection recorded was just 6 parasites.

In both bream and roach populations, prevalence of *E. briani* was greatest in fry, rather than older fish. In both species, prevalence was zero within the oldest year class examined, although very small sample numbers limit significance of this finding. A 46% prevalence in 3 year old bream indicate that this year class remains highly susceptible to *E. briani* infection. These studies confirm that bream fry are highly favoured hosts of *E. briani.*

Table 5.1. Summary data of *E. briani* infections in roach, bream and roach/bream hybrids examined from the Basingstoke Canal during October 2003 and August 2004.

| Date sampled | Host species | Host age | Number examined | Parasite prevalence (%) | Prevalence range 95% confidence | Intensity range (mean) |
|---|---|---|---|---|---|---|
| **October 2003** | Roach | 1 | 99 | 17/99 (17.1%) | (10.8 – 24.7) | 0-4 (1.65) |
| | | 2 | 90 | 7/90 (7.7%) | (3.7 – 15.4) | 0-6 (1.86) |
| | | 3 | 55 | 1/55 (1.8%) | (0 – 9.7) | 2 (2) |
| | | 4 | 3 | 0/3 (0%) | | - |
| | | All | 247 | 25/247 (10.1%) | (6.8 – 14.5) | 0-6 (1.72) |
| | Bream | 1 | 74 | 61/74 (82.4%) | (71.8 – 89.9) | 0-19 (4.43) |
| | | 2 | 66 | 22/66 (33.3%) | (22.6 – 45.4) | 0-20 (2.14) |
| | | 3 | 13 | 6/13 (46.1%) | (22.4 – 73.9) | 0-34 (10.3) |
| | | 4 | 2 | 0/2 (0%) | - | - |
| | | All | 155 | 89/155 (57.4%) | (49.4 – 65) | 0-34 (5.31) |
| | Roach bream hybrids | All | 11 | 2/11 (18.2%) | (3.3 – 50) | 0 – 2 (1.5) |
| **August 2004** | Roach | NA | 0 | NA | | NA |
| | Bream | 1 | 5 | 5/5 (100%) | (50 – 100) | 7– 62 (30.2) |
| | | 2 | 12 | 12/12 (100%) | 75.7 – 100) | 1– 26 (11.7) |
| | | 3 | 0 | - | - | - |
| | | 4 | 9 | 7/9 (77%) | (44.2 – 95.9) | 0 – 6 (2.9) |
| | | All | 26 | 24/26 (92.3%) | (75.4 – 98.6) | 1-62 (12.9) |
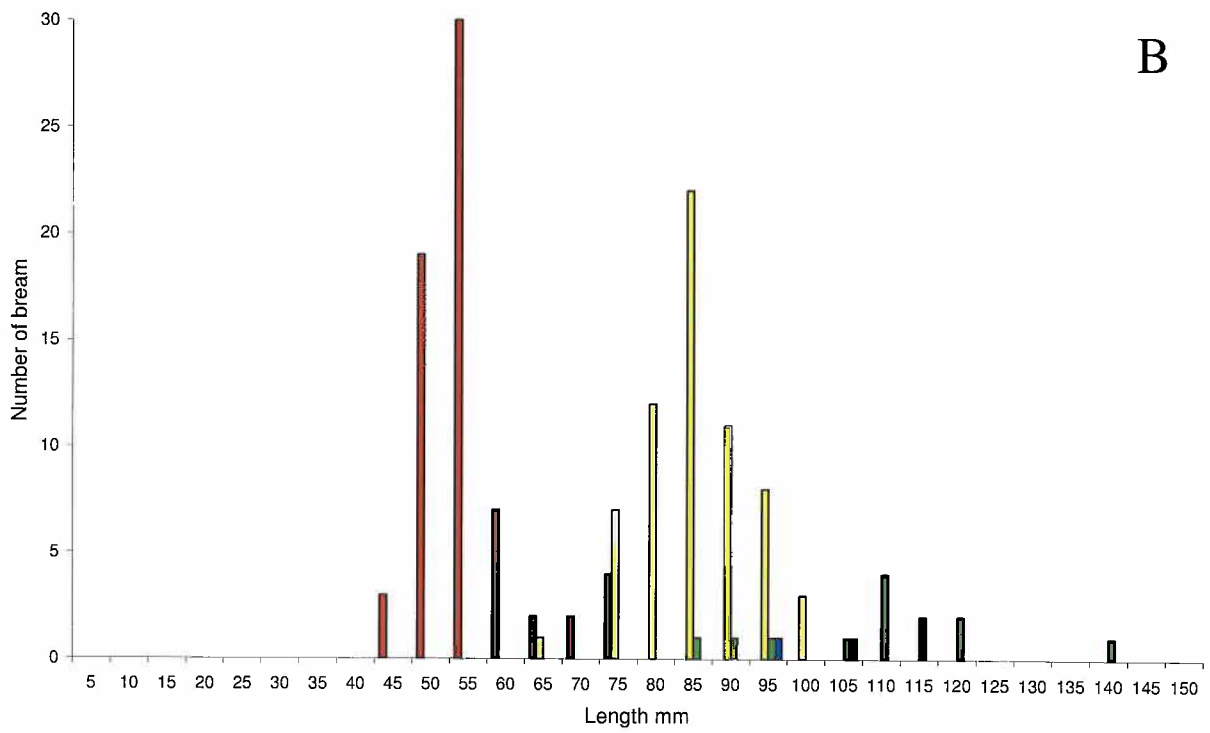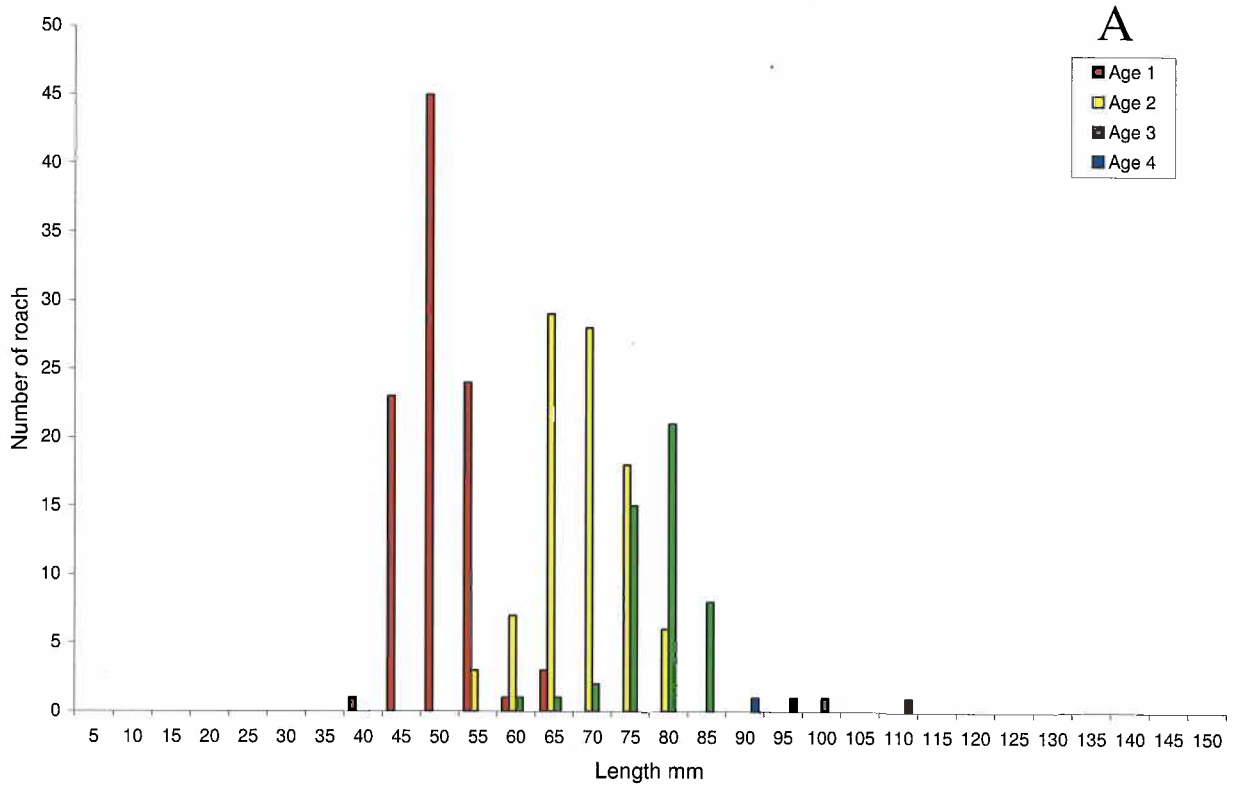
Fig 5.23. Length frequency histogram for roach (A) and bream (B) sampled during 2003 from the Basingstoke Canal

Table 5.2. Summary data of *E. briani* infections in 1 and 2 year old fish from the Basingstoke Canal between October 2003 and August 2004.

| Site | Host species | Age | Number examined | Prevalence (%) | Prevalence 95% confidence | Intensity (mean) |
|------|------|------|------|------|------|------|
| Ash Lock (Oct 2003) | Roach | 1 | 0 | - | - | - |
| | | 2 | 51 | 5/49 (10.2%) | 5.5 – 24.3% | 1-6 (2) |
| | Bream | 1 | 1 | 1/1 (100%) | 5 – 100% | 19 (1) |
| | | 2 | 16 | 3/16 (18.8%) | 5.3 – 43.6% | 0-20 (8.3) |
| Queen Ann Bridge (Oct 2003) | Roach | 1 | 94 | 15/93 (16.1%) | 9.5 – 24.9% | 0-4 (1.5) |
| | | 2 | 16 | 0/16 (0%) | - | - |
| | Bream | 1 | 73 | 59/73 (80.8%) | 70.9 – 89.6% | 0-18 (4.4) |
| | | 2 | 45 | 15/45 (33.3%) | 21.3 – 49.2% | 0-14 (5.9) |
| A325 Bridge (Oct 2003) | Roach | 1 | 5 | 2/5 (20%) | 7.6 – 81% | 0-4 (3) |
| | | 2 | 26 | 1/26 (3.8%) | 24.8 – 98.7 | 1-6 |
| | Bream | 1 | 1 | 1/1 (100%) | 5 – 100% | 1 (1) |
| | | 2 | 4 | 3/4 (75%) | 24.8 – 98.7% | 0-6 (3) |
| Eelmoor Flash (Aug 2004) | Bream | 1 | 5 | 5/5 (100%) | 50 – 100% | 7-62 (30.2) |
| | | 2 | 12 | 12/12 (100% | 75.7 – 100% | 1–26 (11.7) |
| | Roach | NA | 0 | NA | NA | NA |

- **Prevalence and intensity of *E. briani* within Birkett Hall Pond**

A total of 313 rudd were captured from Birkett Hall Pond during a single netting in April 2004 (Table 5.3). The fish examined ranged from 46-90mm in length. No other species were captured from the fishery. The prevalence of *E. briani* within the rudd population was 21.4% (67/313). Intensity of infection ranged from 0 to 85 parasites per host (mean intensity = 15.4 parasites). Due to time limitations, ageing analysis was not undertaken on the sample of fish from Birkett Hall Pond. However, 55mm was used as an approximate upper limit for a 1 year-old rudd (Britton *pers. comm.*). Using this limit, the prevalence and intensity of *E. briani* were both greatest in rudd exceeding 55mm in length (significance). In the rudd population from Birkett Hall Fishery, the intensity of *E. briani* infection increased significantly with host size (Fig. 5.24). Dividing the examined population into 5mm size groups revealed that mean intensity and prevalence also increased significantly with host size (Table 5.4).

Table 5.3. Summary data for rudd sampled from Birkett Hall Pond in April 2004

| Size class | Number Examined | Length range | Prevalence % (95% Conf) | Intensity range (mean) |
|---|---|---|---|---|
| **All rudd** | 313 | 45 – 90 mm | 21.4% (17 – 26.3) | 0 – 85 (15.4) |
| **< 55mm** | 213 | 45 – 55 mm | 16.4% (11.9 – 22) | 0 – 50 (7.4) |
| **> 55mm** | 100 | 56 – 90 mm | 32% (23.4 – 42) | 0 - 85 (24.2) |

- **Distribution of *E. briani* within fishery populations**

*E. briani* was shown to have an over-dispersed distribution within both fisheries examined (Fig. 4.25A & B). The observed variance to mean ratios indicate that *E. briani* is highly over-dispersed within the bream (7.56), roach (2.84) and rudd (39.07)

populations examined. This indicates that most of the fish examined had either no parasites or low-level infections, whilst a relatively small proportion of fish harboured heavy parasite burdens.

Table 5.4. Infection characteristics of *E. briani* in rudd populations from Birkett Hall Fishery during April, 2004.

| Rudd length (mm) | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | 70+ | All |
|---|---|---|---|---|---|---|---|
| No. examined | 29 | 157 | 54 | 35 | 22 | 16 | 313 |
| Prevalence (%) | 10.3 | 17.2 | 14.8 | 25.7 | 27.3 | 75 | 20.8 |
| Intensity range | 0-14 | 0-50 | 0-28 | 0-40 | 0-28 | 0-85 | 0-85 |
| Mean intensity | 7.7 | 7.9 | 8.9 | 11.3 | 13.5 | 45.2 | 15.9 |
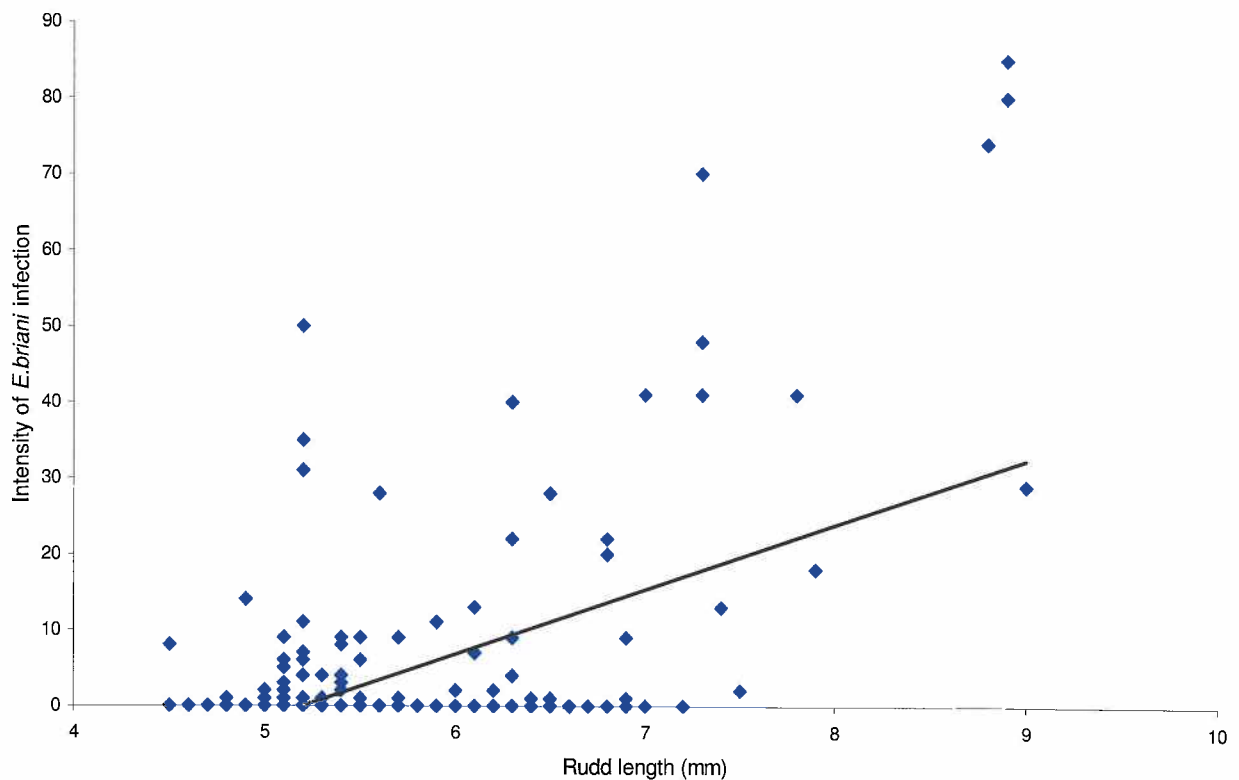


Fig. 5.24. Relationship of rudd length and intensity of *E. briani* within Birkett Hall Fishery during April, 2004.
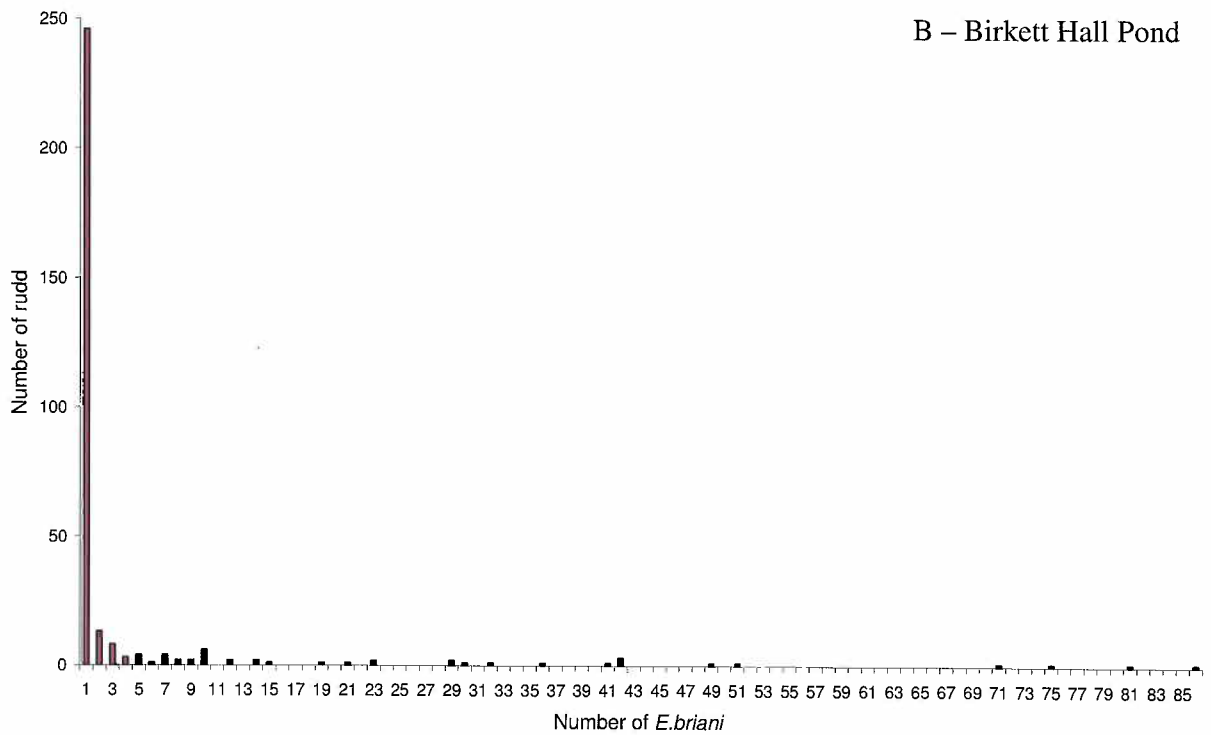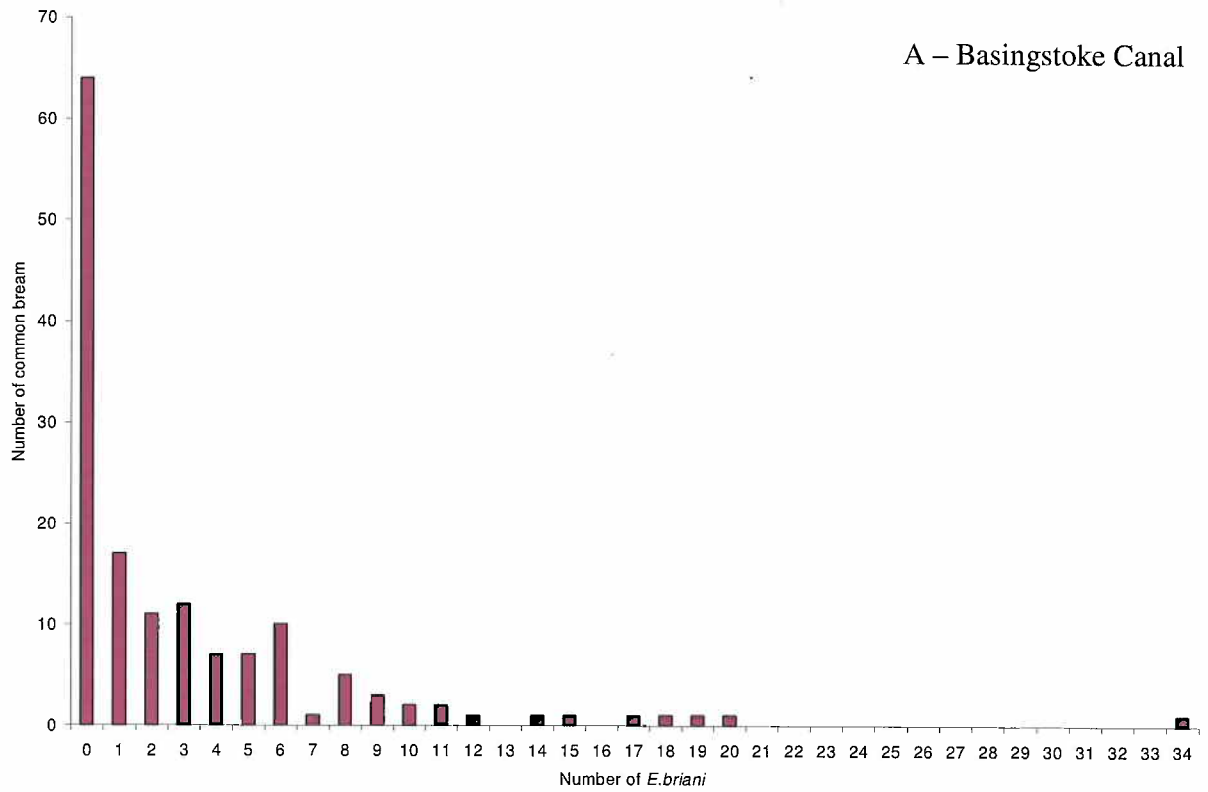
Fig. 5.25 Over-dispersed distribution of *E. briani* within the common bream population of the Basingstoke Canal (A) and the rudd population of Birkett Hall Pond, Essex (B).

- **Effect of _E. briani_ upon condition of infected hosts**

**Roach and common bream fry from the Basingstoke Canal.**

Analysis of all bream sampled from Basingstoke Canal in 2003 revealed that the condition of infected bream was significantly lower than that of uninfected individuals (Table 5.5 Mann-Whitney p <0.05). The mean difference in condition between the infected (K = 1.16) and uninfected bream population (K = 1.24) was 5.4%. When analysing data from bream fry only, the mean condition of infected bream was also lower than that of uninfected fish. However, this was not significantly different. There was no significant difference in the condition of infected and uninfected roach (Mann-Whitney P>0.05). Even fish harbouring the heaviest parasite infections, including those captured during August, 2004 (up to 62 parasites) had condition factor values within the range of uninfected bream.

**Rudd populations from Birkett Hall Pond Essex**

Taking the entire population examined from Birkett Hall Pond Essex, there was no significant difference in the condition of infected and uninfected rudd (Table 5.5). Dividing the infections into light (0-10 parasites), moderate (11-25 parasites) and heavy infections (26+ parasites) there remained no difference in host condition (Table 5.6). Fish harbouring the heaviest parasite burdens had the same level of condition as uninfected individuals. Using 55mm as an approximate upper limit for 1 year old rudd, condition was not adversely affected by the presence of _E. briani_ (Table 5.6).

Table 5.5. Condition of common bream from the Basingstoke Canal (2003 sample)

| Population | Infection | Mean condition factor | Range of condition factor |
|---|---|---|---|
| All bream | Uninfected | 1.22 | 0.92 -1.43 |
| | 1-10 parasites | 1.17 | 0.92- 1.35 |
| | 11-34 parasites | 1.13 | 1.00 – 1.31 |
| 0+ Bream | Uninfected | 1.17 | 0.97 – 1.35 |
| | 1-10 parasites | 1.14 | 0.92 – 1.35 |
| | 11-20 parasites | 1.06 | 1.03 – 1.10 |

Table 5.6 Condition of rudd infected with *E. briani* from Birkett Hall Pond.

| Infection | Number hosts examined | Mean condition factor | Range of condition |
|---|---|---|---|
| All uninfected | 246 | 1.064 | 0.845 – 1.291 |
| All infected | 67 | 1.071 | 0.876 – 1.228 |
| Light (0-10 parasites) | 43 | 1.063 | 0.932 - 1.208 |
| Moderate (11-25 parasites) | 9 | 1.100 | 0.957 – 1.225 |
| Heavy (26 + parasites) | 15 | 1.078 | 0.877 – 1.228 |
| < 55mm uninfected | 178 | 1.048 | 0.932 – 1.146 |
| < 55mm infected | 35 | 1.048 | 0.845 – 1.417 |
| <55mm (26+ parasites) | 3 | 1.064 | 1.003 – 1.109 |

- **Effect of *E. briani* on growth of common bream from the Basingstoke Canal**

The average length of common bream fry in October 2003 was 53mm. Infected fish ranged from 43-73mm in length. There was no significant difference between the average length attained by infected bream and that of uninfected fish. Examination of scales of the most heavily infected fish recorded during the study revealed no obvious abnormalities or evidence of slowed growth (Fig. 5.26).

A



B
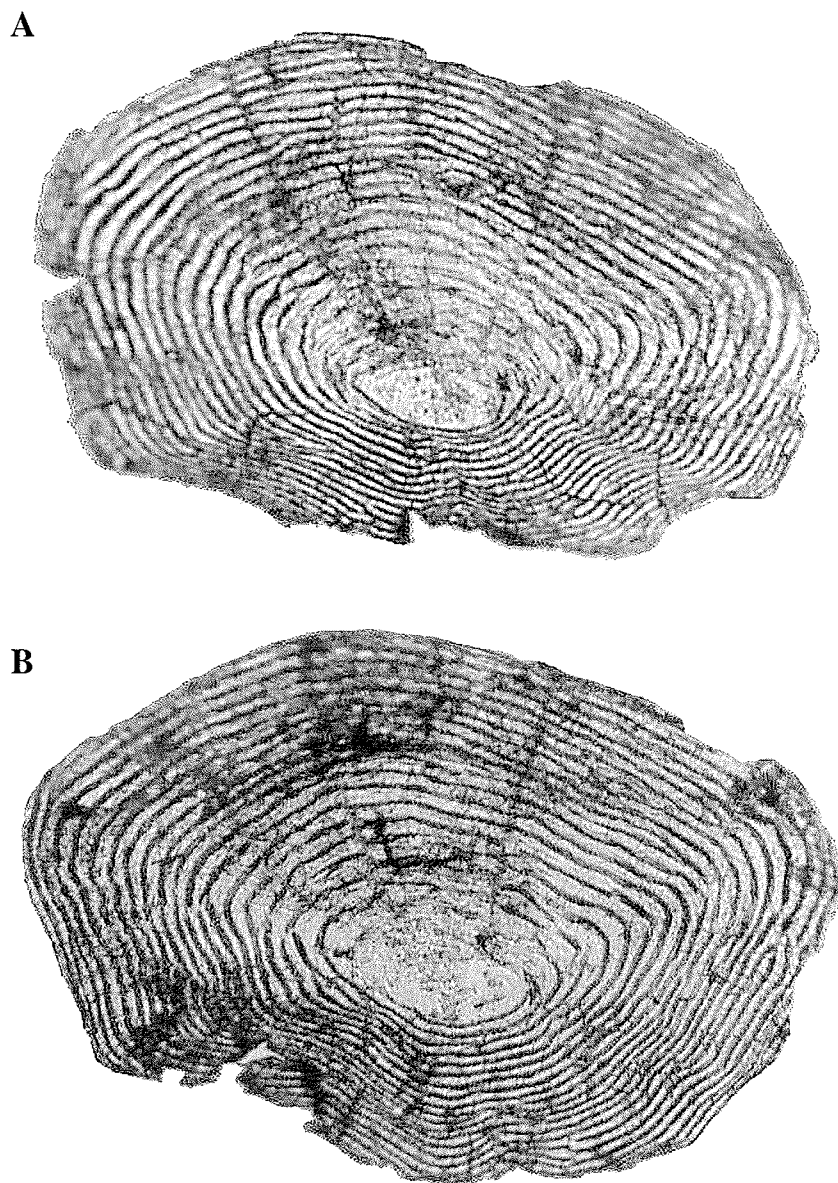


Fig. 5.26. Scales taken from infected bream showing no obvious signs of poor growth or periods of slowed growth, which may be attributed to infection. A – bream length 58mm, 63 parasites. B – bream length 57mm, 42 parasites.

- **Distribution of *E. briani* within England and Wales**

*E. briani* has a widespread distribution within England and Wales. The parasites has been recorded throughout most of England, in particular the south-east and central regions of the country. Distribution records suggest that the parasite maintains a restricted distribution within Wales, the far north and south-west of England. However, this is likely to reflect a lower number of fish movements and thus detection effort within these areas.
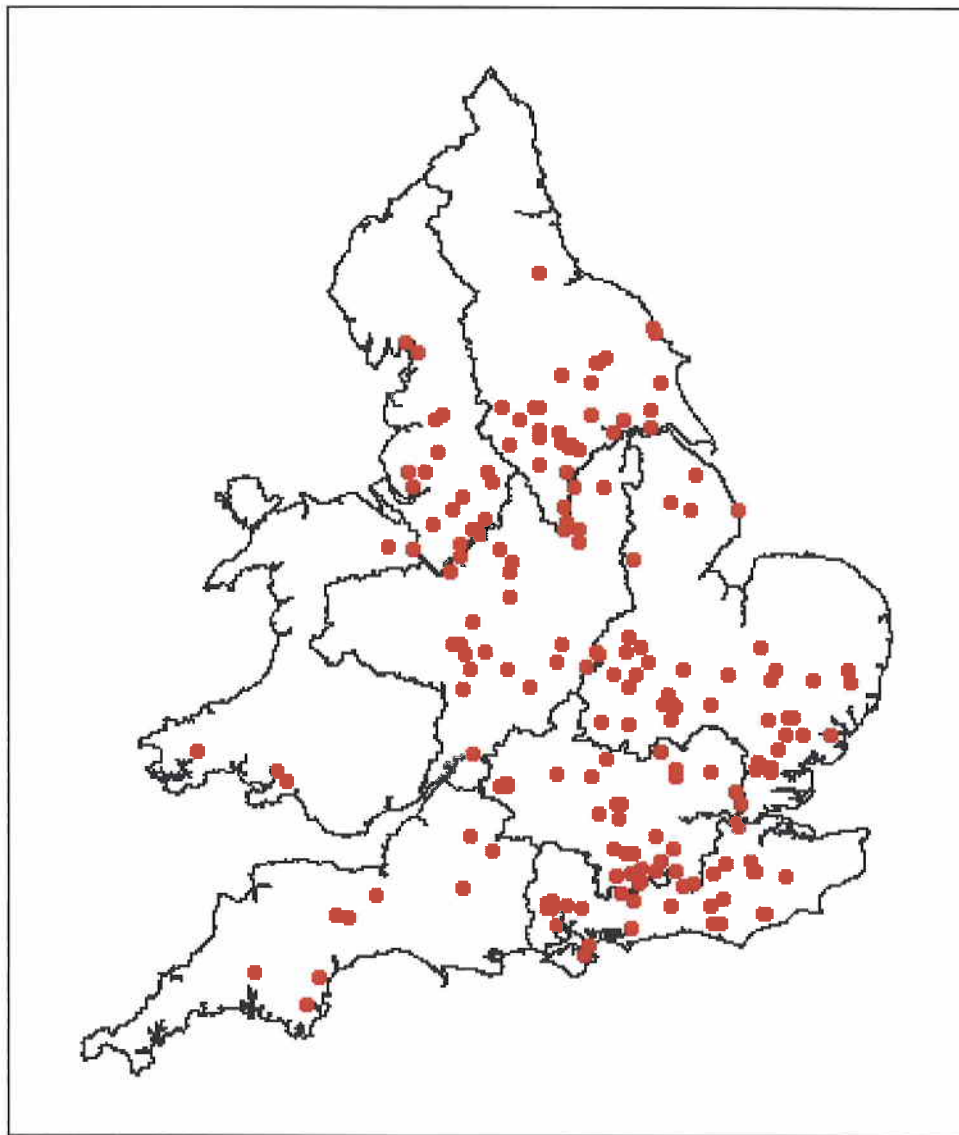


Fig. 5.27. Distribution of *E. briani* within fisheries in England and Wales. Records collated from Environment Agency data 1982 – 2003.

## 5.4 Discussion

### Pathological changes associated with *E. briani* infections

The gills of fish are susceptible to a wide range of disease conditions due to their direct contact with the environment and exposure to a variety of irritants, parasites and pollutants (Eller, 1975; Smith & Piper, 1975; Karlsson, 1983; Hoole *et al.*, 2001; Ferguson *et al.*, 2006). Parasitic crustaceans are among some of the most damaging parasites of fish gills (Kabata, 1970; Paperna & Zwerner, 1982; Abdelhalim, 1990; Domitrovic, 1998; Dezfuli *et al.* 2003). Due to the pathogenicity of some ergasilids, the pathology caused by their feeding and attachment has received considerable attention. Studies include observations of *E. lizae* Krøyer, 1863 (Roubal, 1986a); *E. mirabilis* Oldewage & van As, 1987 (Oldewage & van As, 1987), *E. labrascis* Krøyer, 1863 (Paperna & Zwerner, 1982), *E. australiensis* Roubal, 1986 (Roubal, 1986b, 1989), *E. cyprinaceus* Rogers, 1969 (Rogers, 1969) and in particular *E. sieboldi* (Kabata, 1970; Abdelhalim, 1990; Molnar & Szekely, 1997; Dezfuli *et al.*, 2003). A number of workers have indicated that *E. briani* is likely to be more pathogenic than ergasilids that attach to the fins, skin or nasal tissues. This has been attributed to the relative sensitivity of the gills compared with these other organs (Bauer, 1962; Mann, 1967; Kabata, 1970; Mugridge *et al.*, 1982). However, to date the histopathology of the parasite has not been described. The current study represents the first to describe the histopathological changes caused by *E. briani* to the gills of freshwater fish.

### Damage associated with parasite attachment

According to Kabata (1970) ergasilid parasites cause damage to their hosts through attachment mechanisms, feeding behaviour, mechanical damage from pressure exerted

by the body of the parasite, and reactions of the host to infection. Current observations indicate that the most pronounced pathological changes caused by *E. briani* were associated with attachment. This was characterised by epithelial hyperplasia and pressure changes resulting from use of the parasites antennae. Forceful attachment, combined with the presence of the parasites body between the hemibranchs also caused filament displacement, epithelial compression, lamellar distortion, localised necrosis and hyperplasia. Pressure exerted on the ventral filament surface caused occasional compression of the efferent arteriole. Blood vessel disruption, involving occlusion and haemorrhage is a well documented characteristic of many ergasilid infections, leading to reduced blood flow and respiratory potential (Kabata, 1970; Roubal, 1987; Abdelhalim, 1990; Lester & Roubal, 1995). However, these were not consistent observations for *E. briani*.

Ergasilids exhibit a number of common characteristics in their attachment and feeding behaviour (Abdelhalim, 1990; Lester & Roubal, 1995). It is therefore not surprising that the pathology caused by *E. briani* shares a number of similarities with that of other members of the Ergasilidae. However, despite similarities in the type of changes caused to the gills, the severity of these changes was far less pronounced than that recorded for other species (Paperna & Zwerner, 1982; Abdelhalim, 1990; Dezfuli *et al.*, 2003; *pers. ob.*). This might appear counterintuitive, in view of the predilection of *E. briani* for juvenile fish and the relatively large size of the parasite in proportion to the gill. It is proposed that the morphological and behavioural characteristics of *E. briani* may limit damage to the gills of infected fish. These will be addressed in turn using comparisons of pathological changes caused by the well-described pathogen *E. sieboldi*.

*E. briani* is much smaller than *E. sieboldi* and has a more slender body (Fryer, 1982; Abdelhalim, 1990; Alston *et al.*, 1993). The antennae of *E. briani* are also smaller, narrower and have shorter terminal segments which are inserted into host tissues during attachment (Fryer, 1982; Abdelhalim, 1990; Alston, 1994). It is well documented that penetration of the antennae can severely damage the gills of fish and provoke marked host responses. Records for many ergasilid parasites include descriptions of pronounced hyperplasia, leading to lamellar fusion and thus loss of normal respiratory surfaces (Kabata, 1970; Paperna & Zwerner, 1982; Roubal, 1986b; Abdelhalim, 1990; Lester & Roubal, 1995; Dezfuli *et al.*, 2003). During attachment, *E. sieboldi* inserts the third and fourth segments of the powerful, hooked antennae deep into the gill tissue as far as the cartilage (*pers. ob.*; Abdelhalim, 1990; Dezfuli *et al.*, 2003). In addition to proliferative changes, this causes considerable structural damage to the filaments, haemorrhage and blood vessel constriction (*pers. ob.*; Abdelhalim, 1990; Dezfuli *et al.*, 2003). Conversely, attachment of *E. briani* usually involved insertion of only the antennae tip, reducing the depth of penetration. In most infections, the fully extended antennae were used to embrace the filament with only the terminal segment penetrating the tissue. In very small hosts, attachment to as many as 4 filaments served to further minimise the depth of antennae penetration. This caused less damage to the filament and appeared to provoke only weak hyperplastic responses. In contrast to *E. sieboldi*, attachment of *E. briani* did not result in haemorrhage or rupture of the branchial arterioles. The less damaging attachment behaviour of *E. briani*, smaller size of the parasite, less powerful antennae and different use of this appendage during attachment, therefore appear to reduce damage to the gills and provoke milder host responses compared with other ergasilids.

Whilst many factors influence host selection of parasitic copepods (Kabata, 1991) the morphology and attachment characteristics of *E. briani* may explain the susceptibility of different hosts to infection. The relatively small size of the parasite's antennae and a preference for embracing the filaments during attachment rather than deep penetration may explain the preference of the parasite for small hosts (Fryer, 1982). It is feasible that the size of gill filaments, or water flow through the branchial chamber are too great in large fish to enable firm, sheltered attachment. Similarly, the preference of the parasites to attach tight to the interbranchial septum, may effect the ability of the parasite to attach to different host species depending upon gill morphology. According to Alston (1994) the favoured hosts for *E. briani* include crucian carp, tench and bream. All of these species possess a prominent gill septum. Conversely, the gills of perch and pike have a greatly reduced septum, which extends for less than one quarter of the filament length. This feature, and the corresponding changes in water flow across the gills may explain the near absence of *E. briani* in pike and perch, despite their susceptibility to ergasilids of the skin, fins, nares and dorsal regions of the gill filaments (Environment Agency, unpublished; Kirk, 2000a). These observations suggest that gill morphology and water flow may be important factors in host selection of *E. briani* (Walkey *et al.,* 1970; Abdelhalim, 1990; Kabata, 1991; Alston, 1994).

**Importance of site specificity within the gills**

Current studies confirm that *E. briani* exhibits very strict site specificity within the gills of juvenile fish (Fryer, 1982; Alston & Lewis, 1994). These observations also indicate that only during very heavy infections are parasites forced to other regions of the gill due to space limitation. Whilst many parasites express preferred microhabitats within the gill (Fryer, 1965; Ramasamy *et al.,* 1985; Roubal, 1995), few ergasilids attach in

such such highly specific regions of the gill. Whilst this position may benefit the parasite, providing sheltered attachment, direct water flow for rapid dispersal of nauplii and close contact to gill tissue for nutrition, it may also serve as an important influence upon gill damage.

Attachment of *E. briani* usually involved contact with two gill filaments. This behaviour combined with the dorso-ventrally flattened body of the parasite may reduce resistance to water flow, whilst allowing attachment in the narrow space tight to the gill septum (Fig. 5.28). This characteristic appeared to reduce the downward force applied directly to the gill filament surface. It is well documented that *E. sieboldi* and *E. lizae* typically attach to a single gill filament and generate considerable downward force upon the dorsal surface of gills (Roubal, 1986a; Abdelhalim, 1990; Dezfuli *et al.*, 2003). This attachment pressure combined with the feeding behaviour of the parasite, causes severe epithelial erosion, constriction and exposure of the branchial arterioles and fusion of lamellae along the lateral filament surface. Whilst epithelial compression, vessel narrowing and localised hyperplasia were all recorded consequences of *E. briani* attachment, these changes were far less severe and extensive as those described for other ergasilids. Furthermore, *E. briani* appeared to pull only its head region tight to the filament surface, allowing the body, swimming legs and eggstrings to hang relatively freely between the hemibranchs. The smaller size of *E. briani*, less pressure exerted during attachment and reduced contact of the body within the gill filaments may result in the observation of less severe and extensive damage to the gills (Fig. 5.28).

Crustacean parasites can alter water flow across the gills and disrupt normal branchial ventilation (Leonardos & Trilles, 2003; Kearne, 2005). Consequently, the presence of

*E. briani* between the hemibranchs raises potential for reduced respiratory function by increasing water resistance and turbulence (Hughes & Morgan, 1973). This potential may be increased in very small hosts where parasites extend further along the gill filaments and take up a larger proportion of the gill surface area. However, compared with other gill ergasilids, the strict site specificity of *E. briani* tight to the gill septum and consistent orientation of parasites in a forward direction may limit this effect. The presence of *E. briani* between the hemibranchs may simply serve as an extension of the interbranchial septum, buffering only postlamellar water (Hughes, 1984). This attachment behaviour, combined with the parasites sleek body form may minimise flow disruption and make individuals more hydrodynamic (Fig. 5.28). This is in contrast to other ergasilids including *E. sieboldi,* which, although preferring the dorsal filament surface, may attach to any surface of the gills without strict orientation (Abdelhalim, 1990; *pers. ob.*). This less organised attachment behaviour not only accentuates damage to the gill filaments, but potentially increases disruption to normal water flow within the gills. These behaviours may therefore contribute to the apparent differences in pathogenicity between the species.

During periods of respiratory challenge, teleost fish maintain the ability to change the angle of the gill filaments in relation to water flow within the branchial chamber. The adductor and abductor muscles located within each gill filament fulfil important roles in normal ventilation and respiratory manoeuvres such as 'coughing' (Hughes & Morgan, 1973). During normal respiratory cycles, the filament muscles contract and relax to ensure erection of filaments to form the branchial curtain. This arrangement optimises inter-lamellar water flow as it passes from the buccal to opercular chambers (Hughes & Morgan, 1973). The position of parasites between the filaments may therefore be

analogous to a hard object placed between an open pair of scissors, preventing the filaments from being retracted (Fig. 5.28). Whilst a reduced ability to control filament extension may disrupt gill function, it may also ensure that infected filaments are permanently extended within the branchial curtain (Ojha *et al.,* 1982; Nilsson, 1985). This could increase lamellar flow throughout the filament length and recruit more distal lamellae, which may not be utilised during routine gill ventilation (Fig. 5.28).

The presence of parasites between the hemibranchs may also reduce the ability of hosts to 'cough', a process that involves sudden changes in flow within the branchial chamber allowing irritants or noxious substances to be flushed from the gills (Hughes & Morgan, 1973). During heavy parasite infections, this may act as a safety mechanism for the parasite, allowing attachment to less sheltered regions of the gill. Although parasitism may adversely affect respiratory efficiency of the gills (Alston, 1994), current studies suggest that certain characteristics of *E. briani* attachment may limit the severity of these changes in all but very heavy infections (Fig. 5.28).

The presence of bacterial accumulations surrounding the antennae of *E. briani* provides the potential for secondary infection, especially as integrity of the epithelium is breached during parasite attachment. Gill parasites, including ergasilids may increase susceptibility of hosts to viral, fungal and bacterial infections (Nigrelli, 1950; Dogiel *et al.,* 1958; Lester & Roubal, 1995; Ravichandran *et al.,* 2001; Busch *et al.,* 2003). However, the inability of *E. briani* to transfer between hosts once attached would limit the parasite acting as vector for transmission of these pathogens.
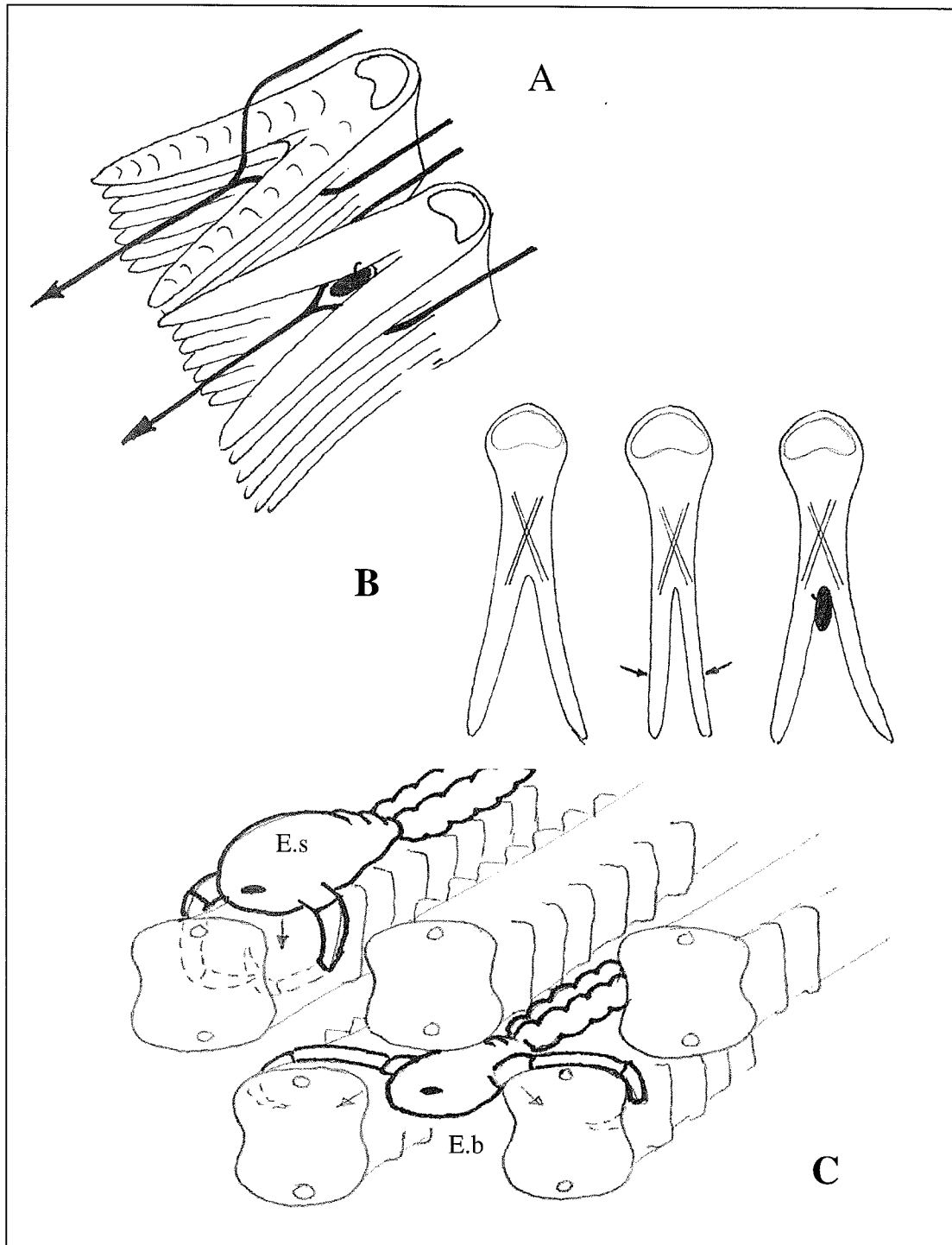
Fig 5.28. Influences of *E. briani* upon gill function of infected cyprinid fish.

A – Position of a single *E. briani* between hemibranchs. Whilst disruption of post-lamellar water may result from infection, this sheltered position may limit impact to the gill. Parasites may therefore act as an extension to the gill septum.

B – Positioning at the gill septum may maintain erection of infected filaments within the branchial curtain, but disrupt normal muscle contractions within the gill.

C – Comparison of attachment behaviour between *E. briani* (E.b) and *E.sieboldi* (E.s). Attachment of *E. briani* involves shallower penetration of antennae, less direct contact to the filaments and lateral pressure to the filament surface. The more aggressive attachment of *E.sieboldi*, involves deeper penetration and direct pressure on the gill.

Chapter 5.

## Damage associated with parasite feeding

A number of workers have emphasised the importance of examining the feeding behaviour of crustacean parasites, before a full appreciation of pathogenicity can be attained (Einszporn-Oreka, 1964, 1965a, b, 1973a, b; Abdelhalim, 1990; Alston, 1994). However confusion has long surrounded the feeding behaviour and nutritional requirements of ergasilid parasites due to difficulties with observing the mouthparts (Einszporn-Orecka, 1965a; Kabata, 1970; Abdelhalim, 1990; Molnar & Szekeley, 1997). The strict site specificity of *E. briani* between the hemibranchs obscured gross observations of feeding behaviour. Similarly, parasites were often recorded between adjacent filaments, with the mouth-parts free of direct contact with host tissues. This made it difficult to obtain good histological sections of the region between mouth and gill tissue. Despite these difficulties, observations of epithelial erosion and compression adjacent to the mouth of *E. briani* were consistent with the feeding activity of ergasilid parasites. This behaviour was primarily associated with the lateral surfaces of the filaments, indicating that *E. briani* may alter position during periods of feeding before resuming a more ventral position on the filament. It is known that certain ergasilid parasites, hang free from host tissues in between periods of feeding (Abdelhalim, 1990).

The absence of severe epithelial lesions on the filaments during most infections indicates that feeding behaviour of *E. briani* may be less damaging than attachment. However, it was often difficult to distinguish changes associated solely with nutrition, as tissue thinning and epithelial compression were likely to be a combined effect of feeding and firm attachment. This requires further investigation through more extensive use of scanning electron microscopy than was made available during the current study.

The severity of damage caused by ergasilid parasites is strongly related to the intensity

of infection (Abdelhalim, 1990; Dezfuli *et al.,* 2003). Alston (1994) confirmed through

experimental studies that juvenile tench, unaffected by light intensities, lost condition

and died with infections between 108-509 parasites. These records combined with

current observations of pathology and site specificity suggest that there is likely to be a

threshold of infection below which fish can successfully compensate for infection and

above which disease occurs (Pathiratne, 1992). Within their normal site of attachment,

*E. briani* cause relatively localised pathological changes. This is accentuated only

when parasites extend onto the lateral and medial filament surfaces. Consequently,

infected fish may have a maximum carrying capacity of parasites dependant upon gill

morphology. With each parasite utilising two filaments during attachment, then it may

be possible to establish the infection threshold for any host dependant upon the number

of gill filaments. Only during heavy infections, are parasites forced to utilise other gill

surfaces, resulting in more serious pathological changes. In such cases, host debilitation

is likely to progress more rapidly. This is consistent with observations of lethal

infections, where parasites were recorded on all surfaces of the filaments (Alston,

1994).

**Difficulties with the interpretation of pathological changes**

The teleost gill is a highly complex organ responsible for respiration, osmoregulation,

acid-base balance and metabolism of circulating hormones (Goss *et al.,* 1998; Olson,

2000). This structural and functional complexity can make it difficult to interpret

physiological consequences of any lesions observed (Eller, 1975; Ferguson, 1989). Fish

may use only a small proportion of gill capacity during normal respiration (Ferguson,

1989). This complexity is compounded by functional differences between species and

the ability of fish to overcome respiratory challenges by increasing branchial irrigation (Ferguson, 1989), raising heart rate (McDonald & McMahon, 1977) or in the case of chronic stressors, altering morphology of the gill to increase diffusion (Laurent & Perry, 1991; Sollid *et al.,* 2005). These compensatory measures can be so successful that infected fish may not appear clinically sick (Ferguson, 1989). It is therefore difficult to assess the pathogenicity of a gill dwelling parasite from observations of pathology alone, unless changes are severe.

Current observations confirm that *E. briani* causes a number of pathological changes to the gills of its host, which during heavy infections are likely to disrupt normal gill function. However, compared with other gill ergasilids this damage appears to be less extensive and severe. It is proposed that the attachment behaviour of the parasite may represent an important evolutionary relationship (May & Anderson, 1990; Schrag & Wiener, 1995; Lambert, 1997), limiting host damage whilst allowing attachment to what is a delicate organ of a delicate host. Due to the parasite's inability to swim if detached from the gills, damage limitation may be an important mechanism for parasite survival. During low-level infections, it is considered unlikely that the observed pathologies would be capable of disease or serious host debilitation.

**Epidemiology of *E. briani* within the Basingstoke Canal**

A total of 442 fish were captured from the Basingstoke Canal during 2003. Prevalence and intensity of infection differed considerably between the roach and bream populations. The low prevalence of parasites in roach, maximum intensity of just 6 parasites and a highly over-dispersed distribution, suggest that *E. briani* is unlikely to have a serious impact upon the performance of the roach fishery within the canal. In

contrast, the higher prevalence and intensity of infection recorded in bream provide greater potential for impact.

The infections observed in roach and bream during the current study are similar to those recorded from the Basingstoke Canal by Alston (1994). This suggests that *E. briani* populations may have remained relatively stable in the ten-year period separating these studies. However, despite small sample numbers, the prevalence and intensity of infections recorded during 2004 were higher than those of 2003. During the current study, the maximum intensity of *E. briani* on a single host was 65 parasites. This was far lower than the maximum of 543 individuals (mean 288) recorded by Alston (1994) in experimentally diseased juvenile tench. The absence of such extreme infections within the fisheries examined suggests two possibilities. Infections of many hundreds of parasites may only develop during artificial conditions or environments where hosts and parasites are confined in very small areas (e.g. a fish farm pond) (Bauer *et al.*, 1969). Or that fish harbouring these infections are removed from wild fish population before detection, either directly from mortality or indirectly through predation of weakened hosts. This remains a difficult area of study to substantiate, especially as the debilitation of fry is likely to be rapid once the disease threshold is exceeded.

### Epidemiology of *E. briani* within Birkett Hall Pond

The prevalence and intensity of *E. briani* in rudd from Birkett Hall Pond confirm the susceptibility of rudd to *E. briani*. This species has not been the focus of previous epidemiological study. These observations also indicate that the parasite can successfully establish in a range of fishery types, including small, heavily stocked, highly managed, discrete stillwaters. Despite considerable differences in the size, depth,

stock composition and management of the two fisheries studied, intensities of *E. briani* were not vastly different. The observed prevalence of 21% (95% confidence range 17-26.3%) is lower than that of common bream in the Basingstoke Canal. Due to the relatively small size of Birkett Hall pond and ability to capture larger proportions of the lake with each netting, these infections are more likely to represent the whole rudd population, rather than the very localised sampling conducted at Basingstoke Canal. The maximum intensity of 85 parasites was again far lower than that recorded during previous disease problems (Bauer *et al.,* 1969; Alston, 1994). These intensities, combined with the highly over-dispersed distribution of the parasites may also limit impact at the population level. Although there are many factors that may influence over-dispersion within parasite populations, current observations may be explained by the heterogeneous distribution of both host and infective stages within the fisheries studied (Anderson, 1993).

**Effect of *E. briani* upon bream growth**

Current studies suggest that infections of *E. briani* do not adversely affect the growth of bream fry. The recorded average growth of 53mm for bream after their first growth season, was very close to the figure of 55mm recognised as good growth for bream populations within the British Isles (Hickley & Dexter, 1979). The observation of infected bream fry exceeding 70mm within a single growing season represents extremely good growth. However, these observations must be viewed with caution. Whilst the presence of parasites with orange pigmentation indicated recent attachment, and those with egg-strings longer establishment, it was not possible to accurately assess longevity of infection in the fish examined. Gnadeberg (1948) suggested that ergasilids may be aged, based upon ovary development, pigmentation and differences in

cephalothorax morphometrics. However, this has not been established for *E. briani* and was far beyond the scope of the current study. It therefore remains possible that fish either gained or lost infection shortly before capture. However, the absence of very poor growth in any of the hosts examined suggests that this is an unlikely consequence of *E. briani* infection at the intensities observed. If growth was not adversely affected within the first year of survival, it is unlikely that *E. briani* poses a threat to older individuals.

## Effect of *E. briani* upon host condition

Ergasilids, including *E. briani* are known to reduce host condition during heavy infections (Schäperclaus, 1991; Abdelhalim, 1990; Alston, 1994). Historic records of *E. briani* suggest that these are primarily associated with confined water bodies, with high numbers of available hosts (Bauer *et al.,* 1969; Alston, 1994). Current observations indicate that the condition of common bream fry was not adversely affected at intensities of up to 62 parasites within the Basingstoke Canal. Similarly, this potential was not realised in the rudd population from Birkett Hall Fishery, despite being a more heavily stocked and confined environment. This suggests that either the conditions necessary for heavy parasite infections were not present, or that such infections do not normally establish within natural environments. However, certain characteristics of these natural environments, including unlimited food availability, low stress and good water quality may enable to fish to tolerate infection.

During experimental infections, Alston (1994) observed no significance difference in condition of 7-8cm tench infected with up to 34 parasites, but a 34% decline in condition for fish infected with 108-509 parasites. Whilst this confirms host impact, these represent very heavy infections. Similarly, records of over one thousand parasites

per host in a Russian fish farm are of a different magnitude to those ever recorded in the British Isles (Bauer *et al.*, 1969; Environment Agency, unpublished). Although these represent severe disease events, current evidence suggests that the likelihood of such infections establishing within fisheries within the British Isles are low.

Many factors influence ergasilid populations, in particular the environment for development of the free-living stages (Alston, 1994). Infections can therefore follow cyclical patterns within host populations, with considerable variation in annual disease potential. Identifying these changes requires long term observations, both within and between years. It is therefore recognised that spot sampling and observations from only a single years data, provided only preliminary indications of impact. Whilst the *E. briani* infections at both fisheries appeared to be tolerated by the cyprinid hosts examined, effects before and after sampling remain unclear. Despite these limitations, the relatively consistent infection levels recorded at the Basingstoke Canal spanning the last decade, and sampling effort covering both autumn and spring periods, suggest that extreme infections are not common in wild fish populations. Even if the heavily infected proportion of both fishery populations were adversely affected by the parasite, the high level of over-dispersion within both fisheries would reduce impact at the population level. Of the 74 bream fry sampled during 2003, only 6 (8.1%) had infections exceeding 10 parasites. Similarly, only 24 rudd (7.6%) from the Birkett Hall Fishery sample of 313 fish had infections over 10 parasites. It could therefore be argued that even if these hosts were lost from their respective populations (which pathological studies indicate is unlikely), this would not adversely affect fishery performance.

Chapter 5.

## Seasonality and role of environmental factors

Environmental conditions, combined with parasite seasonality are important factors influencing diseases of fish populations. Although fish retain the ability to compensate for environmental challenge, it is well recognised that hypoxia can cause greater impacts in hosts harbouring gill parasites, than under normal conditions (Molnar, 1994). Consequently, warm summer conditions or congregation of fish within confined localities may all serve to increase chances of impact from *E. briani*. Similarly, factors like climate change may also influence the disease potential of the parasite, with warmer and longer reproductive periods for the parasite and more stressful conditions for infected fry (e.g. lower flows, algal blooms and dissolved oxygen fluctuations). However, whilst this could potentially elevate intensities of infection to more problematic levels, there are many other factors that together interact before disease epidemics occur (Hall *et al.,* 2006). Such environmental changes are likely to have complex and far reaching influences upon parasites as well as host populations. These may have positive as well as negative consequences. As an example, strong year class strength in cyprinid fish is associated with high water temperatures during the first summer of life (Mills & Mann, 1985; Cowx, 2001; Frear & Cowx, 2003).

Ergasilid populations are known to increase throughout the year, reaching a peak during autumn. The autumn sampling on the Basingstoke Canal would therefore have coincided with the highest prevalence and intensities of *E. briani*. However, this may not represent the most critical period for infection. The respiratory challenges placed upon fish are generally greatest during summer, when temperatures are highest and the oxygen carrying capacity of water at its lowest. This period can be particularly challenging due to the wide diurnal fluctuations that occur in oxygen levels in fisheries

(Seagrave, 2001). During summer, the very small size of fry would mean a larger relative proportion of the gill tissue is infected by each parasite than later in the year. The inability to collect sufficient samples during August 2004 therefore represents a considerable loss to the study. This is particularly disappointing as the highest parasite prevalence (100%) and intensity (62 parasites) occurred during this period. Despite obvious frustrations, this at least highlighted the difficulties and unpredictability with studying wild fish populations. Further sampling efforts are therefore necessary to improve understanding of the seasonal effects of *E. briani* upon the fish host and evaluate any impacts during periods of respiratory challenge.

**Distribution of *E. briani* within England and Wales**

*E. briani* has a widespread distribution within England and Wales. Comparison of current records with those of Alston (1994), Environment Agency (1999) and Hawkins (2001) indicate that the parasite continues to spread with the movement of infected fish. This not only increases the scale of potential disease problems, but also questions justification for the continued control of the parasite. Similarly, the presence of susceptible host species and growing number of coarse fish movements from England into Scotland and Ireland, suggest that *E. briani* is likely to extent its geographical range within the British Isles.

**5.5. Summary and risk assessment**

Current evidence suggests that *E. briani* is not an important pathogen of rudd or common bream in the fisheries examined and at the intensities observed. Pathological descriptions revealed marked, but very localised lesions. Sampling of infected fisheries did not identify any grossly diseased fish or evidence to indicate that mortality is a

likely consequence of *E. briani* infection. Similarly, the over-dispersed distribution of parasites within host populations, a finding consistent with other ergasilids (Abdelhalim, 1990; Alston, 1994) indicates that impacts even at high parasite intensities may involve only a small proportion of the population. Despite this, it is recognised that current studies were based upon relatively small samples and involved only two fisheries over a small period of time. It remains possible that this sampling simply highlighted trends within the 'surviving' fish population and missed important periods for host impact. The difficulties associated with the study of parasite infections in wild fry populations are acknowledged (Longshaw, *et al.,* 2005).

Although current studies have progressed understanding of *E. briani,* the sampling limitations make it unreasonable to conclude with reasonable certainty that the parasite is benign. Further studies are necessary, involving larger sampling effort and a wider cross section of fisheries. The importance of the parasite during periods of environmental stress should be included within these investigations. Literature confirms that condition loss and even mortality, are possible consequences of infection in certain situations. It is therefore important to establish whether these are capable of occurring in wild fisheries. These are urgently required due to the growing economic restrictions placed upon the fish movement industry. The extensive and widening distribution of the parasite within England and Wales is only likely to increase these pressures. However, any parasite that infects the gills of its host, can rapidly multiply in wild fisheries, exclusively infects juvenile fish and comes from a taxonomic family comprising a number of pathogenic species (Kabata, 1970; Abdelhalim, 1990) requires careful consideration. Despite recognition of further studies, a preliminary risk assessment based upon current understanding of *E. briani* is given in Fig. 5.29, 5.30.

Fig. 5.29. Risk analysis process for evaluating the probability of *E. briani* causing undesirable economic and ecological impacts to fisheries. This is based upon available published literature and information gained during the current study.

| Parasite species being assessed – *Ergasilus briani* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact**<br><br>1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise ecologically important fisheries? | | | 0.5 |
| **B Economic impact**<br><br>1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise economically important fisheries? | | 0.3 | |
| **A Ecological impact risk analysis**<br>What is the risk of the parasite having an adverse ecological effect on fisheries? | **= 0.1 x 0.1 x 0.5 = 0.05** | | |
| **B Economic impact risk analysis**<br>What is the risk of the parasite having an adverse economic effect on fisheries? | **= 0.1 x 0.1 x 0.3 = 0.03** | | |

Fig 5.30. Preliminary risk analysis matrix to evaluate the potential impact of *E. briani* upon the ecological and economic development of fisheries.

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low**<br>(0.001 – 0.005) | **Medium**<br>(0.009 – 0.027) | **High**<br>(0.045 – 0.125) |
| **Economic Risk** | **Low**<br>(0.001 – 0.005) | **Low**<br>*E. briani* | Medium | High |
| | **Medium**<br>(0.009 – 0.027) | Medium | Medium | High |
| | **High**<br>(0.045 – 0.125) | High | High | High |

## Chapter 6. Impact of *P. longidigitus* upon fisheries and olfactory function of cyprinid fish.

### 6.1. Introduction

*P. longidigitus* was first recorded in the British Isles in 1994 (Environment Agency, 1999). At the time of detection, very little published information was available on which to assess the potential impact of the parasite on fisheries. Due to the strict site preference of the parasite for the nares, *P. longidigitus* was considered less pathogenic than other members of the Ergasilidae, in particular those found on the gills (Kabata, 1970). However, concern was raised over the ability of the parasite to reduce the olfactory sensitivity of infected fish (Hawkins, 1999; Environment Agency, 1999). Since 1994, it has been perceived that *P. longidigitus* may have the potential to disrupt spawning behaviour of infected fish as a result of damage to the olfactory system (Hawkins, 1999; Environment Agency, 1999). A significant reduction in the ability of fish to detect and respond to odours involved in reproduction could have long term implications for individuals and fisheries (Moore & Waring, 1996a,b). Parasites that affect host reproduction can cause significant impacts upon the equilibrium of infected populations (Hudson & Dobson, 1991; Hudson *et al.*, 2001; Kirk, 2003). However, to date the threat posed by *P. longidigitus* to fisheries has not been substantiated and remains poorly understood.

Freshwater fish display a variety of reproductive behaviours (Liley & Stacey, 1983). For many species this involves the release and detection of pheromones (Stacey, 1981; Liley, 1982; Liley & Stacey, 1983, Sorensen, 1996; Moore *et al.*, 2002). These compounds can either prime the reproductive physiology of conspecific individuals

(primer hormones) or trigger the onset of sexual behaviour (releaser hormones). The release of pheromones prior to spawning synchronises reproduction and thus enhances reproductive success (Moore *et al.*, 2002). The F-series prostaglandins (PGFs) are one group of compounds that have been shown to have such influences (Sorensen *et al.*, 1995a; Moore *et al.*, 2002). PGFs are not only potent odours to certain species of fish, but also have strong reproductive priming effects (Moore *et al.*, 2002). Detection of these pheromones in male cyprinids causes an elevation in the levels of expressible milt (Moore *et al.*, 2002; Lower *et al.*, 2004) as well as plasma steroids, such as androstenedione. Studies using crucian carp (Bjerselius & Olsen, 1993), goldfish (Sorensen *et al.*, 1988, 1989) and roach (Lower *et al.*, 2004) suggest that PGFs act as important olfactory stimulants to male sexual behaviour. In order for fish to react to these chemosensory cues they must be detected by the sensory receptors located within the epithelial lining of the nares. Extensive literature confirms that various waterborne toxins can damage the sensory epithelium of fish (Moran *et al.*, 1987; Bettini *et al.*, 2006), in turn disrupting the detection of spawning pheromones (Moore & Waring 1996a, 2001; Saandhal *et al.*, 2004). However, it is unclear whether parasite-induced damage to the nares can have similar adverse effect (Smirnova *et al.*, 1964; Hawkins, 1999, 2001).

The following chapter details studies undertaken to improve understanding of the impacts of *P. longidigitus* on infected fish. Initial studies involved collation of published literature and confirmation of the pathology caused to the nares of a range of fish species. Experimental studies were conducted in efforts to assess the effect of *P. longidigitus* upon the olfactory sensitivity of infected hosts. This involved testing the responses of infected common carp to the pre-ovulatory primer pheromone 17α20β-

dihydroxy-4-pregnen-3-one (17α20β-P). Due to the need for expert guidance and Home Office-licensed laboratory facilities, these experimental studies were conducted under the supervision of Drs Andy Moore and Nicola Lower, fish reproduction physiologists based at the Cefas laboratories, Lowestoft. Records of *P. longidigitus* distribution were collated to determine the extent of fisheries infected within England and Wales. Two fisheries, heavily infected with *P. longidigitus* were sampled during spring to confirm seasonal variation in the parasite populations during the spawning period of cyprinid fish.

**Aims and objectives of the study**

The current study was undertaken in efforts to satisfy the following aims:

1 - To collate and review current literature on *P. longidigitus* in order to improve understanding of parasite impact, biology, detection and management.

2 - To confirm the pathology caused by *P. longidigitus* to the nares of infected hosts, accounting for variation with host species and parasite intensity.

3 - To assess whether *P. longidigitus* reduces the olfactory sensitivity of infected common carp and thus has potential to disrupt spawning activity within infected fisheries.

4 - To assess the coincidence of seasonal changes in *P. longidigitus* populations with the reproductive activity of infected cyprinids.

5 - To review the current distribution of *P. longidigitus* in fisheries in England and Wales.

## 6.2. Materials and methods

### 6.2.1. Literature reviews

Literature reviews were collated as described in Chapter 3.

### 6.2.2. Fish sampling and maintenance

Fisheries with historic infections of *P. longidigitus* were identified from an internal database of parasite records held at the Environment Agency, National Fisheries Laboratory, Brampton. Waters with recent records of heavy infections were preferentially targeted in efforts to improve chances of parasite detection.

The experimental studies required mature, male common carp in good reproductive condition infected with *P. longidigitus*. Fish were obtained by either seine-netting, or electro-fishing. Fish sex was established on site by presence or absence of expressible milt following gentle pressure to the abdomen. Fish were transported alive to holding facilities at the Cefas laboratories, Lowestoft and maintained in external 1000 litre circular tanks fed with de-chlorinated tap water. Where numbers permitted, small sub-samples of fish were sacrificed and examined from each fishery to establish approximate levels of *P. longidigitus* infection.

For the purpose of histopathological studies, infected fisheries with mixed species populations were identified and seine-netted. Various fish species from a range of sizes were transported to holding facilities at the Environment Agency's National Fisheries Laboratory, Brampton. All fish were maintained in 200 l fibreglass tanks fed with bore-hole water prior to examination.

## 6.2.3. Fish examinations and histopathological studies

All fish were killed by immersion in anaesthetic (benzocaine solution) at a lethal concentration. Each fish was weighed, measured and examined for external abnormalities. The nares of each fish were examined visually by means of a swing-arm dissecting microscope. In all cases, the nasal flap was either opened or removed to reveal approximate parasite intensity. Care was taken to ensure no contact was made with the nasal epithelium. Small numbers of parasites from each fishery were examined under high power magnification to confirm identification. Both nares were removed in their entirety with surrounding area of cranial tissue. These were trimmed following fixation in Bouin's fixative for 24-72 hours. Further decalcification in 10% formic acid was used to soften the cranium of very large fish. Samples were dehydrated in alcohol series, cleared and embedded in paraffin wax. Sections of 3-5µm thickness were stained using Mayer's Haematoxylin and Eosin (H&E). Stained, mounted sections were examined microscopically for pathological changes.

## 6.2.4. Parasite distribution in England and Wales

Distribution records of *E. briani* were collated as descried in Chapter 4.

## 6.2.5. Experimental studies

Drs Andy Moore and Nicola Lower, Cefas Laboratories, Lowestoft are acknowledged for the laboratory facilities and the experimental design described below.

Thirty eight 50 l experimental aquaria were set up within two temperature-controlled rooms. Tanks were individually aerated and fed with de-chlorinated tap-water at a rate of 0.085 l/min. Water temperatures were matched with those of ambient conditions

(12.5°C to 18°C). Each tank was covered to prevent escape of fish and maintained with a natural photoperiod.

Prior to study, each carp was tested for milt production to confirm that only male fish had been selected. Fish failing to produce milt were omitted from the study. Spermiating carp were stripped of all expressible milt by continued massage of the abdomen and introduced to the test tanks to acclimate overnight. The following morning, the priming pheromone 17.20αβ-P (Sigma Products) was added to each tank in sequence, at 15 minute intervals. The pheromone was dissolved in ethanol and added to the tanks at a concentration of $10^{-9}$M. Inflow water to each tank was switched off prior to delivery of the pheromone and left off for a period of 15 minutes to allow fish to detect the pheromone at this concentration. After 15 minutes, gradual water flow (0.085 l/min) was re-instated to each tank to ensure maintenance of good water quality. Fish were left within the pheromone treated water for a period of 6 hours before sampling.

Fish were anaesthetised in 2-phenoxyethanol (0.4ml/l) until sedated. Fork length and weight measurements were recorded. The posterior surface of the fish was wiped with absorbent paper to remove mucus and residues of anaesthetic before all expressible milt was stripped. Milt was collected in a plastic boat and weighed. Approximately 1ml of blood was taken from each fish by means of caudal venepuncture using a heparinised syringe. Blood samples were held in centrifuge tubes on ice until completion of the entire sampling regime (approx 6 hours). Fish were killed by further immersion in anaesthetic and a sharp blow to the head. The gonads were dissected out in their entirety and weighed.

The heart was removed from each fish by dissection in order to stop blood flow and prevent pooling of blood during examination of the narès. The nares of each fish were examined in turn under a dissection microscope for the visual presence of parasites. Care was taken to ensure no contact was made with the nasal rosette. Both nares from each fish were fixed in 10% neutral buffered formalin. After completion of each sample, the anaesthetic water was filtered through a fine plankton net and examined under a low power microscope to ensure parasites had not actively left the nares during the process of anaesthesia.

Blood samples were centrifuged at 4,000 rpm for 15 minutes, serum removed and frozen at $-20^{\circ}$C. Each blood sample was analysed for the steroid androstenedione by means of radioimmunoassay at the Cefas laboratories, Weymouth. The gonadosomatic index (GSI) of each fish was calculated by the equation: 100 x gonad weight / body weight.

### 6.2.6 Seasonality studies

For the purpose of seasonality studies, fish samples were obtained from two stillwater fisheries located in the east of England. These were Swallowbrook Reservoir, Kent and Willow Farm Fishery, Lincolnshire. Three separate samples, each comprising approximately 30 fish were obtained from each fishery between February and July by means of seine netting. All fish were killed by a lethal dose of anaesthetic and examined for *P. longidigitus*. The number and reproductive status of parasites were examined in each host with use of a low-power dissecting microscope and recorded. Temperature readings were taken from a still-water fishery located in the south east of England as these were not directly obtainable from the sites sampled.

## 6.3 Results

### 6.3.1 Literature review of *P. longidigitus*

- **Description and taxonomy**

*P. longidigitus* Yin, 1954 is a parasitic crustacean of the family Ergasilidae. The parasite primarily infects the nares of freshwater fish and was first recorded in China in 1950 (Yin, 1954). Descriptions of adult female parasites have been given by Yin (1954, 1962), Gusev & Smirnova (1962), Do (1982), Gusev (1987); Chernysheva & Purasjoki (1991) and recently Hawkins (2001), the latter author describing British specimens for the first time. Despite considerable attention given to the morphology of paraergasilid parasites, literature specific to *P. longidigitus* is both scarce and fragmentary.

Parasites of the genus *Paraergasilus* are separated from those of *Ergasilus* primarily by the morphology of the second antennae, which terminate in three long, blunt-ended fingers, rather than a single pointed claw (Abdelhalim *et al.*, 1993; Alston & Lewis, 1994; Hawkins, 2001). This attachment feature is unique within the parasitic Copepoda (Yin, 1954). In general, paraergasilids are also smaller than other members of the family Ergasilidae (Fryer, 1982). Adult female *P. longidigitus* are green in colour, have a slender body and measure approximately 500-550μm in length (Do, 1982). Parasites possess four distinct pairs of biramous swimming legs (Do, 1982) and retain the ability to swim after host attachment. During the reproductive season, two conspicuous egg sacs trail from the genital openings of female parasites, each comprising an average of eight to ten eggs (Do, 1982; Hawkins, 2001). This is less than most other ergasilid parasites (Abdelhalim 1990; Urawa *et al.*, 1991; Alston, 1994). Adult male and juvenile stages of *P. longidigitus* are believed to be free-living, but have not been described.

Chapter 6.

Considerable confusion surrounds the taxonomic identity of *Paraergasilus* species (Chernysheva & Purasjoki, 1991). Much of this uncertainty stems from the initial studies of Markewitsch (1937) who erected the genus *Paraergasilus* to accommodate the type-species *P. rylovi* Markevich, 1937, found within a plankton sample from the Caspian Sea. These descriptions were originally in Ukrainian, lacked illustrations and have since been shown to contain numerous inaccuracies (Chernysheva & Purasjoki, 1991). Since then, a number of erroneous recordings have been made, including a description of the parasite as the sub-species *P. rylovi borysthenicus* Sukhenko, 1967 (Sukhenko, 1967; Do, 1982; Chernysheva & Purasjoki, 1991). To date, the genus *Paraergasilus* comprises 14 species described from freshwater, estuarine and marine hosts (Ho *et al.*, 1992; El-Rashidy & Boxshall, 2001). *P. longidigitus* (Yin, 1956) is the only parasite of the genus recorded within freshwater fish of the British Isles (Environment Agency, unpublished; Kirk, 2000a).


- **Host susceptibility**

*P. longidigitus* is a generalist and exhibits low host specificity (Do, 1982; El-Rashidy & Boxshall, 2001). The parasite was first described from common carp *Cyprinus carpio* (Yin, 1954) but has since been recorded from a wide range of fish species (Yin, 1956, 1962; Gusev & Smirnova, 1962; Do, 1982; Purasjoki & Fagerholm, 1987; Hawkins, 2001; Environment Agency, unpublished). Within the British Isles, *P. longidigitus* was first recorded within the nasal cavities of common bream *Abramis brama* and roach *Rutilus rutilus* (Environment Agency, unpublished). Since then, the host range of the parasite has grown considerably and now exceeds 20 freshwater species (Environment Agency, unpublished). Although most records of *P. longidigitus* involve freshwater hosts, infections have also been recorded in brackish environments and even full

strength seawater (Purasjoki & Fagerholm, 1987). However, this requires confirmation. Similarly, the host range of *P. longidigitus* beyond freshwater remains unclear within the British Isles.

Despite having a wide host range, a number of workers have described marked differences in the susceptibility of certain fish species to *P. longidigitus*. Within England and Wales, common bream, pike *Esox lucius* L., common carp and crucian carp appear particularly susceptible to infection (Hawkins, 2001; Environment Agency, unpublished). Conversely, gudgeon *Gobio gobio* (L.) and perch *Perca fluviatilis* L. appear to be less favoured hosts (Tuuha *et al.*, 1992; Hawkins, 2001). Reasons for differences in host specificity of ergasilid parasites have been reviewed by Alston (1994) and include biochemical conditioning of larval stages prior to host location (Bocquet & Stock, 1963), differences in host immunity (Noble *et al.*, 1963), host morphology (Abdelhalim, 1990), attachment-site morphology (Fryer, 1968; Abdelhalim, 1990), host behaviour (Alston, 1994) and parasite behaviour (Bauer *et al.*, 1959; Zmerzlaya, 1972; Abdelhalim, 1990).

- **Life-cycle development**

The life-cycle of *P. longidigitus* has not been adequately described. According to Fryer (1978), it is likely that development and reproduction of *P. longidigitus* follows that of other members of the Ergasilidae. Fryer (1978) states that all species of the Ergasilidae have a direct life-cycle and although the development of the free-living stages is imperfectly known for many species, it is probably similar in all. It may therefore be assumed that the life-cycle of *P. longidigitus* involves six free-living naupliar stages, five free-living copepodid stages and a single parasitic adult stage, each divided by a

moult. Gross observations have confirmed that after attachment to a host, adult parasites quickly develop eggs sacs. On hatching, the free-living nauplii are washed from the nasal cavity into the water. Only post-mated adult females are parasitic, whilst males are believed to die following copulation (Hawkins, 2001).

The seasonality of *P. longidigitus* within the British Isles has not been accurately documented. However, this is believed to be consistent with other ergasilids, following a temperature-dependent pattern of development, maturation and reproduction (Abdelhalim, 1990; Alston, 1994). Ecological studies in temperate climates have shown that the reproduction of ergasilid parasites is seasonal, with reproduction starting in spring and ending in autumn (Tedla & Fernando, 1970; Abdelhalim, 1990; Urawa *et al.,* 1991; Tuuha, *et al.,* 1992; Alston, 1994). Fryer (1978) detailed how the occurrence of free-living ergasilids is also seasonal and strongly governed by climatic conditions. Parasite populations are therefore believed to increase throughout the summer months, reaching a peak in early autumn before declining into winter (Hawkins, 2001). Adult females retain the ability to over-winter whilst attached to a host, although egg development ceases until the following spring. The seasonality of *P. longidigitus,* particularly during the period of fish reproduction, requires further investigation.

- **Distribution and dissemination**

The global distribution of *P. longidigitus* is poorly documented. This has recently been reviewed by Hawkins (2001). According to this author, *P. longidigitus* was first detected in China (Yin 1956, Gusev & Smirnova, 1962) but has since been described within the former USSR (Gusev & Smirnova, 1962), Japan (Do, 1982), Russia (Chernysheva & Purasjoki, 1991), Finland (Purasjoki & Fagerholm, 1987; Tuuha *et al.,*

1992), Slovakia (incorrectly described by Hanek, 1967) and Ukraine (incorrectly described by Sukhenko, 1967). If the accuracy of marine and estuarine records is accepted, *P. longidigitus* has also been described in the Gulf of Mexico, USA (Purasjoki & Fagerholm, 1987). Due to the unusual site of attachment, poor detection effort (Hanek, 1967; Fryer, 1968; Ponyi & Molnar, 1969) and confusion over parasite identity (Chernycheva & Purasjoki, 1991) *P. longidigitus* is likely to have a wider geographical distribution than records indicate, particularly in Europe (Tuuha *et al.,* 1992). This is supported by Purasjoki and Fagerholm, (1987) who described *P. longidigitus* as a common parasite in Finland, despite this being the only record from Western Europe. Current records suggest that *P. longidigitus* has the largest geographical range of any species within the genus *Paraergasilus* (Purasjoki & Fagerholm, 1987; Hawkins, 2001).

*P. longidigitus* was first recorded in the British Isles in 1994, from a stillwater fishery in Derbyshire (Hawkins, 1999; Environment Agency, unpublished). The parasite is believed to be an introduced species (Environment Agency, 1999; Hawkins, 2001) although both time and route of introduction remain unclear. Since 1994, *P. longidigitus* has spread widely throughout England. To date the parasite has not been recorded from Scotland. Reviews of ergasilid distribution within England and Wales suggest that *P. longidigitus* is more widespread than any other ergasilid species (Fryer, 1982; Alston, 1994; Environment Agency, 1999; Hawkins, 2001). Records of *P. longidigitus* in both estuarine and coastal sites (Purasjoki & Fagerholm, 1987) hold important implications for the introduction and dissemination of the parasite within the British Isles. The ability of *P. longidigitus* to transfer between freshwater and saline environments means that saline water would not block the spread of the parasite

between river systems. This extends the potential impacts of the parasite beyond freshwater (e.g. disruption to salmonid migrations), and also causes further problems for the control of its geographical spread.

- **Epidemiology**

Very few epidemiological studies have been conducted on *P. longidigitus*. Consequently many aspects of the parasite's biology, ecology, life-cycle development, population dynamics and seasonality are poorly understood. Despite this paucity of published literature, it is well recognised that *P. longidigitus* primarily attaches within the nasal cavity of its host (Smirnova *et al.*, 1964; Do, 1982; Gusev & Smirnova, 1987; Chernysheva & Purasjoki, 1991; Tuuha *et al.*, 1992; Hawkins, 1999, 2001). Occasional parasites have also been found on the gills (Yin, 1954) of infected fish, particularly during heavy infections. With respect to parasite-induced direct pathology, it may be assumed that the effects of *P. longidigitus* are restricted to the nares (Kabata, 1970).

When *P. longidigitus* was first detected in England, the parasite was found at a prevalence of 100% in common bream and 70% in roach, with mean intensities of approximately 100 parasites and 40 parasites/host respectively (Environment Agency, unpublished). These infections far exceed those given in any published literature. Infections within wild fish populations have since been found that exceeded 600 parasites per host (Environment Agency, unpublished; Hawkins, 2001). Records of 90% prevalence, with intensities of up to 61 parasites per host have also been recorded in riverine dace populations (Hawkins, 2001). These observations confirm that parasite populations can establish within varied environments and that the potential impact of the parasite may not be confined to one type of fishery. Tuuha *et al.*, (1992) made a

comprehensive study of the epidemiology of four ergasilid parasites in a number of interconnected lakes in Finland. However, very low prevalence and intensities of *P. longidigitus* limited detailed analysis of infections of this parasite.

- **Pathology**

Considerable literature exists on the pathology of ergasilid parasites, particularly those that attach to the gills, or are of economic importance to aquaculture (Bauer *et al.,* 1959; Mann, 1967; Kabata, 1970; Paperna & Zwerner, 1982; Roubal, 1986a; Abdelhalim, 1990; Alston, 1994; Dezfuli *et al.,* 2003). However, the pathology caused by *P. longidigitus* has received little attention. During a review of ergasilid parasites in British freshwater fish, Hawkins (2001) provided histopathological descriptions of damage caused by *P. longidigitus* in common bream and common carp. However, these records were limited to observations from a very small number of fish. Hawkins (2001) concluded that *P. longidigitus* can cause serious pathological damage to the olfactory tissues of infected hosts.

- **Impact on fish populations**

Current literature provides very little information on the effects of *P. longidigitus* on fish populations. Schäperclaus (1991) includes *P. longidigitus* in a list of important ergasilid parasites. However, no reason is given for the inclusion of this parasite in this list, or why the parasite may be considered important. To date, no evidence of disease has been recorded at either host or population level as a result of *P. longidigitus* infection. However, it has long been perceived that damage caused by the parasite to the nasal rosette could reduce the olfactory sensitivity of infected hosts, in turn limiting spawning success through failed detection of reproductive pheromones (Environment

Agency, 1999; Hawkins, 2001). This hypothesis has not been explored and represents an important area for further investigation if the impact of *P. longidigitus* is to be better understood.

- **Management and control**

Although considerable attention has been given to the treatment and control of many ergasilid parasites, no specific efforts have been given to *P. longidigitus*. The best and most effective way of controlling the spread of ergasilid parasites is to prevent introduction to water bodies (Hoffman, 1977; Cressey, 1983; Environment Agency, 1999). Once established within a fishery, ergasilids can be extremely difficult to eradicate (Alston, 1994). Many chemotherapeutics, in particular organophosphate insecticides, have been used for treatment of farmed fish populations (Kabata, 1970; Hoffman, 1977; Kabata, 1985; Kirk & Lewis, 1992). However, there are currently no drugs licensed within the British Isles for the control of ergasilid parasites. The application of any drug to extensive water bodies also poses many practical limitations. Brewster (2000) suggested that the treatment of nare-dwelling parasites may be affected by the ability of the fish to close the nasal flap, thus minimising contact with water-borne therapeutics. The only recognised means of eradicating ergasilid parasites from infected fisheries is by draining, drying and liming (Alston, 1994). Parasite populations may be controlled through fishery management measures, which include the reduction of host densities and manipulation of fish stocks in favour of less susceptible species (Schäperclaus, 1991, Gusev & Smirnova, 1987; Hoole *et al.*, 2001). However, until the impacts of *P. longidigitus* are better defined, the importance, or indeed need for management or intervention remain unclear.

## 6.3.2 Pathology caused by *P. longidigitus* to the nares of infected hosts

- **General observations and attachment characteristics**

During the study, pathological observations were made from a total of 34 infected fish. These included samples from roach *Rutilus rutilus*, common bream *Abramis brama*, pike *Esox lucis* and common carp *Cyprinus carpio*. Intensity of infection ranged from 1 to over 150 parasite per nare. *P. longidigitus* were almost exclusively found attached within the nares of the hosts examined. However, in two common bream parasites were located on the gills, and in a single infection on the flanks of the body. Examinations of the nares revealed that *P. longidigitus* attached primarily to the nasal rosette, although the nasal flap and walls of the nasal cavity were also utilised. Attachment involved insertion of the parasite's antennae (Fig. 6.1, 6.2) into the surface of the nasal epithelium. This resulted in relatively superficial attachment allowing parasites to be easily removed for identification.

In small hosts, removal of the nasal flap allowed relatively easy detection of parasites within the olfactory pit (Fig. 6.3). However, infections in larger hosts required more detailed examinations including dissection of the nasal lamellae. During very heavy infections, parasites were found throughout the nares (Fig. 6.4) extending into the nasal opening and even the external skin surface of the head (Fig. 6.5). Whilst attached to the nasal tissues, parasites often displayed a 'flicking' behaviour, with rapid movements of the swimming legs followed by periods of inactivity. This characteristic movement combined with the parasites' green colouration and presence of conspicuous white egg-strings (during the reproductive season) aided detection.
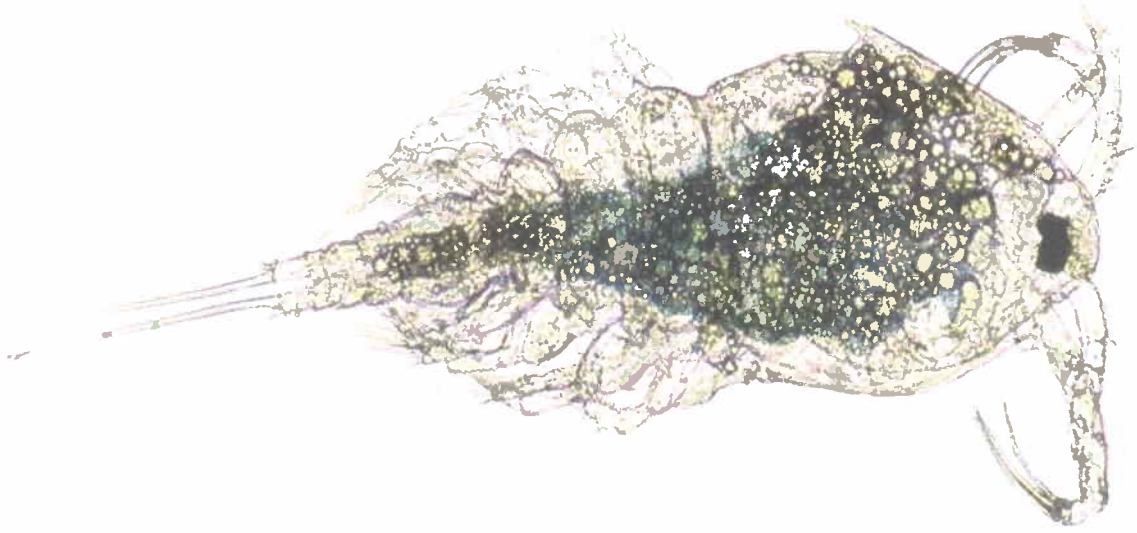
Fig. 6.1. A single *P. longidigitus* (without egg strings). Parasites are typically green in colour and possess conspicuous antennae (arrows) used for attachment to the nasal tissues.
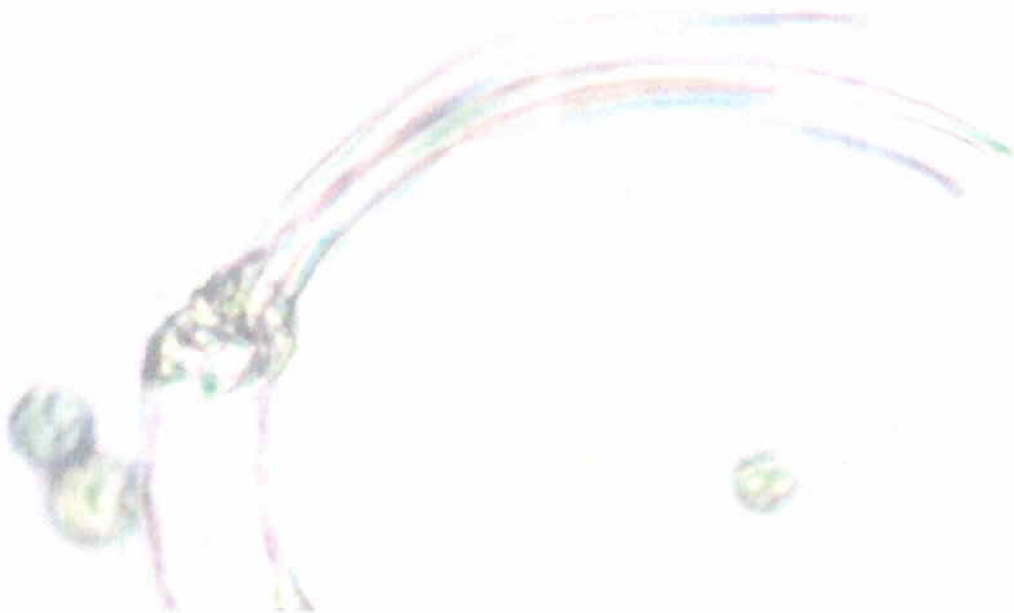


Fig. 6.2. Antennae of *P. longidigitus,* showing the terminal segment split into three, blunt ended fingers.

Fig. 6.3. Head of roach, showing position of nare with nasal flap removed. Three *P. longidigitus* may be seen at the margin of the nasal cavity (arrow).



Fig. 6.4. A heavy infection of *P. longidigitus* within the nare of a juvenile common bream. Numerous green-coloured parasites, each possessing a single black eye spot, may be seen surrounding the lamellae of the nasal rosette (*)
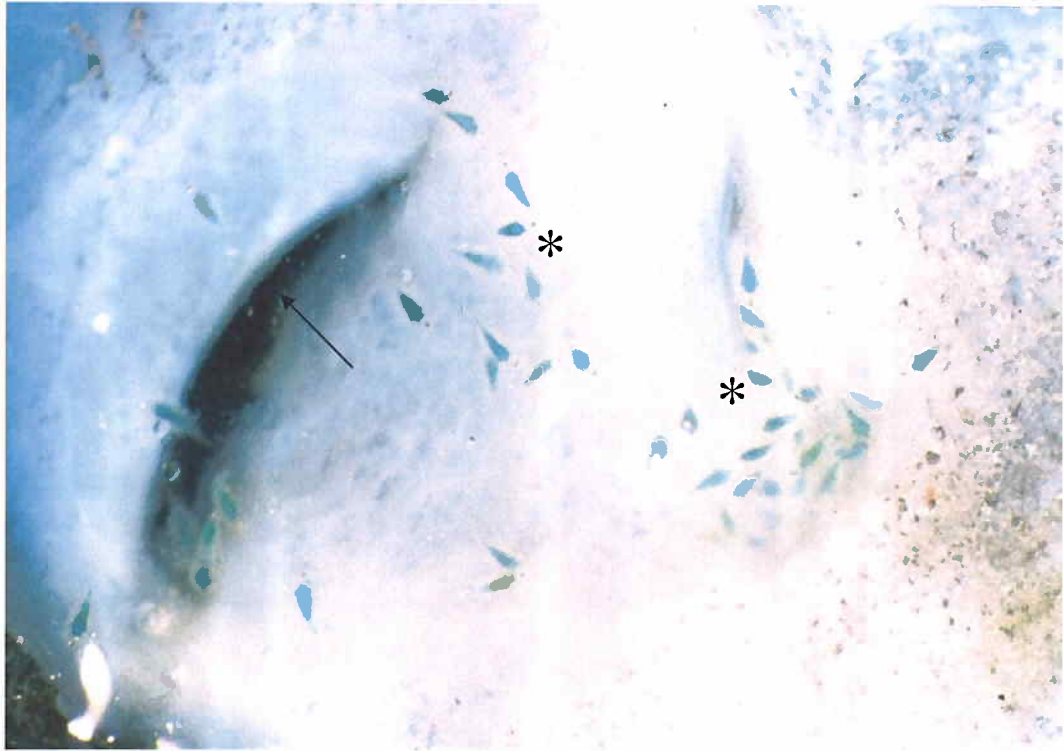
Fig 6.5. A heavy infection of *P. longidigitus* showing a number of parasites surrounding the opening of the nare (arrow) extending onto the external surface of the hosts head (*).

- **Gross pathological changes and clinical signs of infection**

During the study period no gross external pathologies or clinical signs of infection were recorded as a result of *P. longidigitus* infection. Even during infections exceeding 150 per host, infected fish appeared in good condition and were indistinguishable from uninfected individuals. Occasionally, a build-up of mucus within the nasal cavity accompanied very heavy parasite infections. However, this was observed in only two infected common bream and was not a consistent finding.

- **Histopathological changes caused by *P. longidigitus* to the nares of infected hosts.**

*P. longidigitus* were primarily found attached to the lateral surfaces of the nasal lamellae (Fig. 6.6). However, individuals were found on all surfaces of the olfactory pit, especially during heavy infections where most regions of the nares were infected. Parasites were consistently found lying adjacent to the olfactory tissues, allowing the mouth-parts and swimming legs to come into close contact with the epithelium (Fig. 6.7). Attachment of *P. longidigitus* involved varied orientation of individuals (Fig. 6.8) and frequently led to mechanical distortion of the lamellae as tissues were forced to accommodate the body of the parasite (Fig. 6.9).

The pathological changes caused by *P. longidigitus* involved mechanical damage to the nasal tissues as a result of attachment and feeding behaviour. During attachment the parasite's three-fingered antennae were used to grasp the surface layers of the epithelium. This led to the formation of small indentations in the surface of the nasal lamellae (Fig. 6.10) with localised damage and displacement of epithelial cells. In a small number of cases, attachment involved deeper penetration of the antennae, which in some instances approached the basement membrane (Fig. 6.11). This more aggressive attachment behaviour was usually accompanied by an influx of lymphocytes and eosinophilic granular cells (EGC) around the antennae.

The most pronounced and consistent pathological change associated with *P. longidigitus* was that of epithelial erosion resulting from the feeding behaviour of the parasite. This was associated with loss of the normal epithelium and ciliated processes that cover the nasal rosette (Fig. 6.12, 6.13). Similarly, the epithelium adjacent to

attached parasites was occasional devoid of mucus cells (Fig. 6.14). Observations of live parasites combined with pathological characteristics suggest that the feeding behaviour of *P. longidigitus* involves displacement of epithelium from activity of the swimming legs followed by ingestion of tissues by the parasite's serrated mouthparts.

The extent and severity of epithelial damage was strongly related to intensity of infection. Epithelial disruption was generally sparse with light parasite burdens and often localised to areas directly adjacent to attached parasites (Fig. 6.15). In such cases, the majority of the olfactory surface remained normal (Fig. 6.16). More extensive damage with loss of ciliated epithelium occurred with heavy parasite infections (Fig. 6.17). Epithelial erosion was occasionally combined with hyperplasia of underlying undifferentiated basal cells (Fig. 6.18). Very heavy parasite infections gave rise to a number of additional pathological changes, including oedema, localised pyknosis, necrosis, vascular damage within the connective tissues and cellular exfoliation, leading to accumulations of cellular debris either between the lamellae or within the lumen of the nasal cavity (Fig. 6.19). In extreme cases, epithelial erosion extended almost as far as the basement membrane, approaching a state of ulceration.

The size and species of host were important influences upon the pathological changes caused by *P. longidigitus*. In very small fish, limited space between the nasal lamellae appeared to limit damage to regions at the base of the nasal folds. This was also evident with species like pike, where the morphology of the nasal rosette appeared to prevent parasites from feeding upon epithelial surfaces located deep between the nasal lamellae. This limited damage within these regions, but accentuated the epithelial erosion within more exposed areas (Fig. 6.20). In large fish and most cyprinid species studied,

pathological changes were not restricted to any specific regions of the nares. Infections within these hosts included damage to all surfaces of the lamellae including the lamellar pits (Fig. 6.21).

During extreme parasite burdens, large areas of the nasal rosette were severely damaged as a result of *P. longidigitus* infection (Fig. 6.21). This included almost complete destruction of the nasal epithelium with loss of normal olfactory architecture (Fig. 6.22). In such cases, the nasal cavity was filled with large amounts of cellular debris and the lamellae were reduced to pillars of supportive tissue (Fig. 6.22). These pathological changes represented an extreme and relatively uncommon consequence of *P. longidigitus* infection.
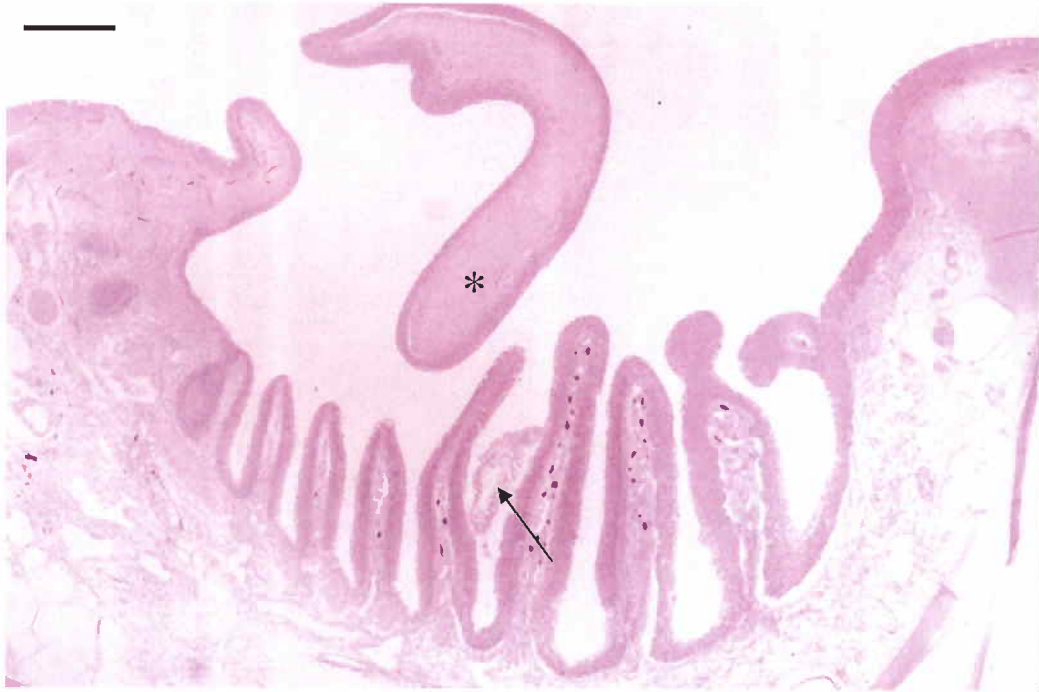
Fig. 6.6. Transverse section through nare of a roach with nasal flap (*) attached. A single *P. longidigitus* (arrow) can be seen between the lamellae of the nasal rosette. Scale bar = 0.5mm.
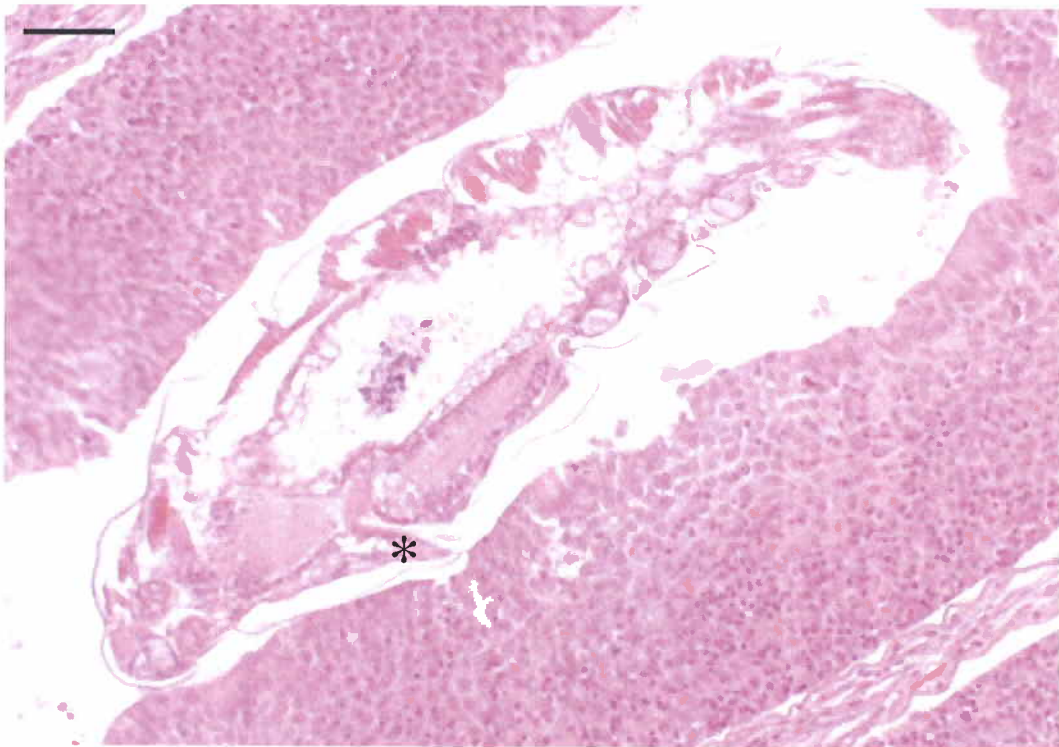


Fig. 6.7. A single *P. longidigitus* attached between folds of the nasal rosette. Close association with the nasal tissues allows the mouth-parts of the parasite (*) to come into close contact with the epithelium. Scale bar = 40μm.
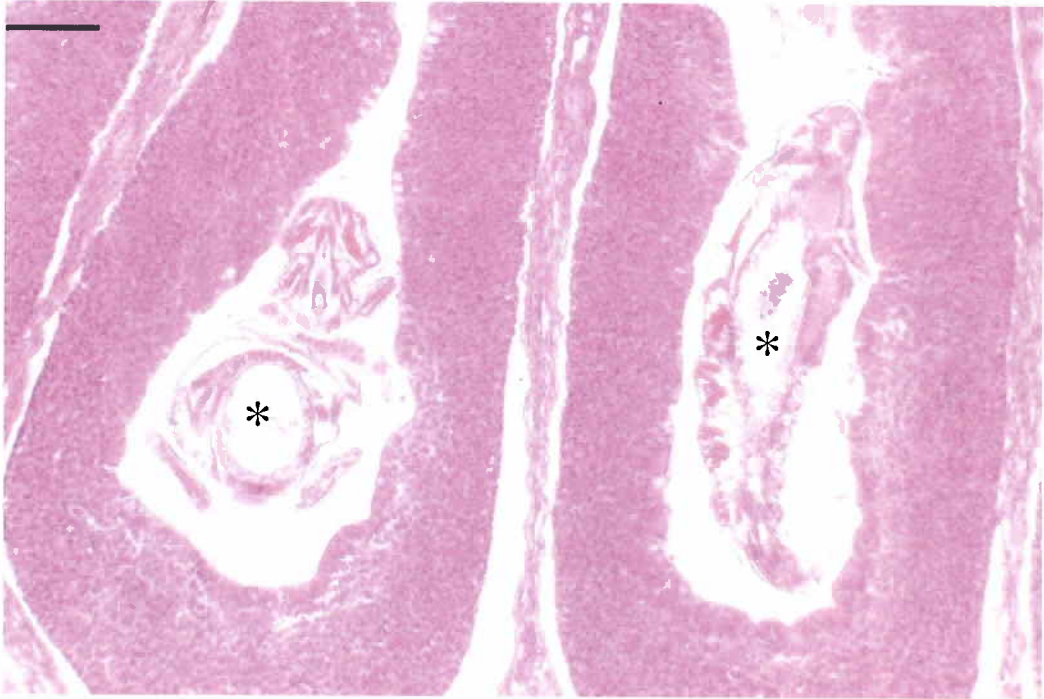
Fig. 6.8. Two *P. longidigitus* (*) attached between adjacent lamellae of the nasal rosette, showing orientation of parasites in both longitudinal and transverse planes. Scale bar = 80μm.
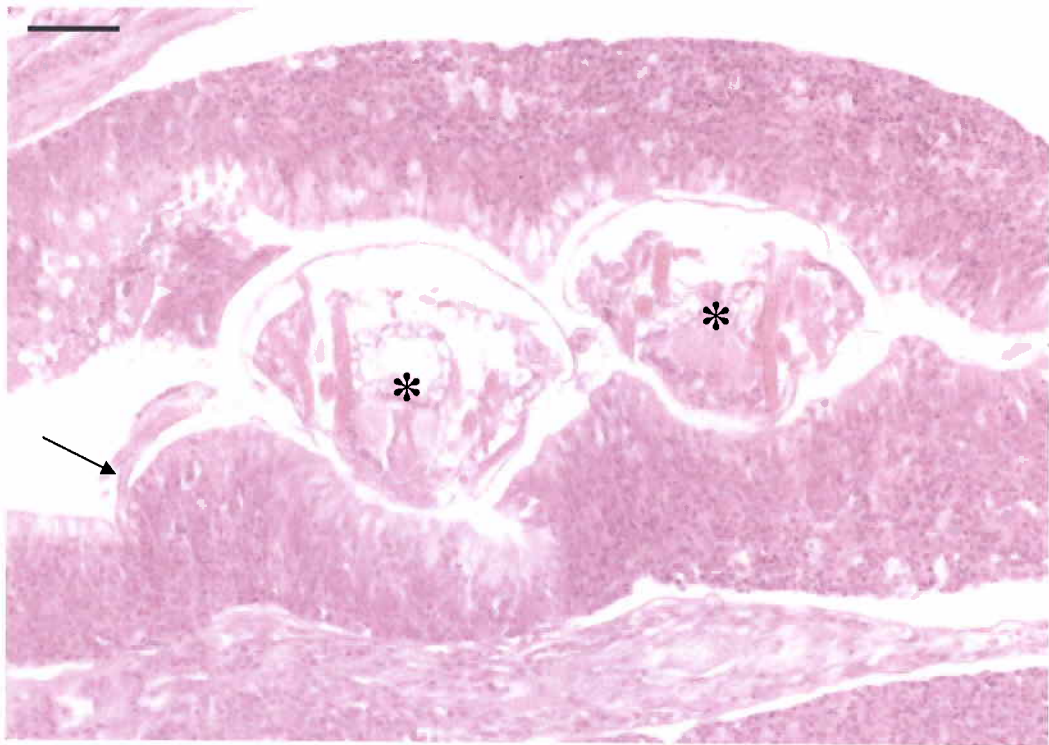


Fig. 6.9. Two *P. longidigitus* (*) attached between the folds of the nasal rosette showing use of the antennae (arrow) for attachment. The presence of the parasite's bodies between the lamellae has resulted in clear distortion of adjacent tissues. Scale bar = 40μm.

Fig. 6.10. Section of bream nare showing insertion of *P. longidigitus* antennae (*)
into epithelial surface. During most infections this caused localised indentation of the
olfactory tissues (arrow) rather than deep penetration into the nasal folds. Scale bar =
20 μm.
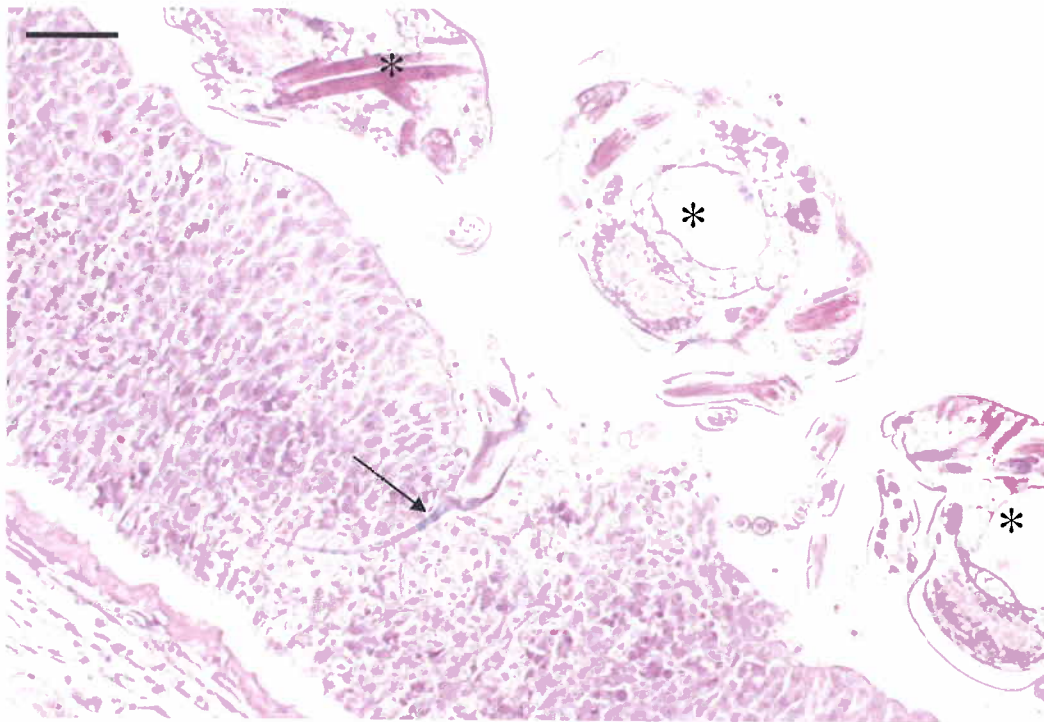


Fig. 6.11. Attachment of numerous *P. longidigitus* (*) to nasal rosette of roach.
Attachment has involved penetration of the antennae (arrow) deep into the nasal
lamellae with resultant damage of epithelium. This form of attachment characteristic
was only occasionally recorded. Scale bar = 40μm.
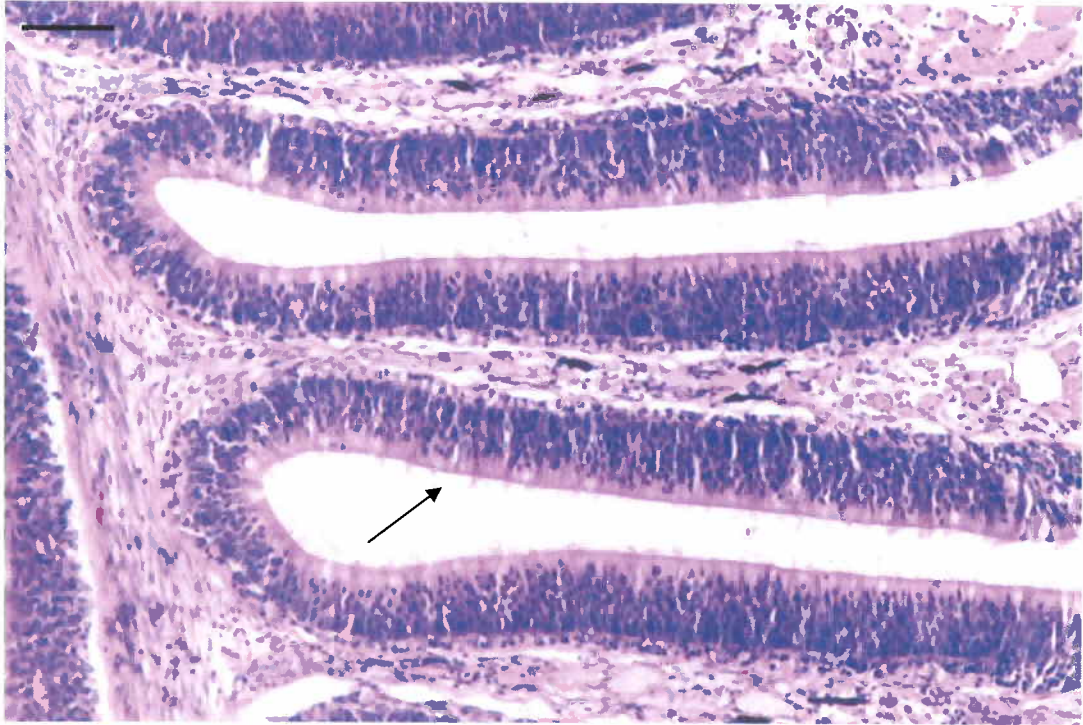
Fig. 6.12. Section showing normal structure of common bream nasal tissue. The eosinophilic ciliated epithelium can be seen covering the lamellae surface (arrow). Scale bar = 80μm.
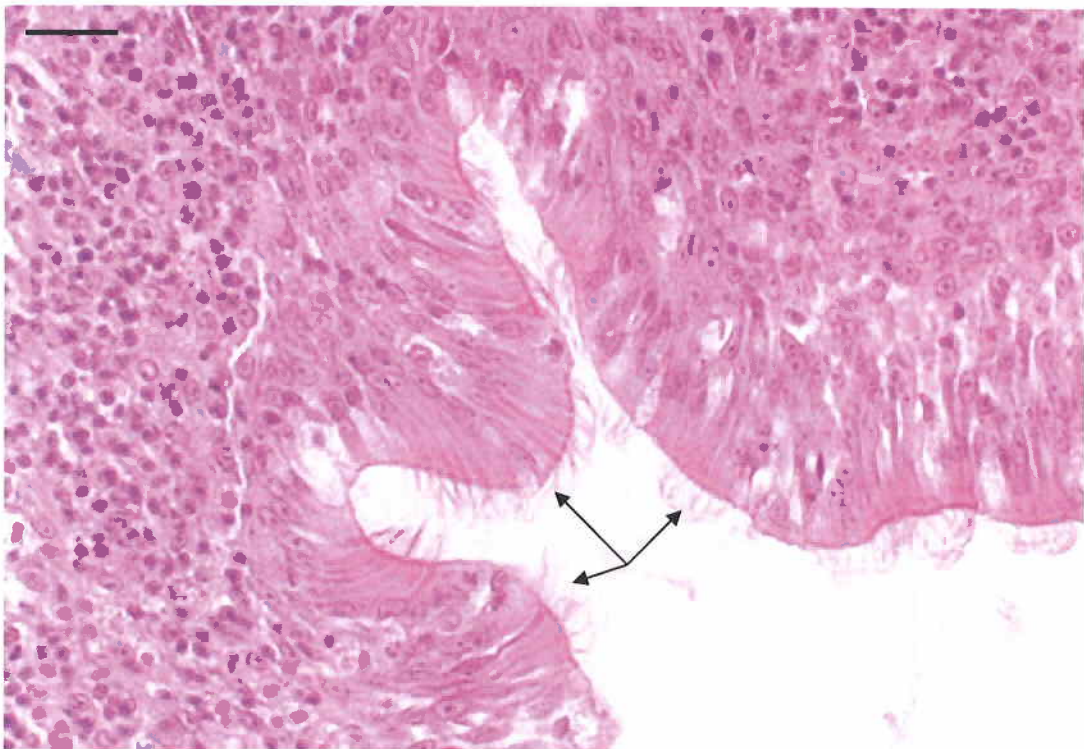


Fig. 6.13. High power magnification of olfactory epithelium of roach. The ciliated processes (arrows) can be clearly seen covering the epithelium surface. Scale bar = 20μm.
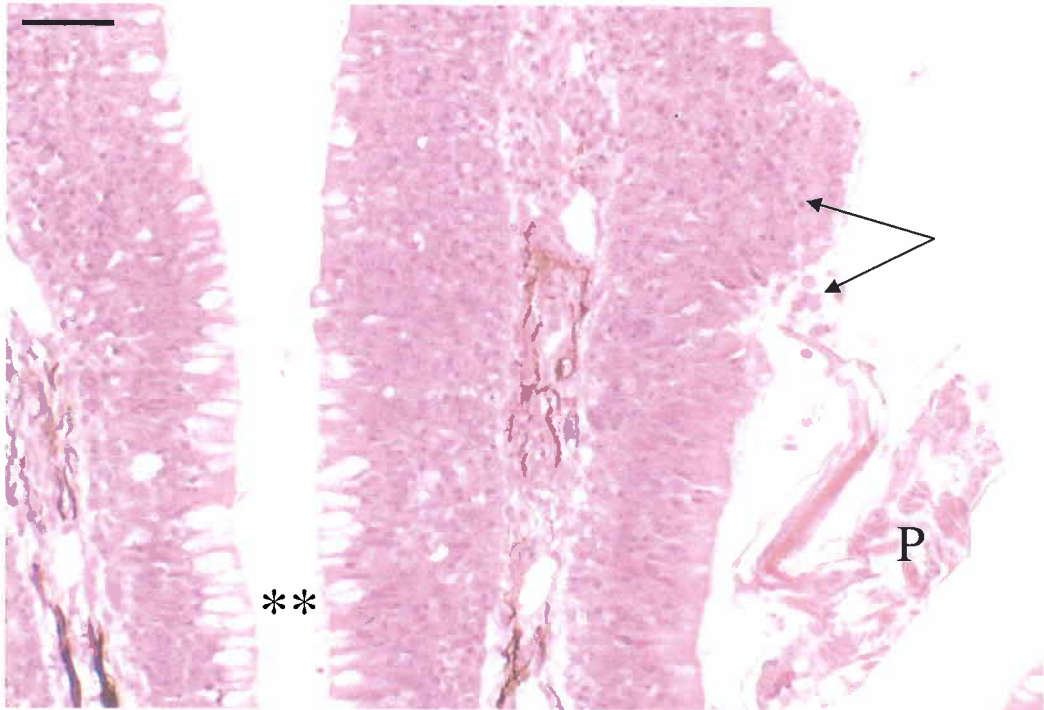
Fig. 6.14. Transverse section through nasal rosette of roach. A single *P. longidigitus* (P) can be seen attached to the nasal epithelium, resulting in indentation, hyperplasia and localised displacement of epithelium (arrows). Absence of mucus cells can be seen adjacent to the parasite compared with uninfected regions of the lamellae (**). Scale bar = 60μm.
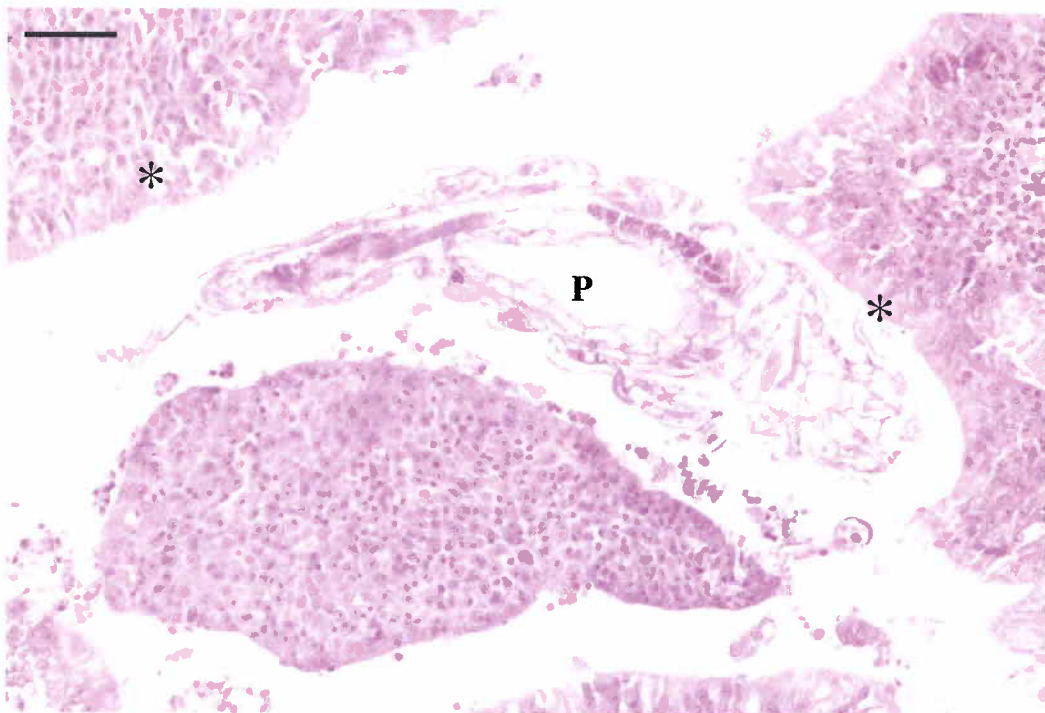


Fig. 6.15. A single *P. longidigitus* (P) attached to nasal tissues of common carp. This has caused erosion, desquamation and localised necrosis of the ciliated epithelium underneath the parasite. Normal epithelium may be seen beyond the immediate sites of parasite attachment and feeding (*). Scale bar = 60μm.
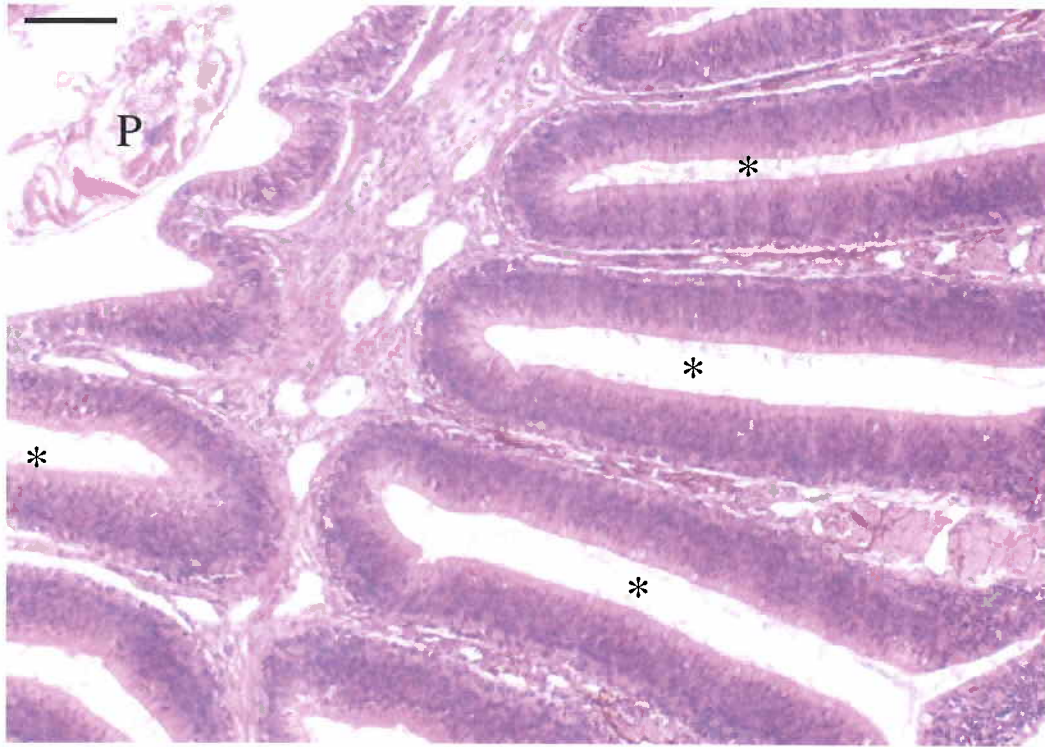
Fig. 6.16. Transverse section through nasal rosette of roach with light infection of *P. longidigitus*. Beyond the immediate site of the parasite (P) the majority of the olfactory epithelium is intact and normal (*). Scale bar = 100μm.
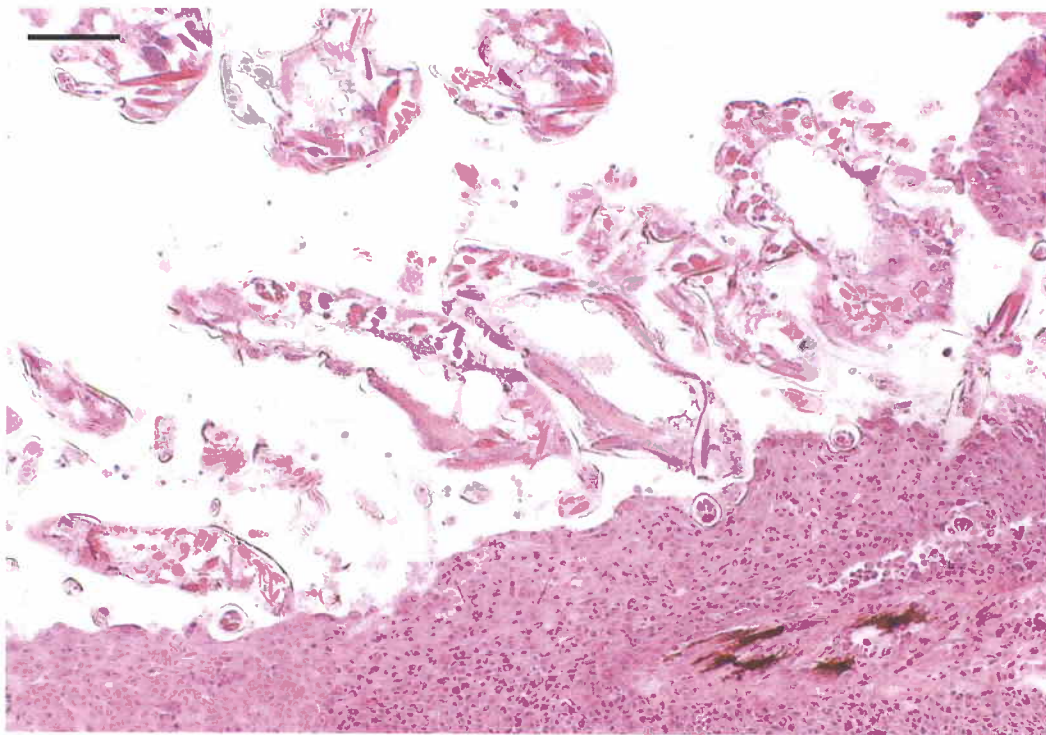


Fig. 6.17. Very heavy infection of *P. longidigitus* within nare of common bream. Extensive loss of ciliated epithelium and microvilli is evident within this highly disrupted region. Parasites are also surrounded in cell debris. Scale bar = 80μm.
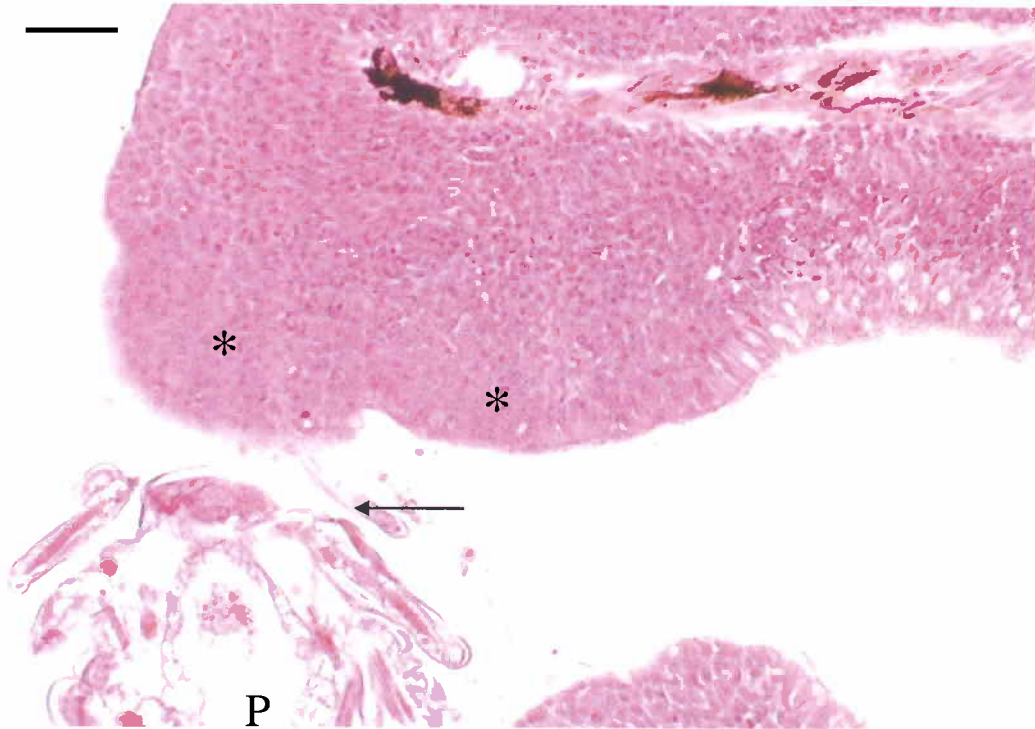
Fig. 6.18. A single *P. longidigitus* (P) attached to nasal lamellae of roach. Close contact of the parasite's swimming legs (arrow) with the epithelium can be seen. This region shows hyperplasia and loss of ciliated epithelium, including mucus cells (*). Scale bar = 40μm.
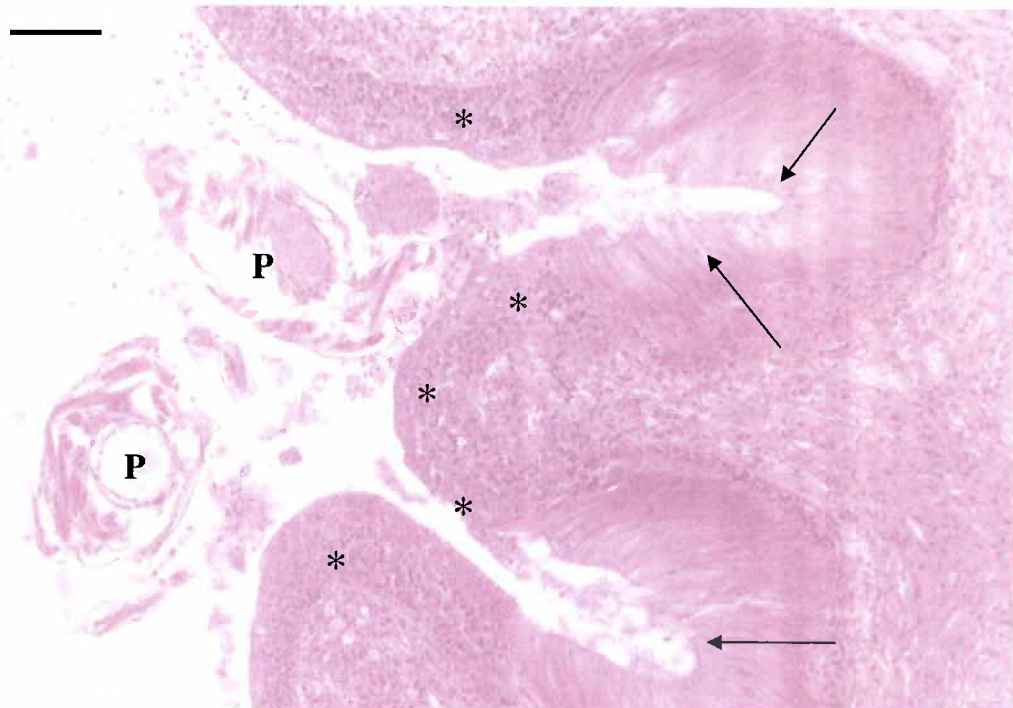


Fig. 6.19. Section through nasal rosette of pike. Extensive erosion of ciliated epithelium (*) has resulted from the feeding behaviour of *P. longidigitus* (P). Normal epithelium (arrow) remains within the pits of the lamellae. Increased inflammatory cells are present throughout the olfactory tissues (*). Scale bar 60μm.
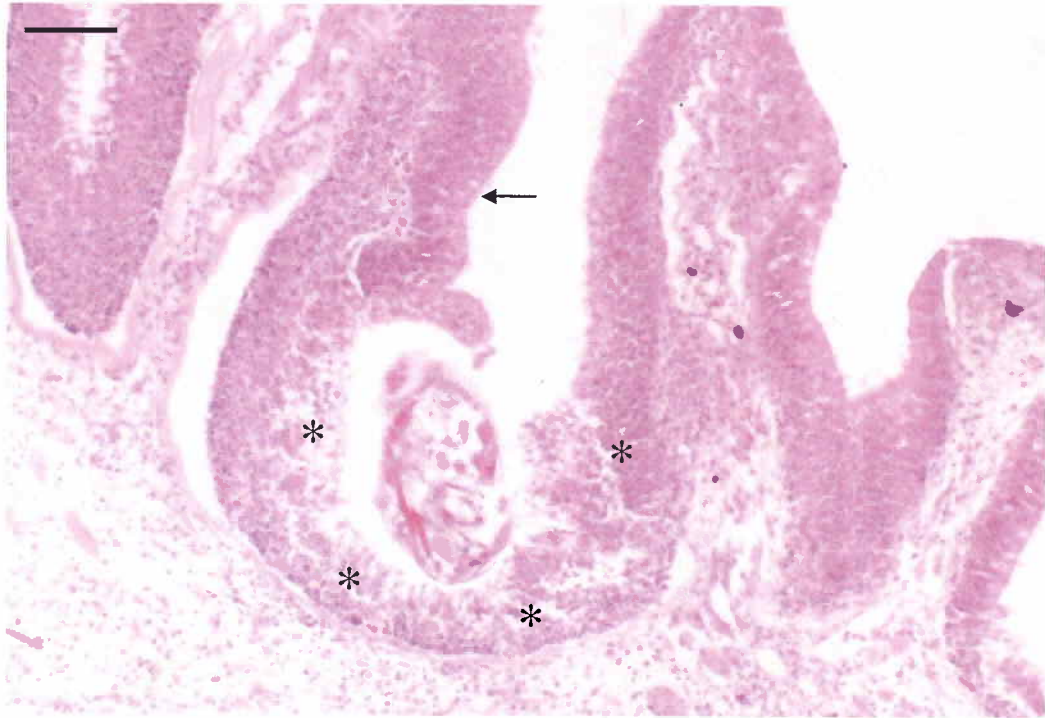
Fig. 6.20. *P. longidigitus* (P) attached within the lamellar pit of bream nare. The intact ciliated mucosa covering the lamellae surface (arrow) has been eroded in the region surrounding the parasite (*). Such damage provoked marked inflammatory responses within these regions (primarily lymphocytes). Scale bar 80μm.



Fig 6.21. Extensive loss of epithelium resulting from a very heavy infection of *P. longidigitus* within the nares of pike. The sides and top of every lamellae (*) have been eroded with loss of ciliated processes. In some areas, epithelial erosion approached the basement membrane (arrow). Accumulations of cell debris extend between the lamellar folds (x). Scale bar = 80μm.
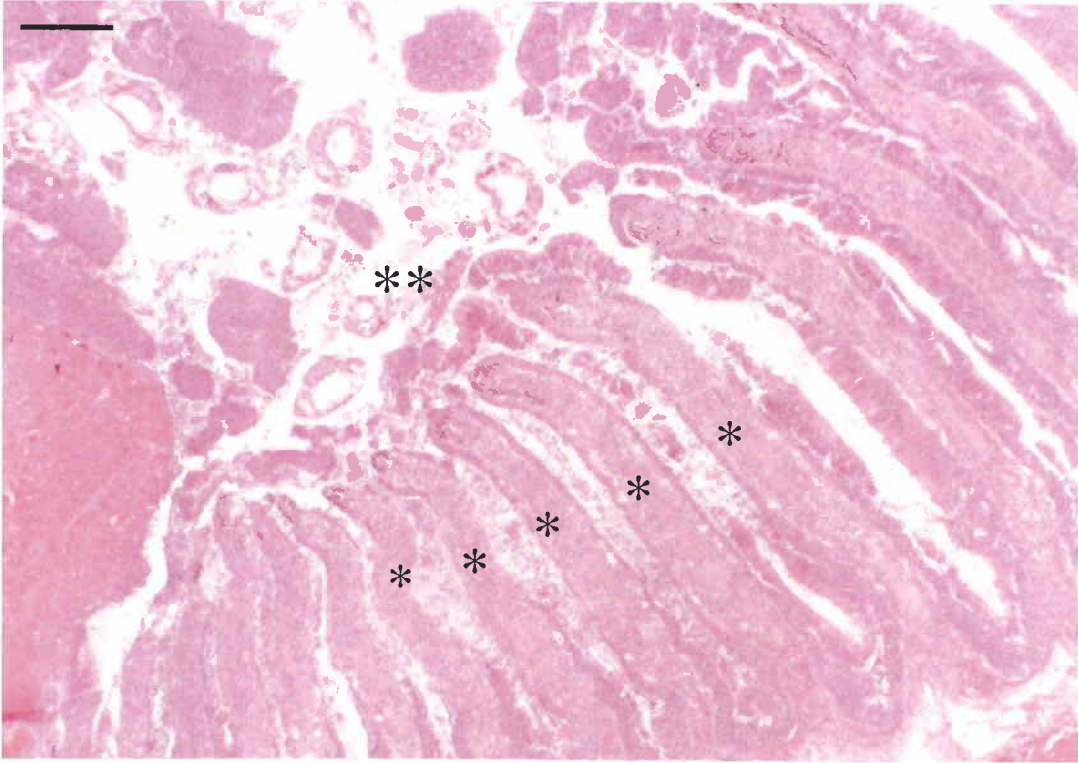
Fig. 6.22. Extreme infection of *P. longidigitus* within nare of common bream. This led to loss of normal rosette structure. Extensive loss of nasal epithelium can be seen along most lamellae (*), with numerous parasites and cell debris present within the lumen of the olfactory pit (**). Scale bar = 200μm.

### 6.3.3. Experimental studies of olfactory sensitivity

During the study a total of 144 common carp were obtained from 10 fisheries throughout England, all of which had histories of heavy *P. longidigitus* infections (Table 6.1). Samples were obtained between mid-April and the beginning of June 2004. Samples ranged from 1-26 common carp depending upon what could be caught and what fishery owners were willing to donate to the study. The fish studied ranged from 26.5cm to 55cm, weighing 455g to 2998g respectively. Most fish were successfully sexed at the time of capture through expression of milt, although this was not always the case. Parasite infections within these samples ranged from a complete absence of *P. longidigitus* to extremely heavy burdens comprising over 100 individuals per nare. The most heavily infected sample was that obtained during early April from Hulborough Pond, exhibiting a prevalence of 86%, with individual intensities exceeding 200 parasites per host (Table 6.1). All other samples revealed either very light infections or an absence of parasites. At no time during the study were any parasites found within the anaesthetic solutions following fish examination.

The effect of *P. longidigitus* on the olfactory sensitivity of common carp was investigated on a total of 80 fish over the study period. Unfortunately, the first sample obtained from Hulborough Pond, comprising many heavily infected fish was lost from the holding tanks due to suspected chlorine contamination of the water supply. After this event, no further samples were held within these facilities. No losses were recorded within the experimental aquaria during the study period. The gonadosomatic index of fish examined varied considerably during the study, reflecting the varied size of gonads and amounts of milt produced. As would be expected, expressible milt levels were positively correlated with gonad weight (P<0.00508) as well as GSI (P< 0.0170). The

levels of expressed milt increased as the reproductive season progressed being significantly greater in June than in May (P < 0.0692).

Table 6.1. Summary data of fisheries sampled, male fish collected and observed infections of *P. longidigitus*.

| Fishery | Date sampled | Number of carp | | | Prevalence (%) Intensity range |
|---------|--------------|----------------|--------------------------|----------------------------------|--------------------------------|
| | | Obtained | Spermiating on capture | Examined for olfactory responses | |
| Hulborough Pond Essex | 30 April | 26 | 21 | 0 | 86% prev. 1-200 parasites |
| Paul Pond Essex | 15 May | 11 | 5 | 3 | No parasites detected. |
| Preston Fishery | 17 May | 11 | 8 | 6 | No parasites detected |
| Makins Fishery | 18 May | 14 | 11 | 11 | No parasites detected |
| Leamington Lakes Leamington Spa | 20 May | 19 | 13 | 11 | 18% prev. 1-2 parasites |
| Bishops Bowl Fishery Leamington | 20 May | 19 | 17 | 17 | 7% prev. 0-1 parasites |
| Kirkby Golf Course | 24 May | 6 | 4 | 4 | 25% prev. 0-2 parasites |
| Hulborough Pond Essex | 3 June | 24 | 22 | 19 | 10% prev. 0-1 parasite |
| Dean Farm, Sussex | 8 June | 2 | 1 | 1 | No parasites detected |
| Lakeside Bielby, York | 10 June | 12 | 9 | 8 | No parasites detected |
| Total | | 144 | 111 | 80 | |

The design of the experimental study relied upon comparing milt production and steroid concentrations with intensity of *P. longidigitus* infection. Consequently, the loss of the only heavily infected sample obtained during the study posed a considerable problem. With an absence of baseline data for common carp it was therefore not possible to determine thresholds for normal and abnormal olfactory responses. Similarly, the absence of infection from a number of waters, exposure of very small numbers of carp from most fisheries and large variation in steroid and milt levels recorded within and between fisheries (Fig. 6.23) made it difficult to draw any general conclusions from the data. In efforts to reduce some of this variation, particular attention was given to samples examined from Hulborough Pond. This water was highlighted because of the large number of carp examined, evidence of heavy infections of *P. longidigitus,* parasitological observations from two separate samples gained in April and June and
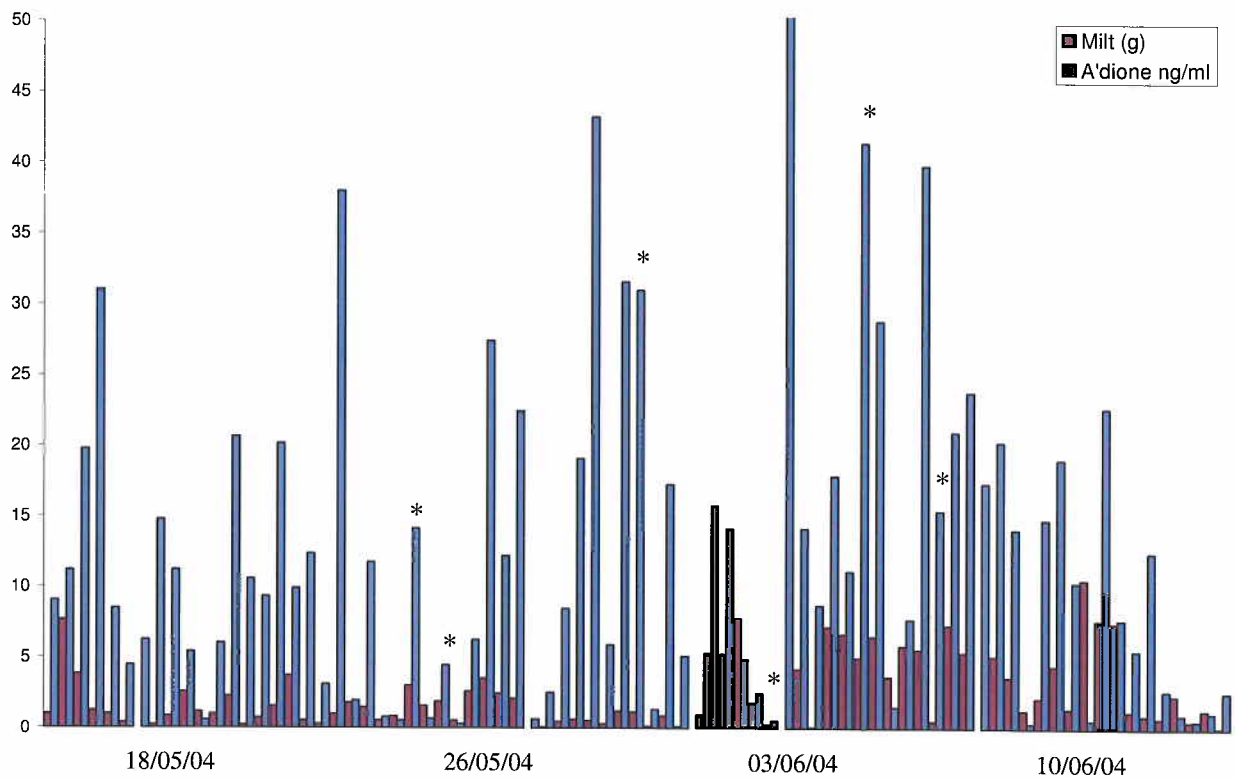


Fig. 6.23. Milt production and androstenedione values for all of the common carp exposed to 17.20αβ-P during the study period. (*) - fish with recorded infections of *P. longidigitus.*

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very high levels of androstenedione and milt recorded from the majority of fish.

- **Case Study - Hulborough Pond**

The first sample of carp, comprising 26 fish, was initially obtained from Hulborough Pond during early April. Examination of a small number of these fish revealed a parasite prevalence of 85.7% and mean intensity of 75.1 *P. longidigitus* per host (n = 7). Two of the fish examined had very heavy parasite burdens comprising over 100 parasites per nare. Although the loss of this initial sample prevented an assessment of olfactory responses, a second sample of fish comprising 19 carp was obtained in early June. In contrast, the prevalence of infection within this sample was 10%, with maximum intensity of 1 parasite per host (Table. 6.2). The observed differences in parasite infection between samples was highly statistically significant (Mann-Whitney p = 0.0002). This finding prompted the further seasonality studies (see Seasonality studies).

Table 6.2. Observed differences in *P. longidigitus* infections recorded from Hulborough Pond during two spring sampling periods.

|  | Numbers of *P. longidigitus* per host (mean / median) |
|---|---|
| April sample (n=7) | 0, 2, 20, 24, 80, ~200, ~200  (75.1 / 24) |
| June sample (n=19) | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1  (1 / 0) |

During the experimental studies, the majority of carp from Hulborough Pond showed very high steroid levels and expressed large quantities of milt (Fig. 6.24). The quantities

of expressible milt recorded by these fish were considerably higher than those recorded for all other waters. The mean volume of milt produced within the 6-hour exposure period was 5.11ml (sd 0.589). The mean concentration of androstenedione recorded was 16.75ng/ml. This was also higher than all other fisheries examined with the exception of Kirkby Golf Course. A small number of carp, in particular fish 5, 8, 13 and 17 revealed very low milt or steroid levels. Histopathological examination of carp showing both very high and very low levels of milt and androstenedione, revealed no significant difference in the damage to the olfactory epithelium. No consistent pathological changes were recorded that could be attributed to *P. longidigitus* infection.
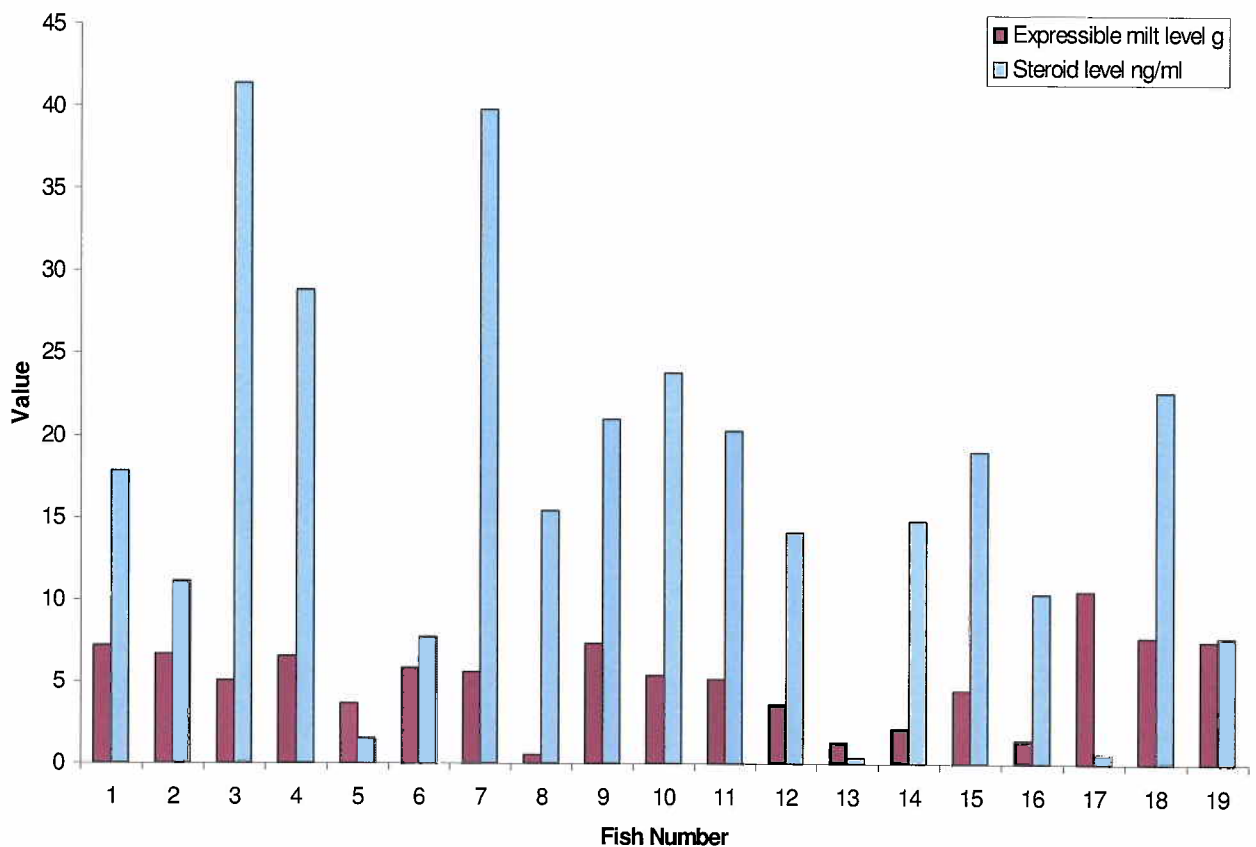


Fig. 6.24. Observed milt and androstenedione levels from common carp obtained from Hulborough Pond during the June sampling period.

## 6.3.4 Seasonality studies

Samples obtained from Swallowbrook Reservoir and Willow Farm Fishery both showed changes in *P. longidigitus* infection consistent with those of Hulborough Pond gained during the experimental studies (Fig. 6.25). These were characterised by a marked decline in the parasite population in late spring, following periods of high prevalence and high mean intensities (Table 6.3). The sudden decline in parasite prevalence and intensity occurred during May. This coincided with water temperatures of approximately 12-15°C. The observed changes in *P. longidigitus* prevalence and intensity were highly statistically significant for all of the waters examined (Mann Whitney P<0.01). Egg string production in *P. longidigitus* was first recorded during early April. Samples obtained prior to this date revealed an absence of any parasites possessing eggstrings. The proportion of *P. longidigitus* possessing eggstrings increased from 0% in March to over 90% by May. One hundred percent of parasites examined in June were gravid although this sample comprised only two individuals (Table 6.3).

Table 6.3. Changes in prevalence, intensity and reproductive status of *P. longidigitus* infection during the spring period.

| Water | Date | No. fish examined | Prevalence (%) | Intensity range | Mean intensity | Parasites with egg strings (%) |
|---|---|---|---|---|---|---|
| Swallowbrook Reservoir | 21 March | 30 | 100 | 1 - 81 | 14.9 | 0 |
| | 4 May | 33 | 79 | 0 - 126 | 13.8 | 90 |
| | 7 June | 24 | 8 | 0 - 1 | 1 | 100 |
| Willow Farm | 9 March | 31 | 80.1 | 0 - 41 | 7.96 | 0 |
| | 4 April | 27 | 85.1 | 0 – 20 | 5.1 | 65 |
| | 14 July | 30 | 23 | 0 - 4 | 2 | 42 |
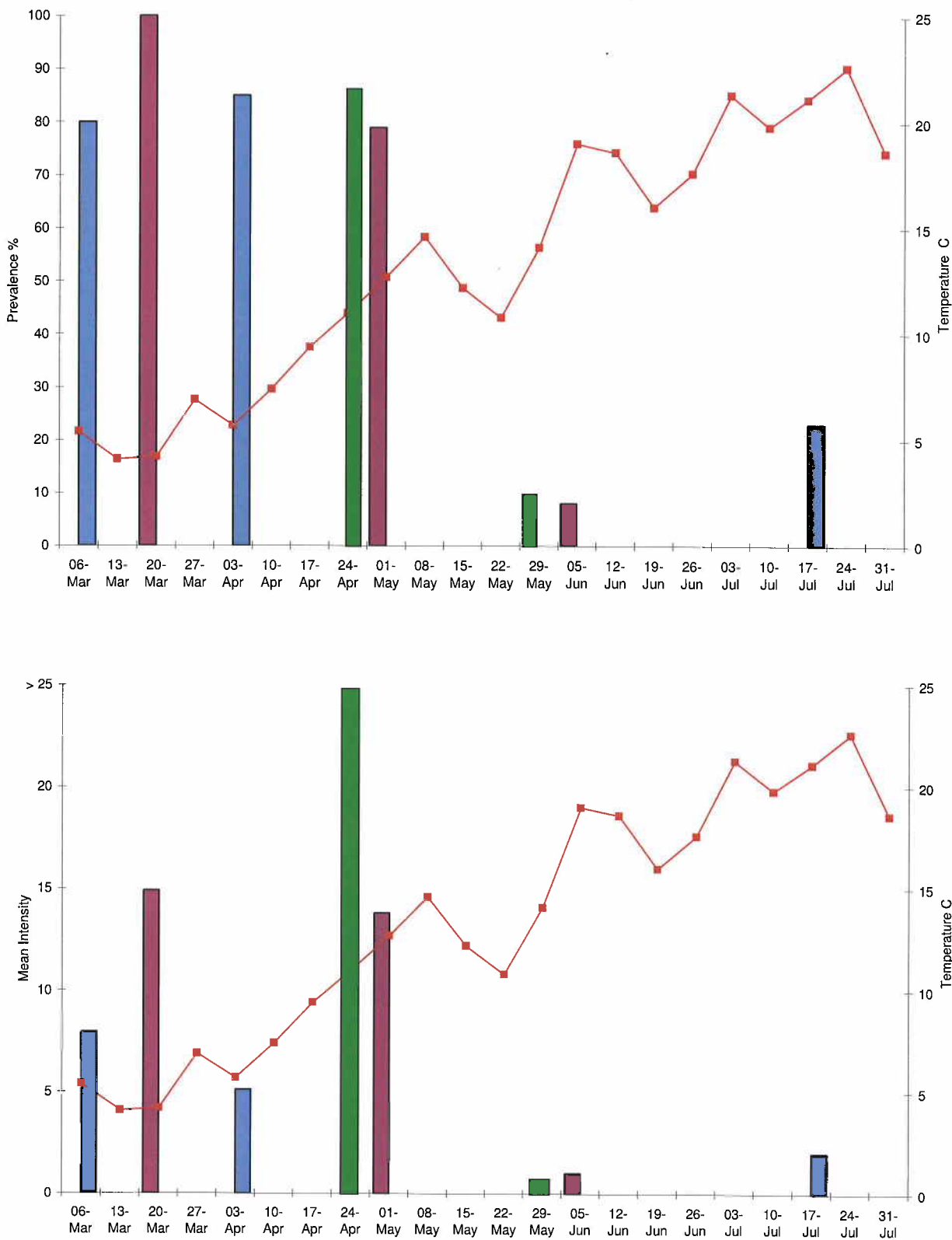
Fig 6.25. Seasonal changes in the prevalence (top graph) and mean intensity (bottom graph) of *P. longidigitus* with temperature (red line) at three stillwater fisheries in England during spring. Willow Farm [ ] Swallowbrook reservoir [ ] Hulborough Pond [ ]

## 6.3.5 Distribution of *P. longidigitus* within England and Wales

Distribution records confirm that *P. longidigitus* has a widespread distribution throughout England and Wales (Figs. 6.26 - 6.27). Environment Agency records showed that between 1994 and January 2007, the parasite had been recorded from 508 fisheries, the majority of which are stillwaters. However, the parasite has also been recorded from river catchments, fish farms and canal systems. Although *P. longidigitus* was first detected in October 1994, distribution records at this time suggest that the parasite had long been present within England and Wales. In the three months of 1994 following first detection of the parasite (Fig. 6.26), 11 fisheries were found to harbour parasite populations. In the following year, 43 infected fisheries were recorded (Fig. 6.24). Initial distribution records of *P. longidigitus* reveal a focus within the south east of England. However, to date *P. longidigitus* has been recorded from all regions of England and Wales. The distribution of the parasite in the north-west of England and Wales remains sparse (Fig. 6.27).
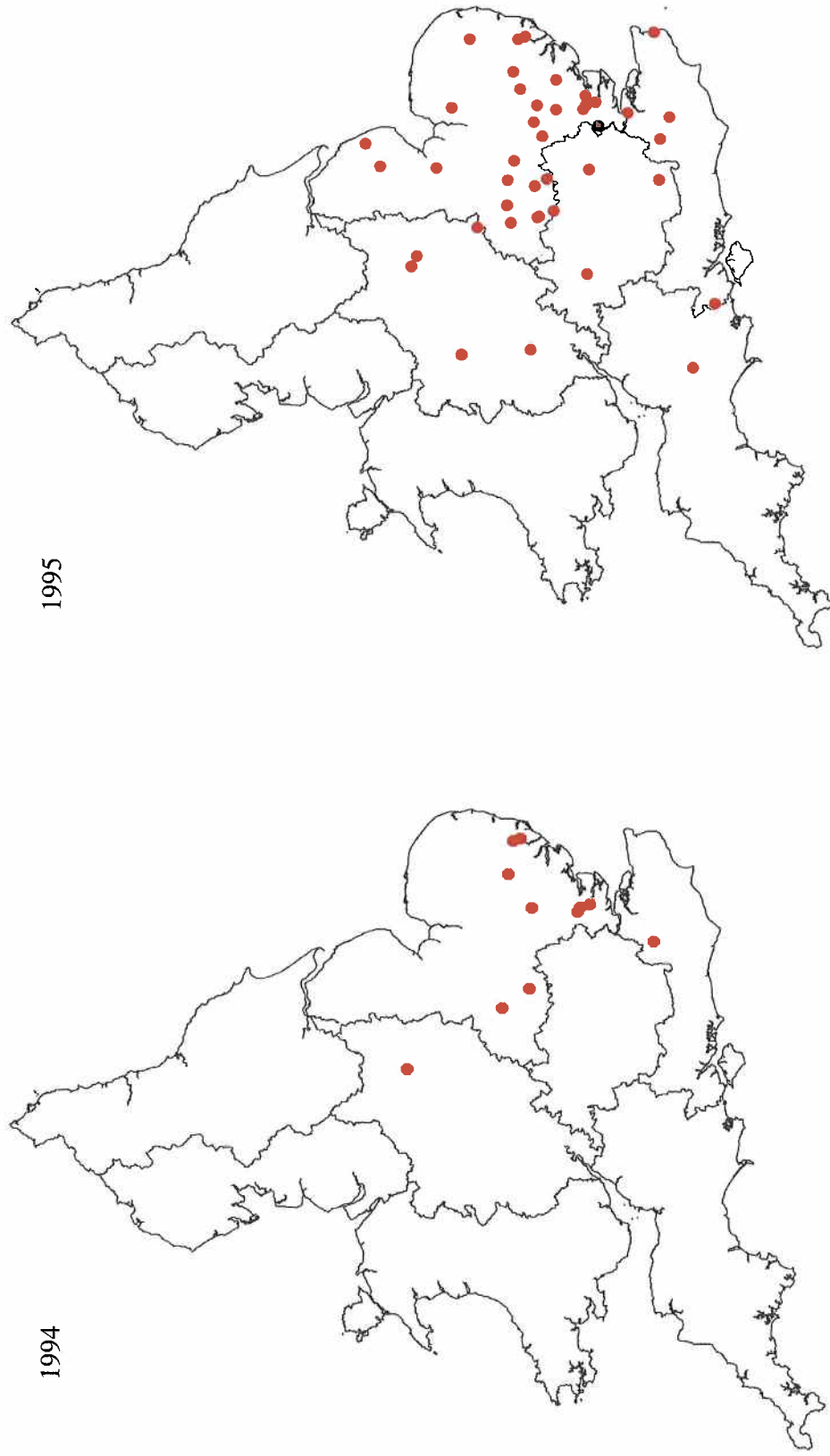
1995

1994

Fig 6.26 Distribution of *P. longidigitus* in England and Wales in the year the parasite was detected (1994) and the following year (1995) showing rapid increase in the number and geographical spread of infected waters recorded.
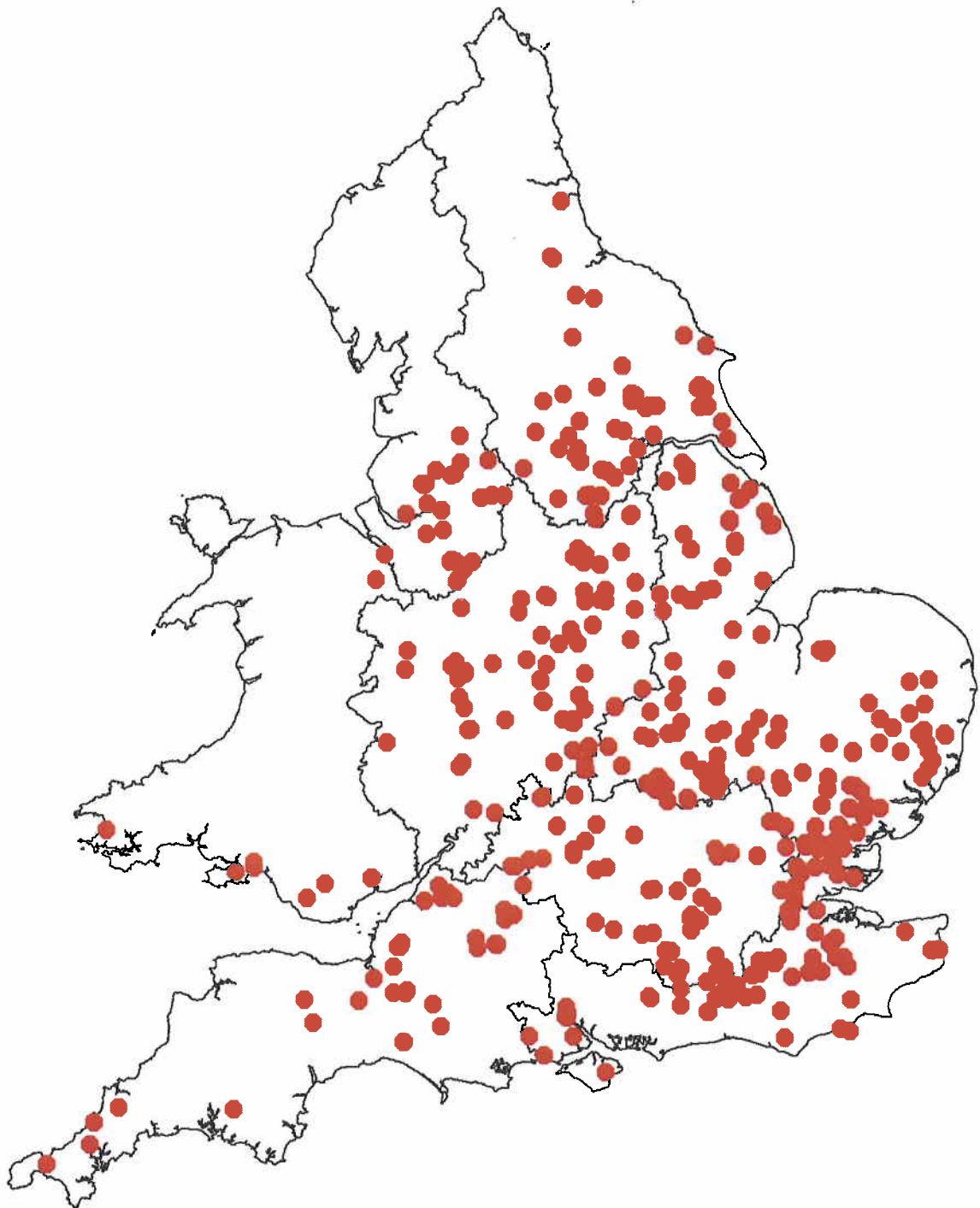
Fig 6.27. Current distribution of *P. longidigitus* in England and Wales (from Environment Agency records 1994 - 2007).

## 6.4 Discussion

**Pathological changes associated with *P. longidigitus* infections**

Considerable attention has been given to the effects of crustacean parasites upon fish. Literature provides descriptions of damage to the gills (Goodwin, 1999; Dezfuli *et al.,* 2003), skin (Rogers & Hawke, 1978; Shariff & Roberts, 1989), fins (Abdelhalim, 1990), skeleton (Bauer, 1959; Quignard, 1968) mouth (Smith, 1975; Colorni *et al.,* 1997) and eye of infected hosts (Bennet, 1964; Kabata & Forrester, 1974). However, despite descriptions of ergasilid and branchiuran parasites from the nares of fish, little attention has been given to the damage associated with these parasites upon olfactory tissues (Kabata, 1970; Burris & Miller, 1972; Williams & Jones, 1994; Muzzall & Hudson, 2004). Hawkins (2001) is the only author to have described the pathology associated with *P. longidigitus*. However, this work was based upon a very small number of fish and included observations from only two host species. The current study is the first to describe the pathological changes caused by the parasite in a range of host species, host sizes and parasite intensities.

Histopathological observations confirm that *P. longidigitus* causes damage to the olfactory epithelium as a result of both attachment and feeding behaviour. However, in contrast to many members of the Ergasilidae, the damage caused by the feeding behaviour of *P. longidigitus* far exceeded that of attachment (Abdelhalim, 1990; Dezfuli *et al.,* 2003). Similarly, compared with many ergasilid species, in particular those associated with the gills, the pathological changes caused by attachment of *P. longidigitus* were relatively mild and superficial. Literature on *E. sieboldi* (Abdelhalim, 1990; Dezfuli *et al.,* 2003), *E. mirabilis* (Oldewage & van As, 1987), *E. lizae* (Roubal,

1986a), *E. colomesus* (Thatcher & Boeger, 1983) and *Thersitina gasterostei* (Pagenstecher, 1861) (Donoghue, 1989) all detail marked damage to host tissues resulting from the deep penetration of the parasite's sharp, claw-like antennae. Conversely, the attachment behaviour of *P. longidigitus* was characterised by only shallow insertion of the blunt antennae tips, resulting in localised pitting and shallow epithelial indentation. Hawkins (2001) described the formation of 'deep wounds' as a typical consequence of *P. longidigitus* attachment. However, neither the images provided in that work, nor the observations made during the current study support this.

The difference in the attachment behaviour of *P. longidigitus* compared with other ergasilid species may be explained by the morphology of the parasite's antennae, feeding characteristics and morphology of the nasal rosette. Firstly, the gripping capabilities of three blunt-ended antennae rather than a single pointed claw, may represent a specific adaptation for attaching to the soft, undulating nasal lamellae. Such superficial attachment may provide parasites with the mobility to graze freely upon the olfactory tissues. The sheltered location within the nares may also negate the need for deep insertion of strong antennae compared with parasites that attach to more exposed regions of the body (Kabata, 1970, 1981; Abdelhalim, 1990). Consequently, both morphology of the antennae and water flow may be important factors that determine the strict site specificity of *P. longidigitus*. The observation of parasites upon the flank of a single host represented a new site record for *P. longidigitus*. However, it remains likely that this was a chance finding and may have been the result of temporary settlement prior to parasite establishment within the nares, or following displacement from the area around the nares.

The most pronounced and consistent pathological changes recorded during the study were those associated with the feeding behaviour of *P. longidigitus*. This was characterised by epithelial erosion, a commonly recorded consequence of ergasilid infections (Kabata, 1970; Abdelhalim, 1990; *pers. ob.*). The activity of live parasites, in particular the rapid movement of the swimming legs is likely to be associated with the displacement of epithelium prior to ingestion. A similar feeding behaviour has been recorded for *N. japonicus* on the skin and fins of freshwater cyprinids (Abdelhalim, 1990). This is supported by pathological observations of frequent accumulations of cell debris and the close association of the parasite's swimming legs with host tissues. These observations suggest that the feeding behaviour of *P. longidigitus* involves active grazing upon the olfactory epithelium. This nomadic behaviour means that the severity of damage caused to the olfactory tissues is highly dependent upon intensity of infection, as well as the size and morphology of the nasal rosette. It is also recognised that damage to the nares is not only associated with the destructive capacity of feeding parasites, but also the regenerative capabilities of the olfactory epithelium (Kasumyan, 2004; Bettini *et al.*, 2006). During light and even moderate parasite intensities a balance in this relationship appears to limit the extent and severity of damage to the nares. Only at higher intensities is a threshold reached, above which progressive destruction of the olfactory tissues ensues. It is such infections that provide potential for more severe pathological damage, extensive loss of ciliated surfaces and destruction of normal rosette architecture.

Crustacean parasites may increase the potential for secondary infections as a result of attachment or feeding wounds (Kabata, 1970; Roberts, 2004). This may be particularly important for hosts infected with *P. longidigitus,* as the olfactory mucosa of fish is

known to be vulnerable to the entry and early development of bacterial infections (Morrison & Plumb, 1994; Wolfe *et al.*, 1994). Furthermore, non-sensory ciliated epithelial cells aid water flow through the nares and promote clearance of particles and pathogens that may enter the nasal lamellae (Morrison & Plumb, 1994). It is therefore feasible that damage caused by *P. longidigitus* to the olfactory epithelium may increase the potential for entry of bacterial pathogens. Enteric Septicaemia of Catfish (ESC) is a serious disease caused by the gram-negative bacterium *Edwardsiella ictaluri* (Blazer *et al.*, 1985). It is well documented that the olfactory epithelium is a primary site by which bacteria gain entry, colonise and subsequently spread (Wolfe *et al.*, 1994). Initial lesions are characterised by loss of ciliated epithelium, extending to the olfactory bulb, cranium, brain and the central nervous system, leading to imbalance and death of infected hosts (Newton *et al.*, 1989; Morrison & Plumb, 1994). However, at no time during the study period was any evidence gained of secondary infection or damage to olfactory tissues beyond the epithelium. Similarly, no clinical signs of infection or behavioural abnormality were associated with *P. longidigitus* infections despite observations of very heavy infections (Environment Agency unpublished; Hawkins, 2001). Consequently, whilst *P. longidigitus* causes localised disruption to the nasal epithelium, infections do not appear to cause ill-health, condition loss, morbidity or mortality at the host level.

Extreme difficulty exists when trying to correlate pathological observations with the potential for physiological or behavioural disturbances in fish (Ferguson, 1989; Ferguson *et al.*, 2006). Although it is clear that *P. longidigitus* can cause loss of ciliated processes within the nares, these changes do not confirm a loss of olfactory function. The surface of the olfactory epithelium of fish comprises a number of cell types,

namely ciliated receptor cells, microvillar receptor cells, sensory crypt cells and non-sensory ciliated cells, the latter densely covering the olfactory epithelium but not believed to have a sensory function (Zielinski & Hara, 1992; Hansen *et al.*, 1999). The morphological distinction of olfactory receptor cells from non-sensory ciliated cells in histological sections can be problematic (Wheater *et al.*, 1979). Similarly, the number and distribution of sensory cells within the nasal rosette can vary with fish species, environmental conditions, fish age and maturity (Wilson & Westerman, 1967; Yamamoto & Ueda, 1977, 1978, 1979; Yamamoto, 1982; Moran *et al.*, 1987; Carpio, 1988; Stewart & Brunjes, 1990; Hansen *et al.*, 1999; Kasumyan, 2004). It therefore remains unclear at what stage fish lose olfactory acuity, following a reduction in the many millions of sensory neurons present within the nares (Jobling, 1995). Kasumyan (2004) suggests that only a small proportion (as little as one third) of receptor elements found within the nares of grass carp *Ctenopharyngdon idella* are necessary to maintain olfactory sensitivity (Pashchenko & Kasumyan, 1984). This supports the earlier assumption that light and even moderate *P. longidigitus* burdens may not have major impacts upon infected hosts. In such cases, a combination of existing and regenerating neurons may maintain olfactory function unless parasite infections are heavy. Whilst extensive loss of epithelium may feasibly reduce olfactory function of fish, it is impossible to attribute these changes to a loss of sensory function without experimental observations.

## Experimental studies of olfactory sensitivity

The physiology, morphology and neurobiology of fish reproduction have received considerable attention (Liley, 1982; Dulka *et al.*, 1987; Klaprat *et al.*, 1988; Li *et al.*, 1995; Stacey *et al.*, 1994; Finger *et al.*, 2000; Stacey & Sorensen, 2002). It is well

recognised that teleost fish have evolved sophisticated and varied chemical signalling systems that allow recognition (Smith, 1992), attraction (Li *et al.*, 1995), kin recognition (Brown & Brown, 1992), synchronisation of reproductive physiology (Dulka *et al.*, 1987) and synchronisation of reproductive behaviour (Sorensen *et al.*, 1986, 1988, 1989). In many cyprinid species the nasal tissues carry out an important function during spawning, mainly in the reception of chemosensory cues (Liley, 1982; Liley *et al.*, 1991; Stacey *et al.*, 1994; Moore *et al.*, 2002). Damage to the sensory receptors within the nares can have serious adverse effects upon the ability of fish to detect pheromones and perform normal behavioural and reproductive functions (Waring & Moore, 1995; Moore *et al.*, 2002). Despite growing interest in fish chemoreception, in particular the adverse effects of pollutants, no studies have been made to examine the role of parasites upon olfactory function (Barber & Wright, 2006). The current study represents the first attempts to improve understanding in this area.

The current study set out to measure the milt production and blood steroid concentrations of 80 common carp following exposure to the pre-ovulatory primer pheromone $17\alpha20\beta$-P. However, a number of problems were encountered during the study which limited the success of this work. Firstly, the need to obtain high value carp between April and June, that were mature, all male, successfully spermiating, less than 2kg in weight (max size for experimental work) and infected with *P. longidigitus* posed a considerable challenge. These problems, as well as the high commercial value of large carp explained the very small sample sizes obtained from some of the fisheries. Consequently, the loss of a large sample comprising many heavily infected individuals represented a considerable loss to the study. Secondly, the experiment was designed to compare the physiological responses of common carp in relation to parasite intensity.

The absence of observations from heavily infected fish, and lack of base-line data of normal responses of common carp to 17α20β-P therefore prevented an accurate assessment of parasite impact to be made at the individual level.

The choice of common carp as an experimental species represented a compromise of susceptibility to *P. longidigitus,* ease of obtaining samples from fisheries and the relatively good understanding of olfactory physiology (Stacey & Sorensen, 2002; Moore, *pers. comm.*). The hormone pheromone system of goldfish *Carassius auratus* and crucian carp *Carassius carassius* are among the best studied of any fish species (Partridge *et al.,* 1976; Sorensen *et al.,* 1986, 1988, 2005a,b; Dulka *et al.,* 1987; Bjerselius & Olsen, 1993). However, the chances of obtaining these species from infected fisheries during the spring reproductive period would have posed too greater risk to the study. The option of establishing experimental parasite infections was desirable, but considered too greater challenge in the available time period (Abdelhalim, 1990; Alston, 1994). Although the physiological responses of common carp to 17α20β-P have received little attention, the nares of many cyprinid fish are known to be morphologically alike (Yamamoto & Ueda, 1978) with similar sensitivities to the same pre-ovulatory pheromones (Irvine & Sorensen, 1993; Bjerselius & Olsen, 1993; Moore, *pers comm.*).

The expression of milt in both infected and uninfected carp at the time of capture suggests that *P. longidigitus* does not hinder reproductive development prior to spawning. The observation of spawning behaviour (chasing and tight shoaling) in carp fisheries during latter stages of the sampling period also support the assumption that basic reproductive behaviour is not hindered in fisheries with established *P. longidigitus*

infections. Varied levels of milt production and blood steroid concentrations recorded during the study are likely to be a consequence of dealing with wild fish populations that are in varying states of physiological condition, reproductive development and maturity. Reproductive timing and physiology is also likely to be affected by the type of fishery, geographical location, management practices, water quality, nutritional status of fish, temperature and many other biotic and abiotic factors (Winfield & Nelson, 1989; Jobling, 1995). Consequently, the large numbers of fish obtained from Hulborough Pond combined with evidence of heavy parasite infections within this water, proved a particularly valuable sample and enabled a preliminary assessment of impact to be made.

The large volumes of expressible milt and high steroid concentrations observed in the carp sample from Hulborough Pond are characteristic of normal olfactory responses to the pheromone 17α20β-P (Lower & Moore *pers. comm.*). The large volumes of milt produced by the majority of carp from this water are consistent with the reproductive physiology of healthy common carp. Christ *et al.,* (1996) measured milt volumes produced by healthy 2kg common carp in an 18 hour period after inducing spawning. Milt volumes produced by carp during June were directly correlated with fish size and GSI and conformed to the ratio of 3.6ml/kg body weight (mean 5.09ml/fish). Although it is recognised that many variables may influence reproductive physiology in cyprinids, the application of this relationship to the Hulborough Pond sample revealed higher mean volumes of milt (5.11ml, n=19) in just 6 hours. Whilst the observed steroid concentrations may be consistent with normal olfactory function (Lower & Moore *pers. comm.*), this is difficult to confirm without understanding of base-line responses over time. It remains unclear why a small number of fish within the sample failed to express

milt or showed very low androstenedione concentrations. In view of the similarity in pathological changes in both high and low responders, these differences cannot be attributed to *P. longidigitus* infection. Confirmation of base-line responses of healthy fish represents an important area of further investigation in order to improve understanding of pheromone communication in the common carp.

Observations of milt and steroid levels from Hulborough Pond are particularly valuable due to the high prevalence and intensities of *P. longidigitus* recorded within this water. Infections exceeding 200 parasites per host during April represent very heavy parasite burdens (Hawkins, 2001). Consequently, current studies suggest that despite established *P. longidigitus* infections, the majority of common carp sampled from this water maintain the capacity for normal reproduction. These studies provide no evidence to suggest that common carp from fisheries heavily infected with *P. longidigitus* have impaired olfactory function or may be incapable of normal and successful spawning activity. Anecdotal evidence gained from fishery owners suggests that waters infected with *P. longidigitus* maintain successful recruitment, spawning activity and fishery performance (Page & Challenger *pers. comm.*). Furthermore, a number of fish movement applications have been received by the Environment Agency to crop infected fisheries due to natural recruitment of resident stocks. Consequently, whilst further studies are necessary to confirm the effects of *P. longidigitus* upon olfactory responses of infected fish, current observations suggest that the parasite is not a serious pathogen of fisheries. Even if heavily infected individuals were compromised by *P. longidigitus* infection, the highly over-dispersed distribution of the parasite within host populations (Hawkins, 2001) is likely to limit impact at the population level. The inability of a small proportion of heavily infected hosts to spawn is unlikely to have serious implications to

the performance of most fisheries. However, it is recognised that this may not be true for all fishery types and that current studies do not address potential impacts to non-cyprinid species. In percids and salmonids, a range of sensory signals may be used to synchronise reproduction, including visual cues (Takeuchi *et al.*, 1987), vibration (Satou *et al.*, 1987), sound (Moore & Waring, 1999) as well as chemical cues (Olsen & Liley, 1993; Scott & Liley, 1994). Evaluating the effects of *P. longidigitus* upon the reproduction of different fish species within different fishery types represents a huge and complex challenge. Consequently, further studies in this area need to be focussed upon fish species of particular economic or ecological interest.

**Seasonality studies**

Current observations suggest that *P. longidigitus* populations undergo marked seasonal changes during the spring period within England. The high prevalence and intensity of *P. longidigitus* during March and April, followed by a rapid decline in parasite numbers during May represent significant seasonal changes within the fisheries studied. It is well documented that members of the Ergasilidae follow a seasonal cycle of maturation, development and reproduction (Bauer *et al.*, 1969; Zmerzlaya, 1972; Abdelhalim, 1990; Abdelhalim *et al.*, 1991; Alston, 1994; Alston & Lewis, 1994; Molnar & Szekely, 1997). For many species, seasonality studies have focussed upon the reproductive development of parasites and establishment of peaks in intensity, most of which occur during late summer and autumn (Hanek & Fernando, 1978; Abdelhalim, 1990; Alston, 1994). Despite detailed epidemiological studies of *E. briani*, *N. japonicus* and *E. sieboldi* in stillwater fisheries in England (Abdelhalim, 1990; Alston, 1994) the current study, although limited by short-term sampling, represents the first to address the seasonality of *P. longidigitus*.

The high prevalence and intensity of *P. longidigitus* during early spring confirms that parasites retain the ability to successfully over-winter within the nares of infected hosts. The onset of egg development during March and April is consistent with other ergasilid species (Abdelhalim, 1990; Alston, 1994). The subsequent decline in the parasite population during May is likely to coincide with the release of larvae from over-wintered female parasites. The sudden loss of parasites during this period may therefore be associated with the metabolic costs of over-wintering, egg-development and reproduction. Although environmental factors, including food availability are known to strongly influence development and survival of copepods (Kuperman & Shulman, 1977; Fernandez, 1979a, b; Green *et al.,* 1991; Paperna, 1991; Alston, 1994), this would not effect the adult parasite population which gain nutrition solely from their host. Taylor (2000) provided a detailed account of the population dynamics of *Argulus foliaceus* in England, confirming that spring coincides with low parasite prevalence and intensity compared with summer and autumn. Pojmanska (1984) described a distinct decline in infections of *E. sieboldi* during June in Poland, compared with late summer and autumn. Similarly, Abdelhalim (1990) described relatively low prevalence and intensities of *E. sieboldi* in spring prior to a rapid increase in the population throughout the summer. Whilst current observations did not extend into summer or autumn, it is considered likely that the seasonality of *P. longidigitus* is consistent with that of other ergasilid parasites. However, whilst the impacts of many ergasilid parasites are associated with late summer (Lahav & Sarig, 1967; Alston, 1994; Molnar & Szekely, 1997; Tildesley, 2006), spring represents the most important period for potential effects of *P. longidigitus* upon host reproduction.

The reproduction of most stillwater cyprinid fish in Britain occurs during May and June, depending upon spring water temperatures (Giles, 1994; Maitland, 2004). Current observations of *P. longidigitus* seasonality reveal that this period coincides with the lowest levels of parasite prevalence and intensity. Consequently, the reduction in parasite activity within the nares during spring may minimise host impact and provide a window for regeneration of the olfactory epithelium. Extensive literature confirms that the olfactory system of fish has a natural regenerative capacity with the receptor cells being constantly renewed (Hansen *et al.*, 1999; Kasumyan, 2004; Bettini *et al.*, 2006; Sandahl *et al.*, 2006). The restoration of olfactory function has been studied in a number of fish species including goldfish (Rekowski & Zippel, 1993; Zippel *et al.*, 1997; Zippel, 2000), trout (Moran *et al.*, 1987), salmon (Sandhal, *et al.*, 2006) and tilapia (Bettini, *et al.*, 2006). The studies indicate that regeneration of the olfactory epithelium may occur in as little as ten days following necrosis of olfactory sensory cells.

Kasumyan (2004) confirmed that restoration of olfactory function may be so rapid as to outstrip the regeneration of other cellular structures within the epithelium. This renewal process is considered to be an adaptation to impairment caused by environmental hazards experienced during the normal life of a fish (Hara, 1993). Hawkins (2001) acknowledged that a reduction in *P. longidigitus* infection may allow epithelial regeneration within the nares. However, due to a poor understanding of seasonality, this author suggested that the only scope for this to occur was through treatment of infected hosts. Current observations suggest that seasonal changes in the *P. longidigitus* population may provide a natural opportunity for epithelial regeneration to occur. A reduction in parasite activity within the nares of fish during spring may therefore promote restoration of olfactory sensitivity, allowing successful reproduction. Due to

the weak pathogenicity of *P. longidigitus* at the host level, the subsequent increase in parasite intensity during the remainder of the year may be relatively unimportant as long as host reproduction is ensured. However, it is recognised that this relationship is highly dependant upon temperature and may not be favourable for species that spawn early or late in the year e.g. pike or salmon (Maitland, 2004).

**Distribution of *P. longidigitus* within England and Wales**

*P. longidigitus* has a widespread distribution in fisheries throughout England and Wales. The wide geographical separation of infected waters may reflect the frequent and wide-scale movement of fish within England and Wales (LFMD, 2006). Although *P. longidigitus* is the most recently recorded member of the Ergasilidae within the British Isles, its distribution now exceeds all other species (Fryer, 1982; Abdelhalim, 1990; Environment Agency, 1999; Hawkins, 2001). The scarcity of records in the far north, south-west of England and Wales is likely to reflect the smaller number of fisheries and reduced fish stocking activity within these regions (LFMD, 2006). The current distribution of *P. longidigitus* is therefore likely to be influenced strongly by the distribution of coarse fisheries containing susceptible host species and thus detection effort through health checks. Due to the inclusion of only Environment Agency records in this work, it is likely that current estimates of distribution represent an underestimate of the actual distribution of the parasite.

It is unclear whether *P. longidigitus* is an introduced species to the British Isles, or a native parasite that simply evaded detection prior to 1994. The non-native status of parasites can be difficult to determine unless observations are made at the time of introduction or comprehensive base-line data exists from extensive monitoring of

fisheries (Gibson, 1993; Kennedy, 1994; Peeler *et al.*, 2006a). Prior to 1994, routine parasitological examinations of fish did not involve inspection of the nares (Chubb, 1979; Kennedy, 1993, 1994; Environment Agency, unpublished). Consequently, the detection of *P. longidigitus* in over 40 fisheries within the first year of being recorded suggests that the parasite was present within the British Isles long before 1994. Although it is believed that *P. longidigitus* is a non-native addition to the parasite fauna of the British Isles (Environment Agency, 1999; Hawkins, 2001) this is difficult to substantiate. However, it is clear that the parasite is now widely distributed within England and Wales and continues to extend its geographical range with fish stocking activity. The extensive distribution of *P. longidigitus* raises question over the value, effectiveness and sustainability of future control measures.

Most metazoan parasites, including ergasilids are introduced and disseminated by the movements of infected hosts (Kennedy, 1976; Fryer, 1993; Alston, 1994; Kennedy, 1994). However, the presence of free-living stages provides potential for spread with contaminated water transfer (Fisheries Surveys, 1999). According to Kennedy (1993, 1994) *P. longidigitus* has many attributes of a successful coloniser. These include a direct life-cycle (Environment Agency, 1999), weak host specificity (Do, 1982; Hawkins, 2001), tolerance to a wide range of environmental conditions (Purasjoki & Fagerholm, 1987) and the ability to survive for periods in the absence of a host. The relatively small size of the parasite and obscure location within the nares are likely to have aided the spread of *P. longidigitus* (Hanek, 1967; Fryer, 1968; Ponyi & Molnar, 1968). Similarly, a paucity of published literature, absence of records detailing impact and lack of clinical signs of disease even during heavy parasite infections, do little to raise awareness of infections prior to fish stocking. With growing reservoirs of infection

within England and Wales, the potential for further dissemination of *P. longidigitus* remains high.

## 6.5. Summary and risk assessment

Current studies contribute significantly to the understanding of *P. longidigitus* in fisheries. Histopathological studies confirm damage to the olfactory epithelium, but suggest that the parasite is less pathogenic at the host level than many other ergasilids. The absence of clinical disease, condition loss or mortality in the current study, or any fisheries recorded since 1994 supports this observation. Despite limitations of the experimental studies, indications are that hosts from heavily infected fisheries maintain olfactory function and the capacity for normal reproductive behaviour. These observations, combined with records of seasonality suggest that *P. longidigitus* is unlikely to hinder successful reproduction of infected cyprinids. Anecdotal evidence from infected fisheries supports these assumptions. Finally, the widespread distribution of *P. longidigitus* questions whether control measures continue to be effective and sustainable. With respect to disease risk, there is little evidence to suggest *P. longidigitus* is a serious pathogen to fisheries. On the basis of current understanding, this information makes it difficult to justify the restrictions placed upon a growing number of fisheries. However, due to the complexities of the factors affecting fish recruitment, and the importance of fish reproduction upon fishery performance, an element of caution must be maintained. In particular, it is recognised that for certain fish species and types of fishery understanding of the effects of *P. longidigitus* remain very poor. A risk analysis for *P. longidigitus* is given in Figs 6.28 and 6.29.

Fig. 6.28. Risk analysis process for evaluating the probability of *P. longidigitus* causing undesirable economic and ecological impacts to fisheries. This is based upon available published literature and information gained during the current study.

| Parasite species being assessed – *Paraergasilus longidigitus* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact**<br><br>1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise ecologically important fisheries? | | | 0.5 |
| **B Economic impact**<br><br>1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise economically important fisheries? | | | 0.5 |
| **A Ecological impact risk analysis**<br>What is the risk of the parasite having an adverse ecological effect on fisheries? | **= 0.1 x 0.1 x 05 = 0.05** | | |
| **B Economic impact risk analysis**<br>What is the risk of the parasite having an adverse economic effect on fisheries? | **= 01 x 0.1 x 0.5 = 0.05** | | |

Fig. 6.29. Risk analysis matrix to prioritise the potential impact of *P. longidigitus* upon the ecological and economic development of fisheries. '

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low** (0.001 – 0.005) | **Medium** (0.009 – 0.027) | **High** (0.045 – 0.125) |
| **Economic Risk** | **Low** (0.001 – 0.005) | **Low** *P. longidigitus* | Medium | High |
| | **Medium** (0.009 – 0.027) | Medium | Medium | High |
| | **High** (0.045 – 0.125) | High | High | High |

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## Chapter 7. *Philometroides sanguinea* infections in crucian carp fisheries

### 7.1. Introduction

The crucian carp *Carassius carassius* (L.) is a native freshwater cyprinid of the British Isles (Bolton *et al.*, 1998; Wheeler, 2000). In the last decade, growing demand for crucian carp to stock stillwater fisheries has lead to increasing numbers of fish movements and expanding aquaculture production (LFMD, 2006). Crucian carp populations predominate in central and eastern England, although they have been recorded from most parts of the British Isles as a result of fishery stocking (Wheeler, 1978, 2000; Maitland, 2004). Despite widespread movements of fish to support angling interests, and tolerance of the species to varied environmental conditions, the distribution of crucian carp remains relatively discrete in England (Wheeler, 1977, 2000). In recent years, concern has grown over a perceived decline in the number of fisheries containing thriving crucian carp populations (Bolton *et al.*, 1998; Wheeler, 2000; Häenfling *et al.*, 2005). A recent review of freshwater fishery development in England and Wales suggested that the crucian carp is becoming an increasingly threatened freshwater fish species (Environment Agency, 2004). Reasons given for this apparent decline include hybridisation, competition, loss of preferred habitat and disease (Couchman, 1997; Bolton *et al.*, 1998; Häenfling *et al.*, 2005). This has emphasised the need to protect true crucian carp fisheries (Häenfling *et al.*, 2005) and minimise potentially harmful impacts to native populations (Bolton *et al.*, 1998; Wheeler, 2000).

*Philometroides sanguinea* is a parasitic nematode that infects fish of the genus *Carassius*. The parasite was first discovered in the British Isles in 1982 from a farm

pond in Essex (Moore & Chubb, *pers. comm.*; Williams *et al.,* 2004). Literature suggests that philometrid nematodes can be harmful parasites of fish (Bauer *et al.,* 1969; Paperna & Zwerner, 1976; Moravec & Dykova, 1978; Moravec, 1994; Kaall *et al.,* 2001; Wang, 2002; Moravec *et al.,* 2003; Moravec, 2004a, 2006). Members of the genus *Philometroides,* including *P. sanguinea,* have been described as highly pathogenic and held responsible for disease in both wild and cultured fish populations (Vasilkov 1967; Vismanis & Nikulina, 1968; Uhazy, 1978; Schäperclaus, 1991; Yu *et al.,* 1993; Moravec, 1994; Moravec *et al.,* 2003; Moravec & Cervinka, 2005). Observations of farmed and experimentally infected populations indicate that juvenile crucian carp are particularly susceptible to *P. sanguinea* and die during heavy worm burdens (Moravec, 1994). However, observations of infections in wild fish populations are generally scant and poorly understood.

The detection of *P. sanguinea* within England has raised concern from fishery scientists who believe the parasite may pose an additional threat to an already vulnerable species. Similar concern was reported in the Czech Republic, where detection of *Philometroides* sp. in imported carp lead to a cull of all fish in efforts to eradicate infection and protect native stocks (Moravec & Cervinka, 2005). Despite these concerns, information on *P. sanguinea* is generally fragmented and provides insufficient detail on which to base a reliable assessment of disease risk. To date, the pathology of the parasite has not been described. The seasonality, distribution, epidemiology and pathogenicity of *P. sanguinea* have not been studied within the British Isles. During a recent review of drancunculoid nematodes, Moravec (2004a) stated that studies are urgently required to progress understanding of these parasitic worms.

# Chapter 7.

This chapter describes studies to improve understanding of the pathology and epidemiology of *P. sanguinea* within crucian carp fisheries in England. Literature was collated and reviewed to establish evidence of impact and pathogenicity. The pathology caused by adult female parasites is described for the first time. Observations of life-cycle development were made to assess the importance of seasonality upon parasite development, host impact and parasite detection. Population studies were conducted to identify effects of the parasite upon the growth and condition of crucian carp. Particular attention was given to infections within 0+ hosts, in efforts to evaluate published records of impact within this year class.

The specific aims of these investigations were;

1. To collate and review published literature on *P. sanguinea* to assess current understanding of the parasite and evidence of disease in fisheries.

2. To describe the pathology caused by female *P. sanguinea* within infected crucian carp.

3. To improve understanding of life-cycle development and seasonality within the British Isles, with emphasis on host impact and parasite detection.

4. To investigate the prevalence, intensity and distribution of *P. sanguinea* within infected crucian carp populations.

5. To evaluate the effects of female *P. sanguinea* upon growth, condition and survival of 0+ crucian carp in order to evaluate pathogenicity within this year class.

## 7.2. Materials and methods

### 7.2.1. Literature reviews

Methodologies were consistent with those described in Chapter 3.

### 7.2.2. Fish sample collection

Crucian carp populations infected with *P. sanguinea* were identified from a fish health database held at the Environment Agency's, National Fisheries Laboratory, Brampton. Contact details for each water were obtained from an internal fish movement database (LFMD, 2006) or through Environment Agency local area officers. Due to the focus on crucian carp fisheries, crucian carp were the primary host sampled during the study. However, where present, goldfish and carp hybrids were also retained for examination. All fish were captured by seine netting (Fig. 7.1) or electro-fishing and transported live to holding facilities at Brampton. Fish were maintained in either 25 l or 200 l fibreglass tanks fed with bore-hole water.

### 7.2.3. Parasitological examinations

Crucian carp were killed by lethal anaesthesia (immersion in 5% w/v benzocaine solution), measured, weighed and examined for any visual abnormalities or signs of clinical disease. Scales were taken from the flank of each fish to confirm age. The fins were examined under a low-power dissecting microscope for presence of mature female parasites. The size, position and location of nematodes within the fins were recorded. Greatest emphasis was placed upon the presence of female worms, due to their large size and active migrations within the host. However, internal parasitological examinations were conducted on a small number of fish to establish the occurrence and

location of the different life stages of the parasite. These included microscopic examination of the liver, kidney, spleen, swim-bladder, caudal musculature and intestinal tract. The condition factor (K) of both infected and uninfected fish was calculated by the equation K= 100 x W/L$^3$, (where W = fish weight in grams, L = fork length in centimetres) as stated by Jobling (1995).

## 7.2.4. Histopathology studies and scanning electron microscopy

Methods used for histopathological investigations were as described in Chapter 4.

## 7.2.5. Life-cycle development and population studies

Observations of seasonal development were made from samples obtained from infected fisheries at different times of the year, in particular autumn and spring. Parasitological examinations were conducted to establish life stage development and reproductive status of parasites from infected hosts.

Population studies were conducted from samples captured with a 25m fry seine net to ensure capture of all size groups of fish.  In a single case, an entire population of crucian carp was obtained from a farm pond in Devon. This comprised three distinct size groups of fish (small <60mm, medium 90-150mm and large 180-300mm). This enabled observations of infections within different size and age classes of fish. Due to the large number of fish submitted from this water (~5,000 fish), sub-sampling protocols were employed to gain representative samples of the population. This was achieved through use of the internet based epidemiological software Win Episcope 2.0 (http://www.clive.ed.ac.uk/winepiscope).

A small number of crucian carp (n=10) were maintained in a 1000 l external holding tank during spring to allow observations of the timing of larval dispersal by gravid female worms. Water temperature was recorded weekly and observations made to determine presence or absence of female parasites within the fins.

### 7.2.6. Distribution of *P. sanguinea* in England and Wales

Methods used for the collation of *P. sanguinea* distribution records were consistent with those detailed in Chapter 4.

## 7.3. Results

### 7.3.1. Literature review of *P. sanguinea*

- **Description and taxonomy**

*Philometroides sanguinea* is a parasitic nematode of the family Philometridae (Moravec, 1994, 2004a; Anderson, 2000). The parasite was first described by Rudolphi (1819) under the name *Filaria sanguinea,* based upon specimens found in the 'prussian' or 'gibel' carp *C. auratus gibelio* from Berlin. Literature on *P. sanguinea* is generally scarce and fragmented. Many early records of the parasite were plagued by morphological and taxonomic inaccuracies, leading to considerable confusion and numerous misidentifications with other philometrid parasites (Vismanis & Yukhimenko, 1974; Moravec, 1971, 1994, 2004b). Many published records of *P. sanguinea* are also confined to Russian and Asian literature, raising difficulties with translation and interpretation of findings. Work by Moravec (1971, 1994, 2006) has helped overcome some of these limitations and greatly improved understanding of the parasite. In particular, the comprehensive review of European parasitic nematodes by Moravec (1994), provides a valuable summary of literature detailing the parasite's morphology, ecology and taxonomy.

Morphological descriptions of *P. sanguinea* have been given by Nybelin (1928, 1931) and Moravec (1971, 1994). The morphology of female worms is far better understood than that of males. This is likely to be due to the small size and resultant difficulty in obtaining and studying male specimens, a problem common to many dracunculoid nematodes (Moravec, 2004a; Gollety *et al.,* 2005). Male and female *P. sanguinea* show considerable sexual dimorphism (Moravec, 1994, 2004a). Adult female worms are relatively large, elongated, red-brown, thread-like nematodes which may attain a length

of 40-50mm (Moravec, 1971; Chubb, 1980; Schäperclaus, 1991). Female worms have a cylindrical body covered in numerous papillae-like bosses, which are a morphological characteristic of the genus *Philometroides* (Moravec, 1971, 1994). Male worms are almost transparent, lack external armature and rarely exceed 3mm in length (Moravec, 1971, 1994; Schäperclaus, 1991). Larval stages of *P. sanguinea* within the copepod host have been described by Nybelin (1931).

At the time of writing, the genus *Philometroides* comprised 14 species. However, as the systematics of the Dracunculoidea requires attention, it is unclear if these are all valid species (Moravec, 2004a, 2006). *P. sanguinea* and *P. cyprini* Ishii, 1931 (syn. *P. lu*sii, *P .lusiana*) are the only representatives of the genus recorded from freshwater fish of Europe, the latter being a parasite of common carp *C. carpio* (Moravec, 1994; Anderson, 2000). *P. sanguinea* is the only species to have been recorded within freshwater fisheries of the British Isles (Kirk, 2000a).

## • Host susceptibility

Literature indicates that *P. sanguinea* only infects fish of the genus *Carassius*. According to Moravec (1971, 1994) early records detailing infections in other host species (Ratz, 1897; Kastak, 1956; Meszaros, 1967) are considered invalid due to erroneous species descriptions. Accepting this, crucian carp *C. carassius,* goldfish *C. auratus* and the wild strain of gibel carp *C. auratus gibelio* are the only documented hosts of *P. sanguinea.* However, it is unclear if the 'gibel' of 'prussian' carp, recorded as the type host for the parasite, is actually a species, sub-species, strain, morphotype of goldfish, or even a carp hybrid (Bolton *et al.,* 1998; Häenfling *et al.,* 2005).

Whilst the role of crucian carp as a host for *P. sanguinea* is beyond dispute (Moravec, 1994; Williams *et al.,* 2004) susceptibility of goldfish requires confirmation. The international trade of ornamental goldfish has been held responsible for the dissemination of *P. sanguinea* to some regions of the world (Hoffman, 1970; Hoffman & Schubert, 1984; Moravec, 1994). However, there is a lack of literature that either describes or depicts parasites within this host. Vismanis & Nikulina (1968) suggest that *P. sanguinea* is a specific parasite of crucian carp and that records in other species are incorrect. This confusion may stem from long standing problems in the identification of crucian carp and its associated hybrids throughout many parts of the world (Bolton *et al.,* 1998, Wheeler *pers. comm.*).

Within the British Isles, records of *P. sanguinea* have been restricted solely to crucian carp (Kirk, 2000a; Environment Agency, unpublished; Moore *pers. comm.*). Consequently, any impacts caused by *P. sanguinea* on freshwater fisheries in England and Wales are likely to be restricted to crucian carp populations. Whilst goldfish hold little value to fisheries, the role of this fish in the potential spread of the parasite requires further consideration.

• **Life-cycle development**

The life-cycle development of *P. sanguinea* has received considerable attention. As with many species of *Philometroides,* understanding of parasite development has come mainly from observations of experimental infections (Wierzbicki, 1958, Yashchuk, 1971, 1974a, 1974b, 1975; Nakajima & Egusa, 1977a,b; Wang, 2002). However, many of these records are confined to Russian and Japanese literature. Whilst detailed experimental studies are essential for the determination of nematode migrations in fish

(Adams, 1969; Anderson, 2000) these do not always reflect the pattern of infection in the wild animals or account for influences of seasonality. Field studies have focused on parasite development within the copepod intermediate host (Yashchuk, 1974a). The only study to have documented the seasonality and life-cycle development of *P. sanguinea* in the wild is by Wierzbicki, (1960) from a stillwater in Poland. This work indicates that development, maturation and reproduction of the parasite are strictly seasonal in temperate climates (Wierzbicki, 1960; Chubb, 1980). The seasonal development of *P. sanguinea* within the British Isles requires confirmation in order to highlight host impacts, ensure effective detection and maximise effectiveness of controls.

The life-cycle of *P. sanguinea* appears to be consistent with most philometrid parasites and involves a free-living copepod intermediate host (Nakajima & Egusa, 1977c; Moravec, 1994; Anderson, 2000; Wang, 2002; Moravec, 2004b; Moravec & Cervinka, 2005). Crucian carp are known to become infected through ingestion of infected copepods. Once consumed, juvenile nematodes burrow through the intestinal wall into the body cavity, migrating to the vicinity of the kidneys, swim-bladder and gonads (Wierzbicki, 1958). After copulation, female worms migrate through the musculature into the fins where they increase in size considerably. According to Wierzbicki (1960), gravid females appear in the fins during late summer, with intensities increasing during autumn and winter. Once located within the fins, adult females remain almost static until late spring when they shed their larvae. Male parasites remain within the body cavity and do not undergo migration. Gravid female worms liberate their larvae into the water through the process of 'functional bursting' (Moravec, 1994).

Laboratory studies have focused on parasite development within crucian carp (Wierzbicki, 1958; Ouk & Chun, 1973), larval dispersal by gravid female parasites (Nakajima & Egusa, 1977b), survival of free-living larvae (Yashchuk & Vasilkov, 1970) and larval development within the copepod host (Nakajima & Egusa, 1977a).

• **Distribution and dissemination**

*P. sanguinea* was first recorded from Germany in the early 19th century. Due to taxonomic inconsistencies, early geographical records of the parasite are difficult to confirm. However, *P. sanguinea* is considered a palaearctic species (Moravec, 1994) and is believed to be widespread in much of Europe and Asia. The parasite has been documented from Sweden, Germany, Poland, Czech Republic, Hungary, the USSR and North America (Moravec, 1971, 1994; Bauer & Hoffman, 1976).

*P. sanguinea* was first recorded in the British Isles in 1982 from a stillwater population of crucian carp located in the south-east of England (Moore *pers. comm.* Williams *et al.*, 2004). The parasite is considered by the Environment Agency to be a non-native species. However, this is difficult to confirm with any certainty. It therefore remains possible that the parasite is a native species, which has only been detected as a result of increasing numbers of fish movements.

Assuming that *P. sanguinea* is a non-native species to England, the source and route of introduction remain unclear. Evidence of historic helminth invasions suggests that direct transfer with infected fish is the most likely means of translocation (Kennedy, 1993; Moravec, 1994). A number of authors have attributed the spread of *P. sanguinea* to movements of infected goldfish with the ornamental industry (Moravec, 1994).

However, the importation of infected carp for the purpose of fishery stocking remains an additional avenue of introduction. The spread of parasitic worms, including philometrid nematodes may also be achieved through dispersal of free-living stages or intermediate hosts (Yashchuk, 1974a; Chubb & Yeomans, 1995; Moravec, 2006).

- **Epidemiology**

Little information exists on the epidemiology, population ecology, seasonality, and distribution of any parasites within the genus *Philometroides* (Wang, 2002). Yashchuk (1974a) studied the dynamics of larval infections of *P. sanguinea* within wild copepod populations. This involved the capture and examination of 64 thousand copepods from two ponds in the former USSR, where natural prevalence and intensities of infection were established. Very little attention has been given to the epidemiology of parasites within the definitive host. Furthermore, no information exists on the prevalence, intensity and distribution of infections within crucian carp populations in the British Isles. This clearly represents an important area for further investigation.

- **Pathology and impacts to fish populations**

According to published literature, parasites of the genus *Philometroides* can be responsible for losses in both wild and cultured fish populations (Schäperclaus, 1991; Moravec, 1994, 2006). Literature suggests that *P. sanguinea* is a highly pathogenic nematode, particularly to small crucian carp (Wierzbicki, 1958, 1960; Vasilkov, 1983; Morvaec, 1994). Records of impact from *P. sanguinea* include debilitation and mortality of wild, farmed and experimentally infected hosts (Vismanis & Nikulina, 1968; Vasilkov, 1983; Moravec, 1994).

According to Vasilkov (1983), philometroidosis was responsible for serious losses in crucian carp fry in the Ukraine, where prevalence of infection reached 100% of the population. The disease occurs either in acute form, which results from newly acquired infections in 6-8week old fry, or as a chronic infection, which debilitates infected hosts during migration and development of adult female parasites. Vismanis and Nikulina (1968) reported a mass mortality of crucian carp in Russia in 1966 as a result of 'heavy parasite infections'. Heavy infections in wild fish populations have also been recorded in Poland (Wierzbicki, 1960), Czechoslovakia (Cakay, 1957) and Hungary (Molnar, 1966). These effects are supported by experimental observations, which indicate that juvenile crucian carp are unable to withstand internal migrations of *P. sanguinea* (Wierzbicki, 1958, 1960; Moravec, 1994). Although these published accounts suggest that *P. sanguinea* is an important pathogen of crucian carp, most records are brief and contain little detail on which to base a robust assessment of disease potential. Furthermore, the pathology of the parasite has not been described, limiting understanding of pathogenicity. This represents an essential area for further investigation if the impact of the parasite to crucian carp fisheries is to be evaluated.

- **Management and control**

Due to the economic importance of philometroidosis in the USSR and Asia (Moravec, 1994), efforts have been made to control losses from infection (Vasilkov *et al.,* 1974; Schäperclaus, 1991; Borisova *et al.,* 1987). Nakajima and Egusa (1977b) studied the effects of temperature, drying, freezing and irradiation upon activity and survival of larval stages of *P. sanguinea* (recorded as *P. carassii*) obtained from infected crucian carp. The larvicidal effects of 12 chemotherapeutics were also examined.

Following the introduction of *Philometroides* sp. to Czech Republic with imports of infected common carp, the consignment was destroyed and facilities disinfected based upon potential disease threats (Moravec & Cervinka, 2005). With respect to fisheries, Schäperclaus (1991) suggested that *P. cyprini* should be controlled through restriction of fish stocking, destruction of diseased fish and disinfection of affected waters. The use of chemotherapeutics for the control of parasite infections in wild fisheries is problematic. There are no licensed drugs for the treatment of nematode infections in fish within the UK (Veterinary Medicines Directorate, 2006). The only recognised means of parasite eradication from infected fisheries is through draining, drying and liming (Environment Agency, 1999).



Fig 7.1 Netting a still-water fishery for crucian carp infected with *P. sanguinea.*

## 7.3.2. Pathological changes associated with infections of adult female *P. sanguinea* in crucian carp.

• **General observations and attachment characteristics.**

Adult female *P. sanguinea* were primarily located between the fin rays of infected crucian carp (Figs 7.2, 7.3). The caudal fin was most commonly infected, although parasites were occasionally found within the pectoral fin, anal fin and dorsal fin (Fig. 7.4). Adult female nematodes removed from the fins for identification measured between 14 and 54mm in length. In the majority of infections, parasites were positioned in a U-shape, with the head and tail extending toward the fin tip and the parasites mid-body into the caudal musculature (Fig. 7.3). This often gave the appearance of two worms within the fin, rather than a single individual. Female nematodes normally utilised different positions within the fins, although parasites occasionally shared the same space between the fin rays (Fig. 7.3). Once established within the fins, female parasites remained relatively inactive, although occasional extension and retraction of the head and tail regions were observed.
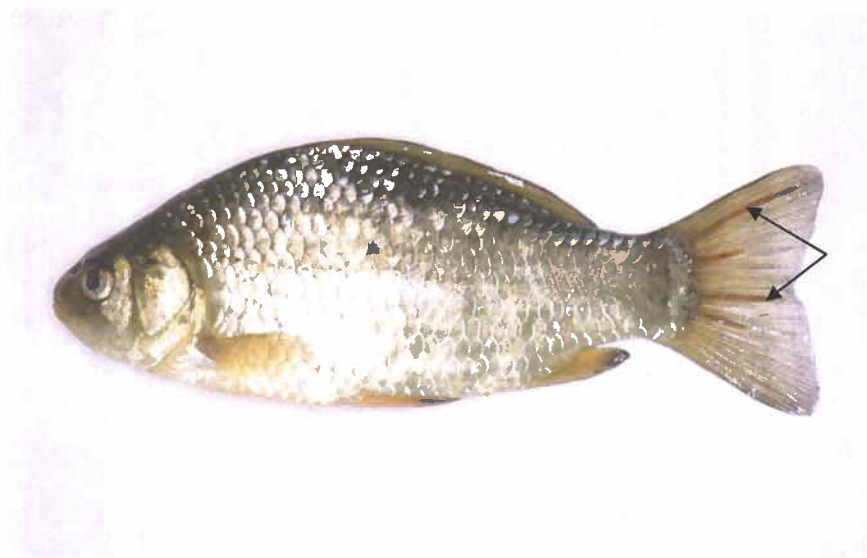


Fig 7.2. A crucian carp infected with two female *P. sanguinea,* clearly showing the red coloured parasites present between the fin rays of the tail (arrows).

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Fig 7.3. Three gravid female *P. sanguinea* within the tail of crucian carp. During most infections the mid-body of each parasite led within the caudal musculature whilst the head and tail extended toward the edge of the fin. Adjacent parasites occasionally shared the same space between the fin rays (*).
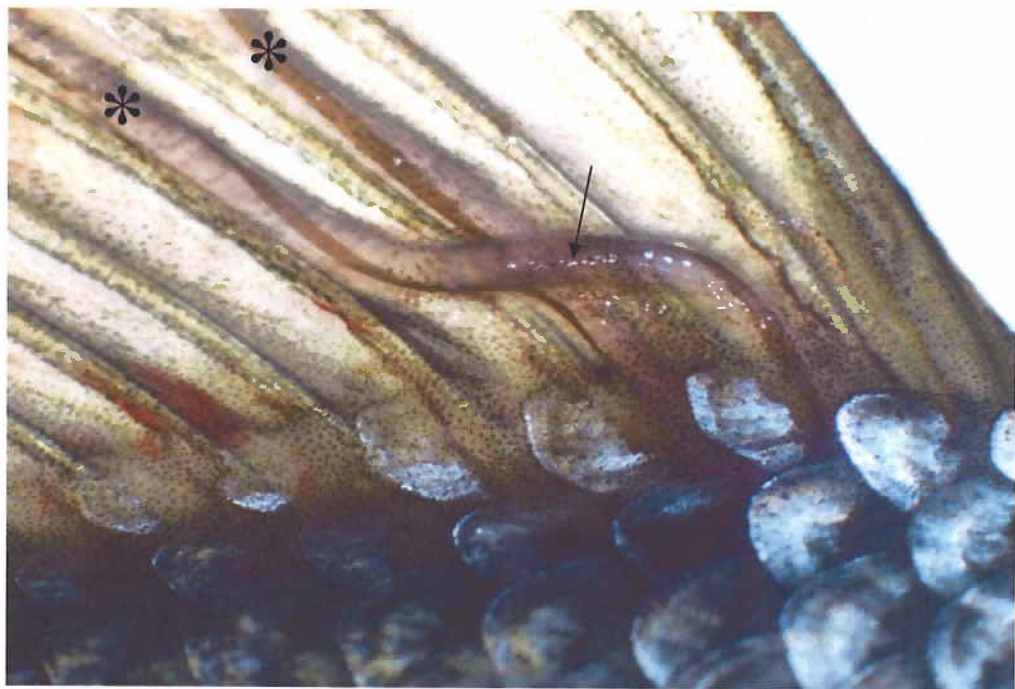


Fig 7.4. A single female *P. sanguinea* established within the dorsal fin of a crucian carp. The head and tail (*) are positioned towards the fin tip whilst the body, which overlaps itself (arrow) extends into the dorsal musculature.

- **Gross pathological changes**

Crucian carp infected with *P. sanguinea* did not show any serious signs of clinical disease or obvious indications of debilitation. The movement of parasites within the fins occasionally caused a build-up of opaque material around the head and tail of the worm (Fig. 7.5). In a small number of juvenile hosts (<70mm), worms located in the dorsal fin (Fig. 7.6) and caudal fin (Fig. 7.7) resulted in a raised lump within the adjacent tissues. However, despite these noticeable swellings, the skin was never broken until the point of larval dispersal (Figs. 7.8, 7.9). The process of larval dispersal involved emergence of the female worm into the water, followed by rupture and death. Deflated parasites trailed behind the host (Fig. 7.8). A number of fish examined from infected fisheries exhibited a range of fin and tail deformities, although these could not be attributed directly to a history of *P. sanguinea* infection.
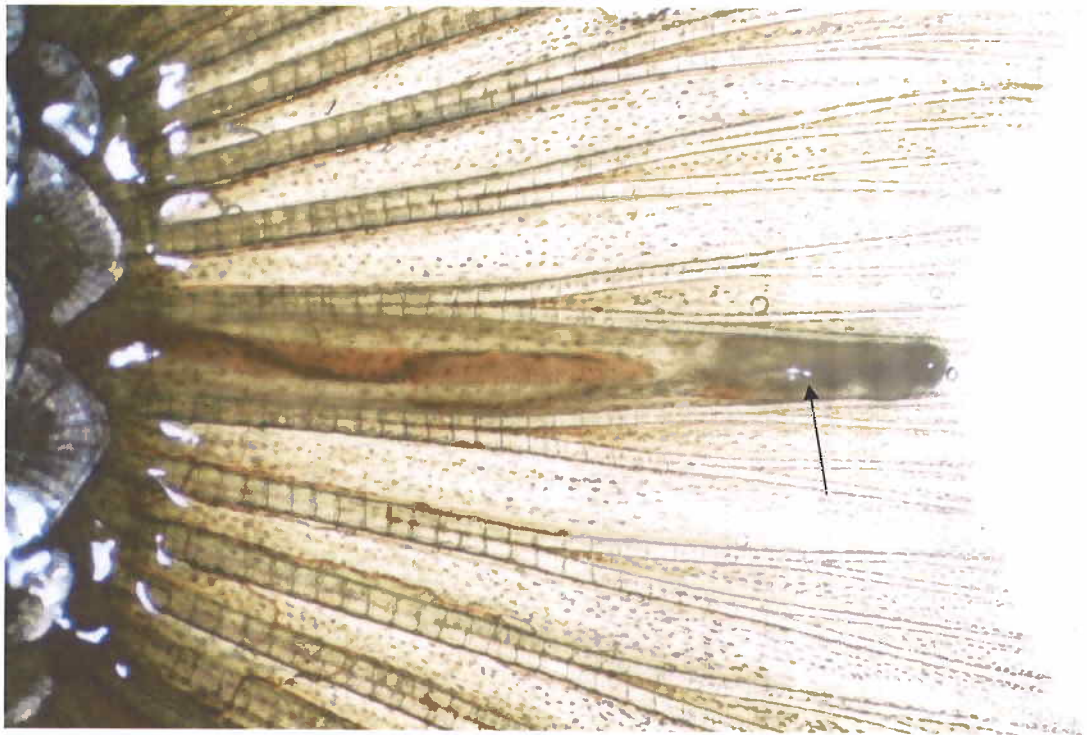


Fig 7.5. Accumulation of cloudy material (arrow) between the fin ray adjacent to a single female *P. sanguinea*.
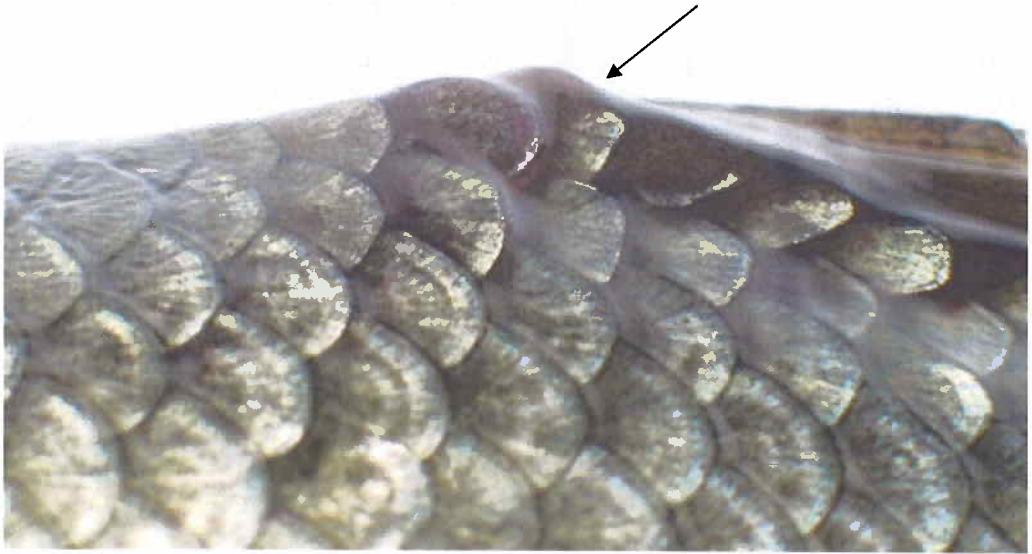
Fig. 7.6. A single parasite located at the base of the dorsal fin of juvenile crucian carp. The presence of the nematode resulted in a pronounced lump within this region (arrow), and distortion of the dorsal fin.



Fig. 7.7. Caudal swelling (arrow) resulting from penetration of a single *P. sanguinea* into the base of the tail of a juvenile crucian carp.

Fig. 7.8. A ruptured female parasite (arrow) trailing from the tail following larval dispersal.



Fig. 7.9. SEM of a spent female *P. sanguinea* (*) following the process of larval dispersal. The tunnel in which the parasite developed can be clearly seen (**).

- **Histopathological changes caused by** *P. sanguinea* **to the fins of crucian carp**

Development of female parasites within the fins led to expansion, thinning and distortion of the fin tissue (Fig. 7.10 – 7.12). This was accentuated when more than one worm established in the same position, leading to displacement of the fin rays (Fig. 7.12). In a number of infected fish, blood vessels were displaced by the presence of the parasite, although these did not appear damaged. Within the tail, parasites were usually located between the fin rays, although individuals occasionally crossed between the paired fin-ray elements. Up to three worms were observed within the same inter-ray space, causing marked distention and pronounced swelling of the fin (Fig. 7.12). The skin was noticeably thinned within these regions. Expansion of gravid worms between the fin rays led to compression and exfoliation of cells, which included numbers of macrophages and lymphocytes. The cuticular bosses covering the parasite's cuticle often led to discrete indentations and compression of surrounding tissues (Fig. 7.11).

Female parasites located within the dorsal and anal fin caused deformity of the fins and displacement of fin rays (Fig. 7.13). Parasites within very small hosts caused formation of raised nodules within this region. The presence of parasites within the fins, particularly at the fin bases, led to localised skeletal muscle degeneration (Fig. 7.14). Muscle tissue was often displaced by the presence of the nematode and replaced by loose connective tissue (Fig. 7.14). This connective tissue commonly surrounded the parasites and was interspersed with capillaries and inflammatory cells. Small accumulations of basophilic material within these regions appeared like bacteria. Parasites located close to the skin surface, or to one side of the fin ray elements, caused pronounced swelling of the dorsal and caudal regions of infected hosts (Fig. 7.15). The

epidermis within these regions occasionally appeared hyperplastic, partly surrounding the parasite (Fig. 7.15). These observations were often accompanied by an influx of inflammatory cells within the epidermis. The severity of these changes was influenced strongly by size of the host. Infections in small hosts caused marked distortion and displacement of tissues, whilst parasites of large crucian carp resulted in relatively minor tissue damage.

Inflammatory changes caused by *P. sanguinea* varied in severity. Initial migration of parasites into the fins provoked a pronounced inflammatory response comprising masses of eosinophils and neutrophils. This reaction was often concentrated along the sides of the parasite (Fig. 7.17) and involved migration of inflammatory cells from blood vessels towards the site of infection. Tissues surrounding the parasites showed localised signs of oedema, hyperplasia, congestion, and compression, although these changes were generally mild. Once established within the tail, the presence of worms led to condensation of tissues around the sides of the parasite, forming a tunnel of connective tissue (Fig. 7.17). In a small number of sections, this appeared to partially encapsulate the nematode within a membrane of host tissue, the thickness of which increased with duration of infection (estimated by time of year) (Fig. 7.18). Fluid, possibly caused by parasite secretions, was often observed between the worm and the surrounding layer of host tissue. This was seen as an eosinophilic layer around the parasite, which often contained inflammatory cells and exfoliated host cells (Fig. 7.19).

The emergence of gravid female parasites from the fin during larval dispersal caused mechanical damage to the fin tissues, and breached integrity of the skin. This reproductive behaviour lead to a noticeable lesion in the skin surface with an influx of

inflammatory cells around the parasite (Fig. 7.20). The presence of large numbers of granular cells, eosinophils, neutrophils and aggregations of lymphoid cells (Fig. 7.20) represented a marked immune response by the host. Larval dispersal resulted in rapid shrinkage of the female parasite and collapse of the cuticle within the fin (Fig. 7.21). In such cases, large numbers of inflammatory cells as well as small numbers of blood cells were recorded adjacent to the parasite's cuticle. In heavy parasite infections, inflammation extended beyond the direct site of parasite attachment with increased numbers of inflammatory cells extending throughout the fin. Examination of fin tissue after parasite emergence, revealed hyperplasia of the epidermis (Fig. 7.22). Necrotic cells were dispersed throughout this tissue, as well as a central mass of connective tissue in the area vacated by the parasite (Fig. 7.22).

Fig. 7.10. A single *P. longidigitus* within the tail of crucian carp. Anterior (*) and posterior (**) sections of the parasite can be seen in adjacent fin ray-spaces. Infections within relatively large hosts resulted in only mild fin distension.   Scale bar = 200μm



Fig. 7.11. A gravid female nematode (*) within the fin of a juvenile host. Presence of the parasite within small fish resulted in pronounced distension of the fin and compression of epithelium (arrow). Scale bar = 200μm



Fig. 7.12. Occurrence of three parasite sections (*) within the same region of the fin resulting in pronounced swelling and distortion. Scale bar = 200μm

Fig. 7.13. Section showing three gravid *P. sanguinea* (*) within the dorsal fin of crucian carp. Infection with these nematodes has caused displacement of fin rays. In some regions, the parasite is surrounded by loose connective tissue. Scale bar =



Fig. 7.14. TS of dorsal fin, showing presence of two worms between adjacent fin rays. Displacement and degeneration of skeletal muscle can be seen within this region (*) as well as disruption and compression of connective tissue. Scale bar = 80μm

Fig. 7.15. Pronounced swelling of caudal region of crucian carp. Presence of a single *P. sanguinea* (*) has caused distortion of fin rays. Scales have become partially displaced and the hyperplastic epidermis (arrow) has partially surrounded the parasite. Scale bar = 150μm



Fig. 7.16. TS of caudal region of crucian carp with sections of three *P. sanguinea*. Parasites located outside (**) of the fin rays have caused pronounced swelling within this region. Scale bar = 150μm

Fig. 7.17. Infiltration of inflammatory cells (*) accumulating around a newly established parasite (P) in the tail. A layer of connective tissue can be seen along the sides of the nematode (arrows) represented the beginning of encapsulation. Scale bar = 80 μm



Fig. 7.18. TS of caudal region of crucian carp, showing a single parasite (P) led perpendicular to the fin rays. The cuticle of *P. sanguinea,* characterised by numerous bosses (arrow) has become surrounded in a tunnel of loose connective tissue (*). Scale bar = 80μm
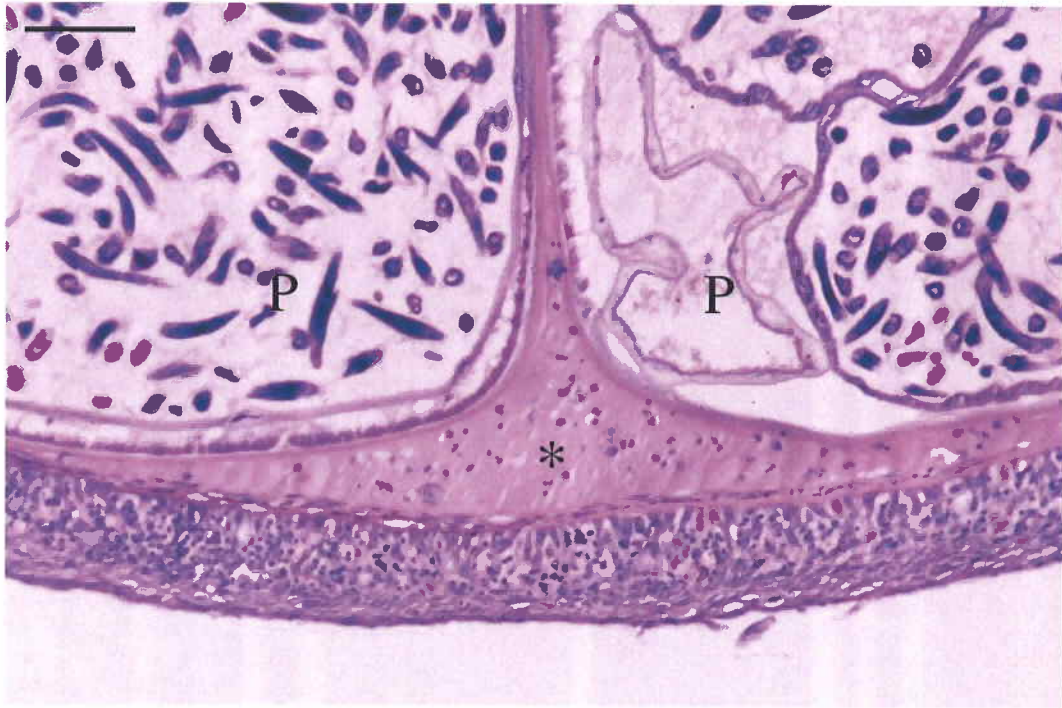
Fig. 7.19. Section of *P. sanguinea* in tail of crucian carp. Homogenous eosinophylic material (*), possibly caused by the release of parasite secretions was often recorded between parasite (P) and host tissue. This contained macrophages, lymphocytes and exfoliated host cells. Scale bar = 40μm
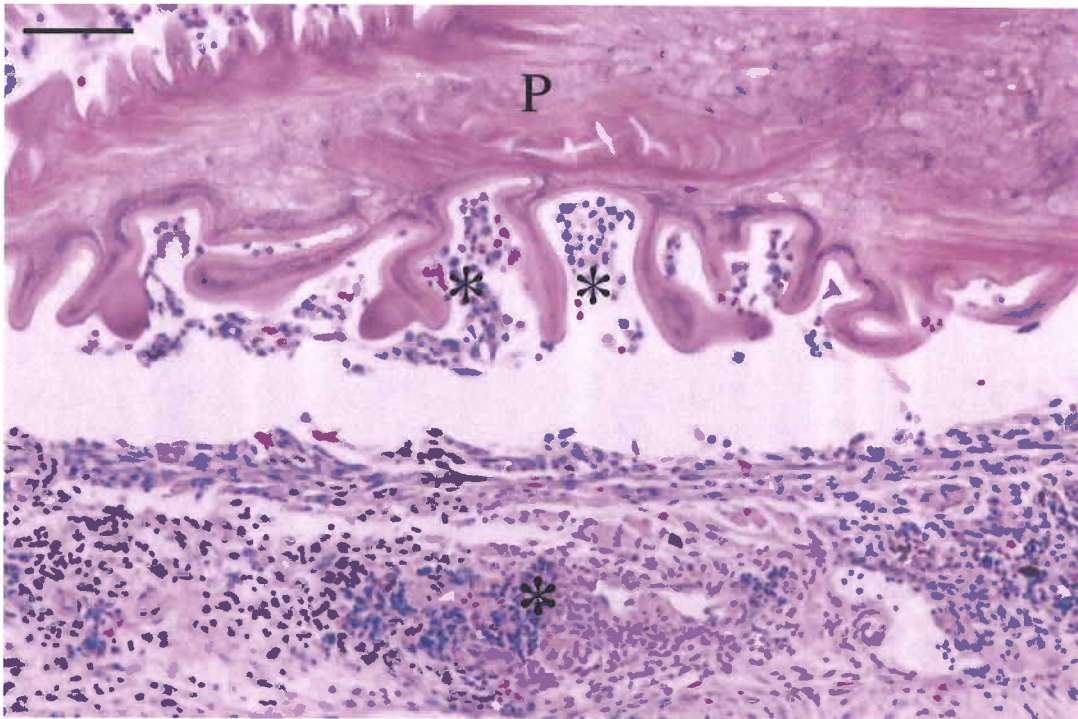


Fig. 7.20. Section of crucian carp tail following larval dispersal. The collapse of female parasites (P) led to rapid shrinkage of the parasite's cuticle. Inflammatory cells infiltrating the host tissues (*) can be seen surrounding the parasite Scale bar = 40μm

Fig. 7.21. TS of crucian carp tail following larval dispersal. The structure of the parasite (P) rapidly collapsed following this event. This was accompanied by a pronounced inflammatory response directed towards the parasite. Scale bar =100μm.
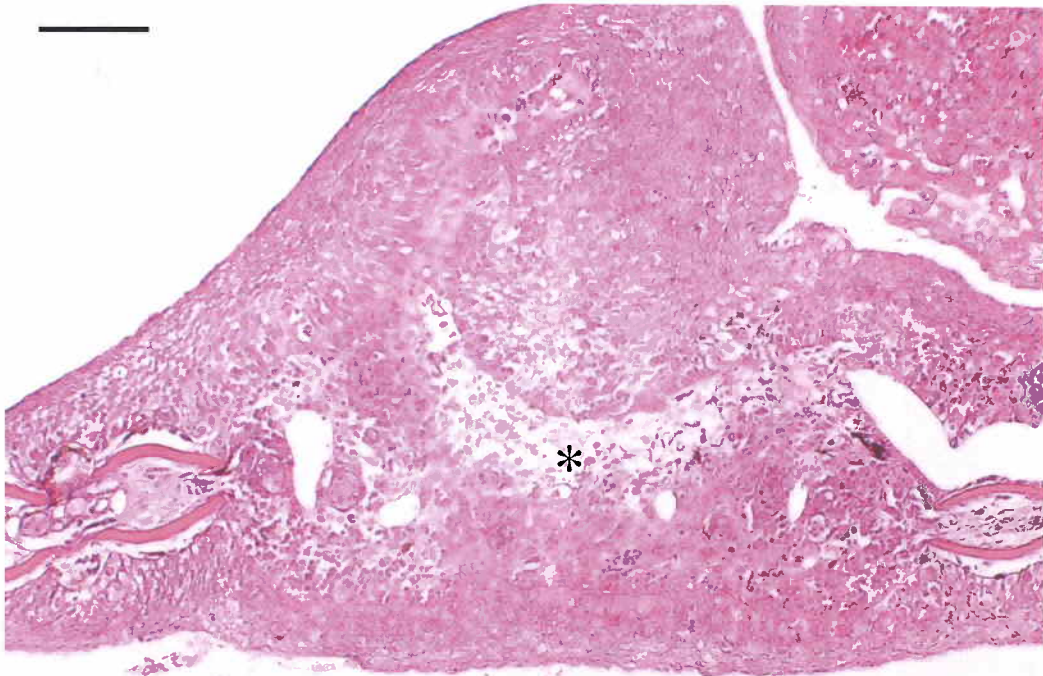


Fig. 7.22. TS of crucian carp caudal fin following process of larval dispersal. Hyperplasia of the tissues between the fin rays can be seen following emergence of the parasite. The collapsed connective tissue that once surrounded the worm can be seen within the centre of the fin (*). Scale bar = 80μm

**7.3.3 Epidemiology of *P. sanguinea* within infected crucian carp populations**

- **Prevalence, intensity and distribution of *P. sanguinea***

During October 2003 and May 2004, a total of 370 crucian were examined from 7 stillwaters in England. The number of fish examined from each water ranged between 14 and 155 fish (mean = 52.4), depending upon the number that could be caught and the number the fishery owners were willing to donate to the study. The waters sampled included 6 stillwater fisheries and a single fish farm. These were located in the south-east, south-west and midlands regions of England. The crucian carp captured during the study ranged from 38 mm to 318mm in length and were aged between 0+ and 5+. Despite efforts to include goldfish and carp hybrids within the sampling, only a single common carp x crucian carp hybrid was captured. This fish was from Halland Park Farm fishery.

Parasites were found in all 7 crucian carp populations examined (Table 7.1). The prevalence of *P. sanguinea* infections ranged between 3.4% (0 – 17.7%, 95% confidence range) and 21% (6.1-50%, 95% confidence range) and intensity of infection from 1 to 4 parasites (mean intensity 1 to 1.9 worms per host). For those waters in which there was sufficient variability in intensity, (>2 parasites) the distribution of *P. sanguinea* was shown to be over-dispersed within the crucian carp populations (variance/mean > 1). However, for waters where only 1 or 2 parasites were the maximum intensities recorded, it was not possible to establish this relationship. The frequency distribution of *P. sanguinea* within Oakside Fish Farm population is shown in Fig. 7.23.

Table 7.1. Records of adult female *P. sanguinea* in the fins of crucian carp from the waters examined during the study.

| Locality (Date sampled) | No of fish examined | Host range examined (mm) | Prevalence (%) (95% Conf) | Intensity range (mean) |
|---|---|---|---|---|
| Halland Park Farm Pond 2 (October 2003 | 69 | 36 – 128 | 12/69 (17.4%) (13.4 – 33) | 1-2 (1.1) |
| Cranleigh AC Water Surrey (October 2003) | 39 | 83-184 | 5/39 (12.8%) (5.2 – 26.7) | 1-2 (1.2) |
| Oakside Fish Farm (February 2004) | 155 | 38 – 308 | 15/155 (9.7%) (5.7 – 15.4) | 1-4 (1.9) |
| Eastfield Lake 1 Spilsby (April 2004) | 14 | 80 – 174 | 3/14 (21%) (6.1 – 50) | 1-2 (1.3) |
| Eastfield Lake 2 Spilsby (May 2004) | 30 | 114 – 191 | 1/30 (3.4%) (0 – 17.7) | 1 (1) |
| Eastfield Lake 3 Spilsby (May 2004) | 30 | 99 – 190 | 5/30 (17%) (6.8 – 34.8) | 1-2 (1.2) |
| Eastfield Lake 4 Spilsby (May 2004) | 30 | 114 – 179 | 3/30 (16.7%) (2.8 – 26.3) | 1 (1) |
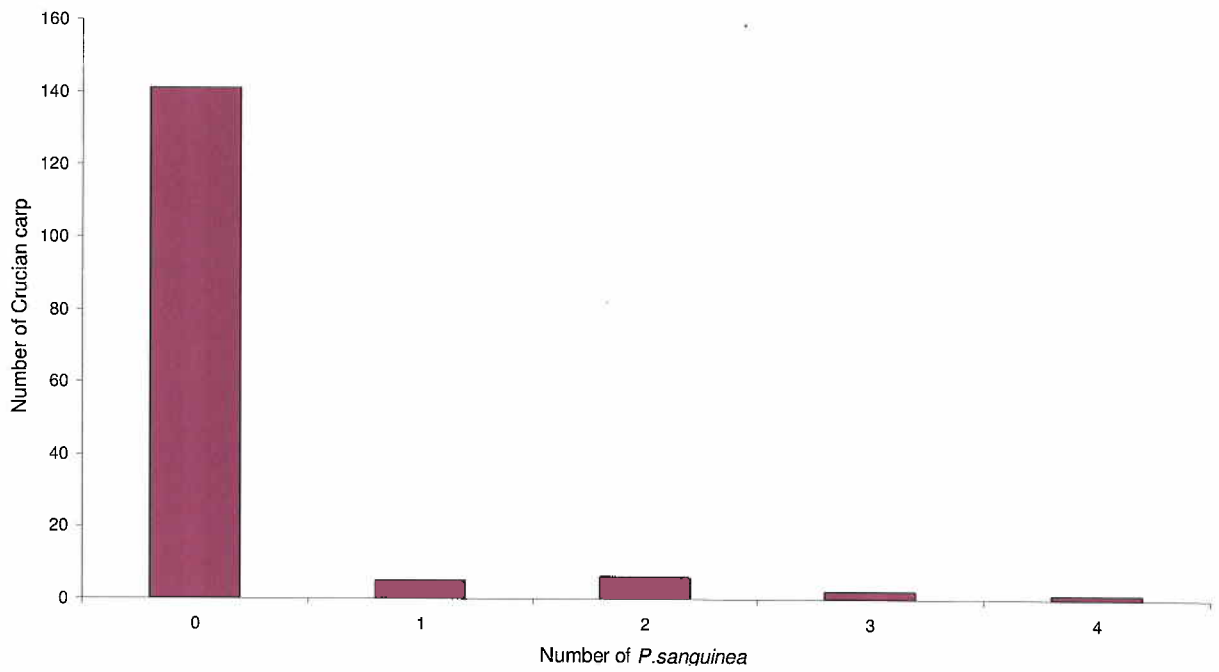
Fig. 7.23. Frequency distribution of *P. sanguinea* for crucian carp examined from Oakside Fish Farm.

- **Host susceptibility to *P. sanguinea***

During the study, the vast majority of *P. sanguinea* were recorded from crucian carp. No goldfish were obtained from any of the fisheries studied. However, examination of a single common carp x crucian carp hybrid, sampled from Halland Park Farm fishery revealed the presence of a gravid female parasite within the tail (Fig. 7.24). The absence of other fish species or hybrids from these studies prevented further assessment of host species susceptibility.



Fig. 7.24. A common carp x crucian carp hybrid infected with a single adult female *P. sanguinea* (arrow).

- **Infection relative to host size**

During the study, *P. sanguinea* were recorded in crucian carp ranging from 66 to 241mm in length. The mean length of infected crucian carp was 133mm. The heaviest parasite infection recorded during the study comprised of 4 female worms, from a crucian carp measuring 145mm in length. Although a general trend of increasing parasite intensity with host size was seen when grouping all crucian carp examined, low variability in intensity levels from most fisheries prevented accurate analysis of this relationship. Due to greater variation in parasite intensity (1-4 nematodes) at Oakside Fish Farm, attention was focused upon this sample. This data confirmed a trend of increasing parasite intensity with host size, centred around 150mm. When grouping all fish examined during the study and dividing size classes by quartiles, prevalence of adult female *P. sanguinea* increased with host size (Fig. 7.26). However, only the smallest size class of crucian carp had a significantly lower prevalence of infection than the other groups examined (Mann Whitney P <0.05).

- **Infections relative to host sex**

Host sex was recorded from two stillwaters during the study period, namely Cranleigh Pond and Oakside Fish Farm. These data revealed that both male and female fish from both fisheries were susceptible to infection with *P. sanguinea*. There was no significant difference between infections of *P. sanguinea* in male and female crucian carp at both Cranleigh Pond (Mann Whitney, P = 0.5188) and Oakside Fish Farm (Mann Whitney, P = 0.0677).
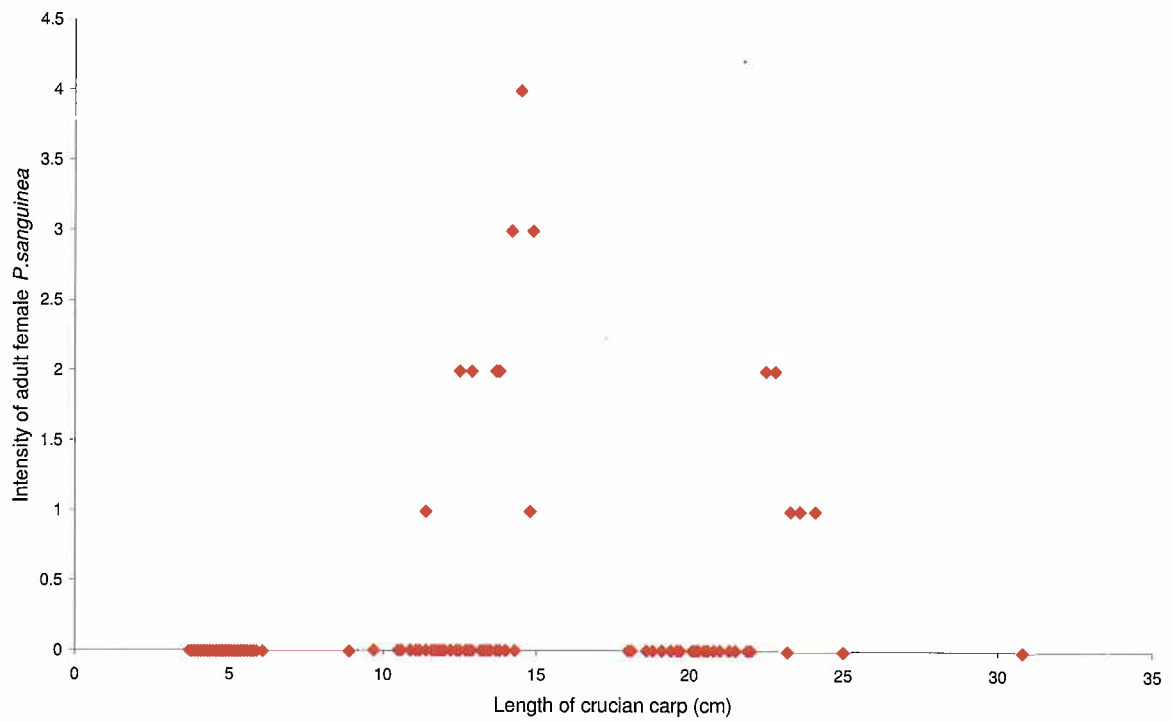
Fig. 7.25. Relationship of host length and intensity of infection of adult female *P. sanguinea* for crucian carp examined from Oakside Fish Farm.
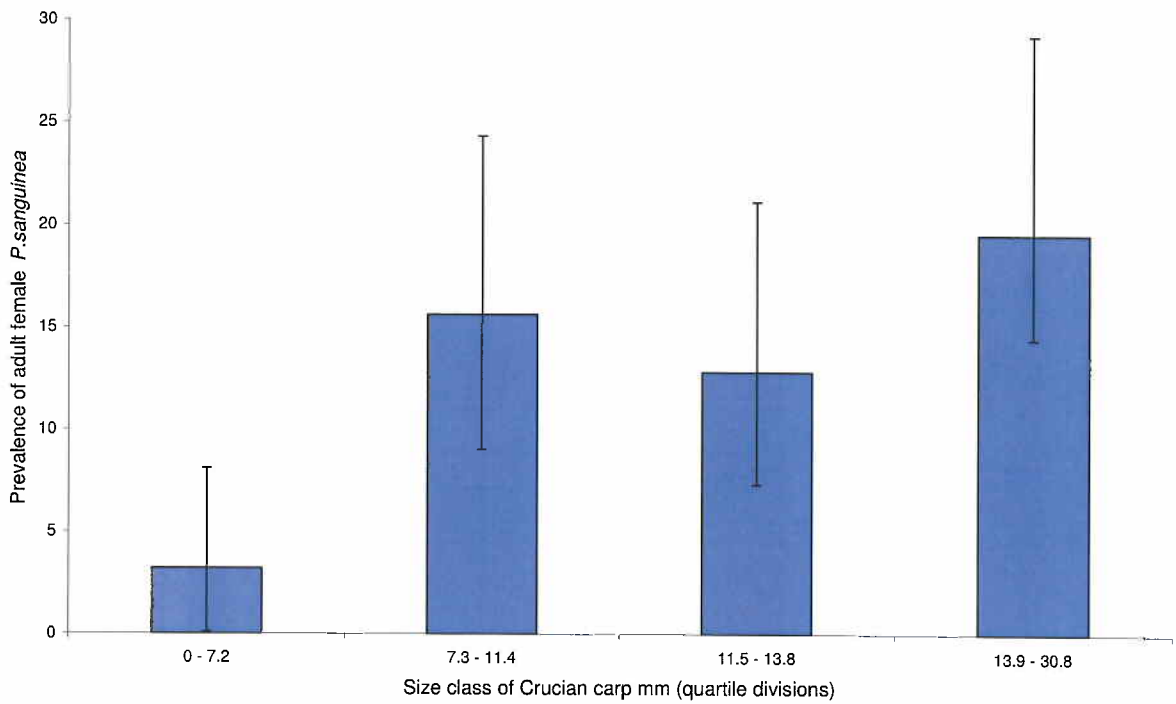


Fig. 7.26. Prevalence of adult female *P. sanguinea* in relation to size class of all crucian carp examined during the study.

- **Infections relative to fish age**

Scales were taken from all fish examined from Oakside Fish Farm to establish the effect of host age upon infections of *P. sanguinea*. During the study period crucian carp between 0+ and 4+ were found to be infected with adult female *P. sanguinea*. Although particular efforts were made to observe infections of *P. sanguinea* in 0+ crucian carp, at no time during the study were any 0+ fish found to harbour any life stage of the parasite (Fig. 7.27). Similarly, no parasites were recorded from any 5+ crucian carp (n = 9). Both prevalence (50%) and mean intensity of infection (2.67 parasites/host) were greatest in 2+ crucian carp. This was significantly greater than all other ages of crucian carp (Mann Whitney, $P<0.05$).
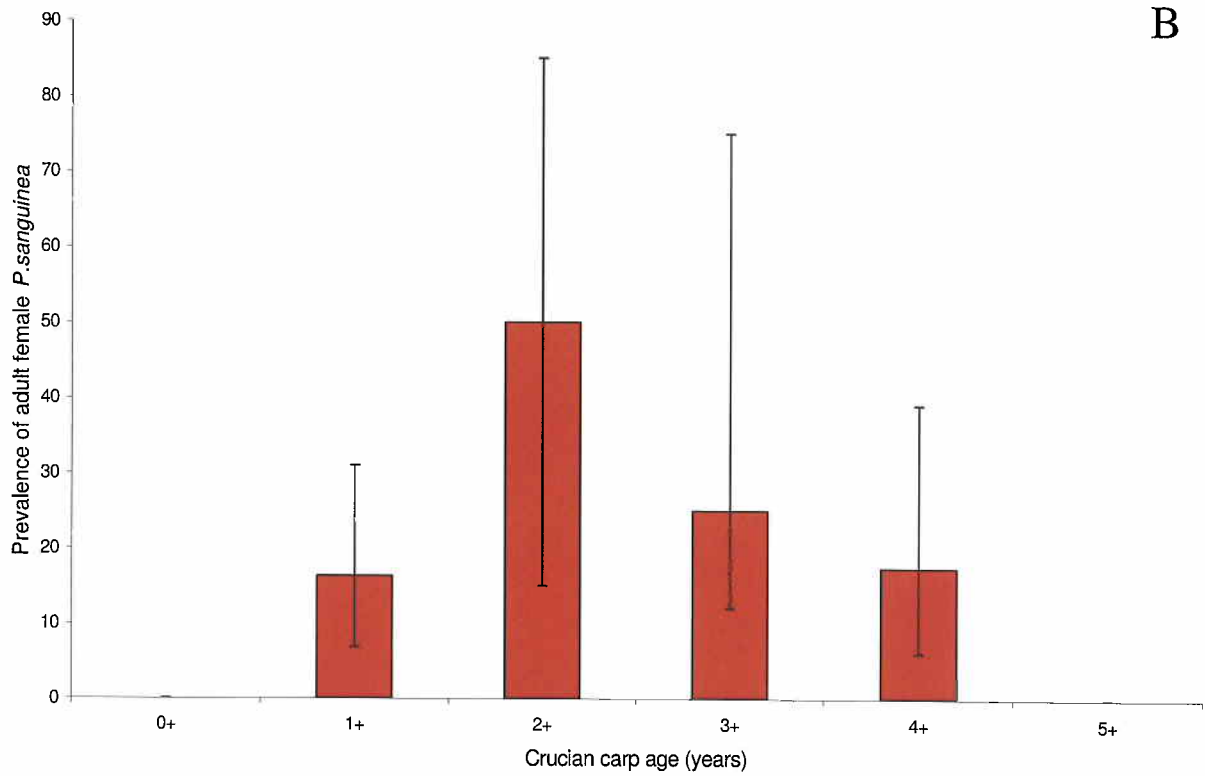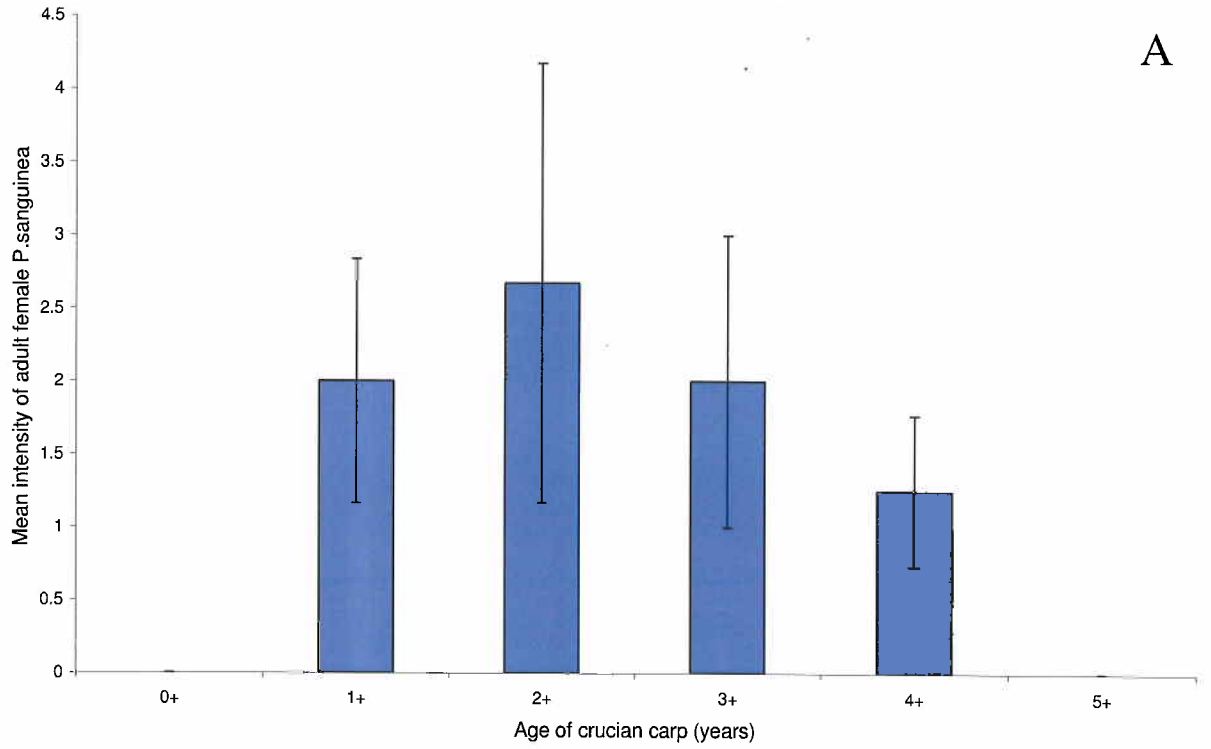
Fig 7.27. Mean intensity (A) and prevalence (B) of adult female *P. sanguinea* in relation to age of crucian carp sampled from Oakside Fish Farm.

- **Infections relative to site of infection**

During the study period, the caudal fin was the favoured site of adult female *P. sanguinea* infection. Of the 44 infected fish observed, 40 (90.1%) possessed worms within the caudal fin, 1 (2.3%) within the anal fin, 2 (4.5%) within the dorsal fin and 1 (2.3%) with the pectoral fin. At no time during the study was the pelvic fin detected as a site of *P. sanguinea* infection. In a single infection, parasites were found in both the caudal fin and dorsal fin.

- **Effects of *P. sanguinea* upon condition of crucian carp**

From the populations of crucian carp examined, *P. sanguinea* did not appear to reduce host condition at intensities of up to 4 parasites. However, due to the absence of nematodes in 0+ fish, an assessment of impact to very small fish was not possible. Similarly, extremely small numbers of infected fish within some populations prevented a reliable assessment of condition loss within these fisheries. This limited comparisons between condition of infected and uninfected individuals.

The condition of crucian carp from Oakside Fish Farm, Eastfield Lakes and Cranleigh Water are shown in Table 7.2. No significant association was found between infection with *P. sanguinea* and the condition of hosts. Even the three most heavily infected crucian carp fish detected during the study, harbouring intensities of 3, 3 and 4 female parasites, had condition factors of 2.25, 2.26 and 2.08 respectively. These were all higher than the mean condition factors of the uninfected populations.

Table 7.2. Condition of crucian carp from three stillwater fisheries infected with *P. sanguinea*.

| Water name | Infection | No of fish examined | Intensity range | Mean Condition | Range of Condition |
|---|---|---|---|---|---|
| **Eastfield Lakes (lakes 1-4)** | Uninfected | 92 | 0 worms | 1.868 | 1.477 – 2.156 |
| | Infected | 12 | 1-2 worms | 1.854 | 1.473 – 2.186 |
| **Oakside Fish Farm (group II)** | Uninfected | 39 | 0 worms | 2.03 | 1.737 – 2.476 |
| | Infected | 10 | 1-4 worms | 2.11 | 1.871 – 2.267 |
| **Cranleigh Water** | Uninfected | 34 | 0 worms | 1.974 | 1.769 – 2.139 |
| | Infected | 5 | 1-2 worms | 2.106 | 1.949 – 2.426 |

## 7.3.4 Life-cycle development and parasite detection

Samples of infected crucian carp were obtained through autumn, winter and early spring, allowing observations of the main developmental stages of *P. sanguinea*. An absence of sampling opportunity during late spring and early summer limited observations of newly established parasites within the crucian carp. Efforts were not made to study development of larval worms within the intermediate copepod host.

Current studies confirm that the life-cycle of *P. sanguinea* follows a seasonal cycle of development within fisheries in England. Gravid female parasites (Fig. 7.28) were visible within the fins of infected crucian carp from September until the middle of June. The migration of worms to the fins was not synchronised, with worms of differing

developmental status being recorded within the fins of the same host. Maturity of female worms within the fins could be measured visually through an increase in size, change in colour and development of larvae. Parasites recently established within the fins were a light translucent brown in colour and were not easily detected. Female worms became darker and redder with state of development and duration of infection (Fig. 7.28). A more accurate assessment of larval development was achieved through microscopic examination of female parasites following rupture of the body wall. During January and February, female parasites contained only rounded eggs (Fig. 7.28). These developed through a number of clear developmental stages into highly active larvae (Fig. 7.28). By mid April most gravid female parasites contained very active larvae.

The process of larval dispersal was observed in two infected crucian carp. This process began with raised activity of gravid worms within the fins, characterised by regular contractions between the fin rays and occasional emergence of the parasite's head into the water. Larval dispersal involved progressive emergence of the female worm from between the fin rays, until over half of the worm was hanging free into the water. At this stage the female worm became noticeably turgid, and then burst open releasing a visible cloud of larvae into the water that was dispersed by movement of the host's tail. Female worms instantly ceased movement, although deflated remnants remained attached to the host. Freshly dispersed larvae (L1) collected from the remnants of spent females measured approximately 400μm in length and were highly motile, continually contracting and straightening (Fig. 7.28).

Parasitological examinations revealed only a small number of adult male parasites within infected hosts. These were found within the body cavity, primarily on the

serosal surface of the swim bladder. On a single occasion, a male parasite was observed on the surface of the kidney. Adult male parasites were virtually transparent and required careful dissection and high power magnification to detect. Male worms possessed a round head, smooth cuticle and conspicuous orange, barbed spicule (Fig. 7.18). Despite detailed examinations, no immature female parasites were detected either within the internal organs or during migration to the fins. Adult male parasites were recorded on the swim bladder con-currently with adult females in the tail, suggesting that males survive the process of reproduction. The presence of degenerated male parasites on the swim-bladder of some hosts, indicated that these worms remained within this location for the remainder of their life and do not undergo tissue migrations. The life-cycle of *P. sanguinea* observed during the study is summarised in Fig. 7.28. Developmental stages that were not observed during the study, have been included according to Moravec (1994). For detection purposes, characteristic features of both male and female worms from crucian carp are also shown (Figs. 7.29-7.30).

# Chapter 7.

When positioned within the fins, newly established female parasites are light coloured and almost transparent (1*). Larval development within the body of the parasite (2,3,4) makes females become larger, redder and more conspicuous (5).



5

During spring, females break out of the fin to shed larvae. The sudden change in osmotic pressure causes the uterus of the parasite (*) to rupture (6) shedding masses of larvae (7) into the water.



6
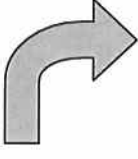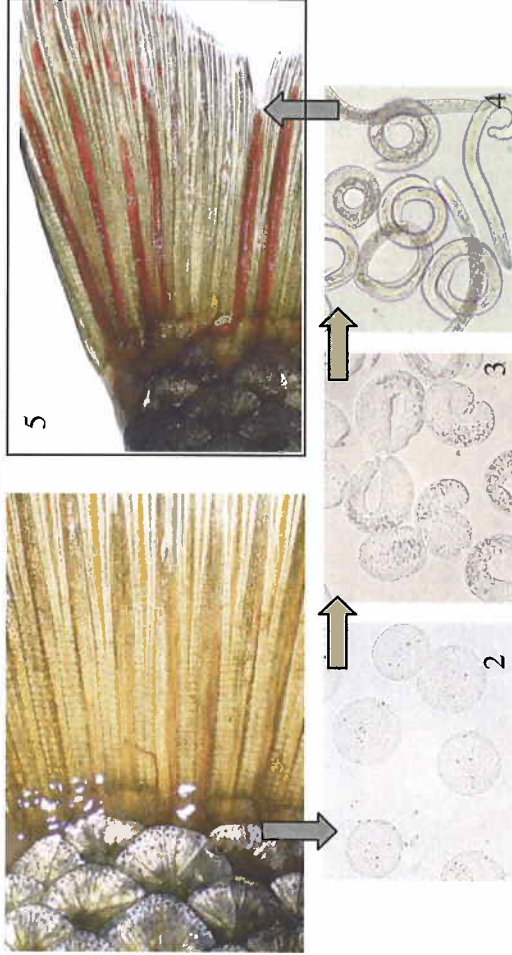
Crucian carp gain infection through ingestion of infected copepods. Male and female worms migrate to and mate on the swim bladder (10, 11). Females then migrate to the fins.



11



10
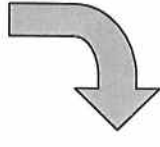
When released into the water, larvae (0.2mm) (8) repeatedly coil and uncoil (8). Literature suggests that copepods (9) act as an intermediate host to the parasite. Larvae development within this host takes place until parasites are viable to the definitive host.

Fig. 7.28. Life-cycle development of *P. sanguinea*
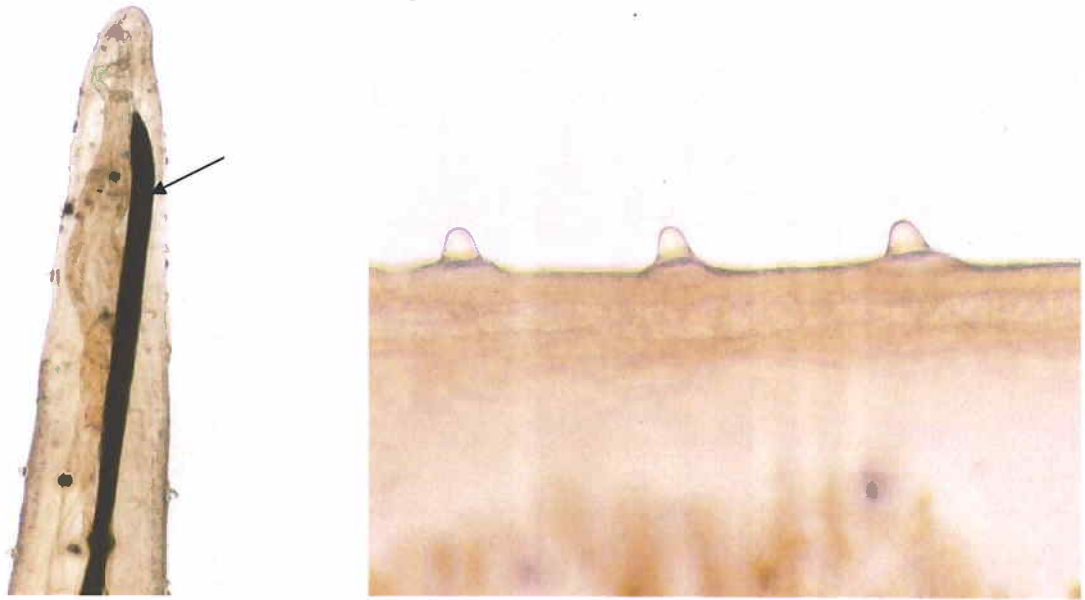
Fig. 7.29 (a) An adult female parasite removed from the fins, showing rounded head and dark uterus which runs the length of the body (arrow). Examination of the parasites cuticle (b) revealed the presence of numerous bosses that appeared to be randomly distributed.



Fig. 7.30. A male *P. sanguinea* (2.6mm total length) from the swim-bladder, showing relatively transparent body, rounded head (*), smooth cuticle and characteristic broad, yellow-orange spicule (arrow) located at the blunt, posterior end of the parasite.

Observations of infected crucian carp maintained in semi-natural conditions during the spring confirmed that the process of larval dispersal was associated with rising temperatures during mid April (Fig 7.31). Gravid worms released their larvae from mid April until the end of July. A cold period experienced during May appeared to result in the temporary cessation of larval dispersal, which began as temperatures rose again during June and July. All gravid females successfully achieved larval dispersal.
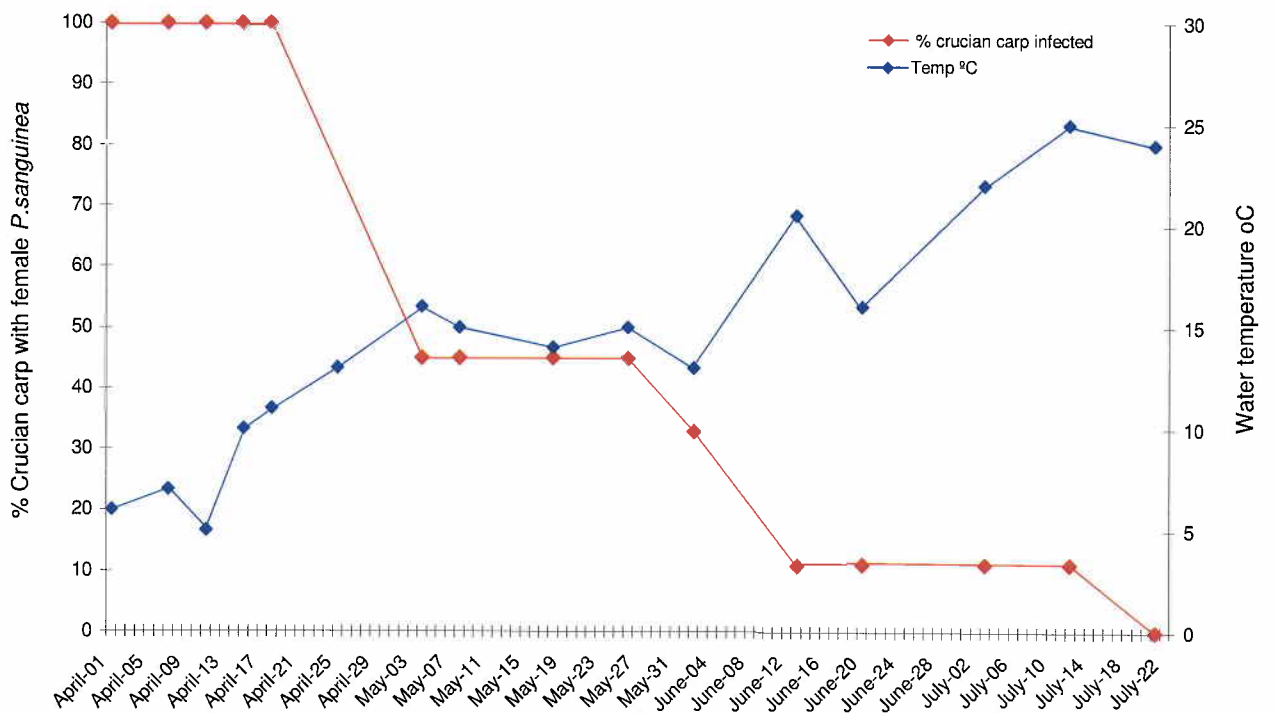


Fig. 7.31. Relationship between dispersal of female *P. sanguinea* and water temperature in crucian carp maintained in semi-natural conditions.

## 7.3.5 Distribution of *P. sanguinea* within England and Wales

*P. sanguinea* has been recorded from a total of 6 stillwater fisheries and a fish farm within England and Wales (Fig. 7.32). The occurrence of the parasite at Eastfield lakes includes infection within 4 adjacent ponds. Detection of the parasite at Oakside Fish Farm, lead to eradication through the process of liming. This water has not been included in the current distribution records (Fig. 7.32). With the exception of this water, all sites know to be infected by the parasite are located within south-east England.
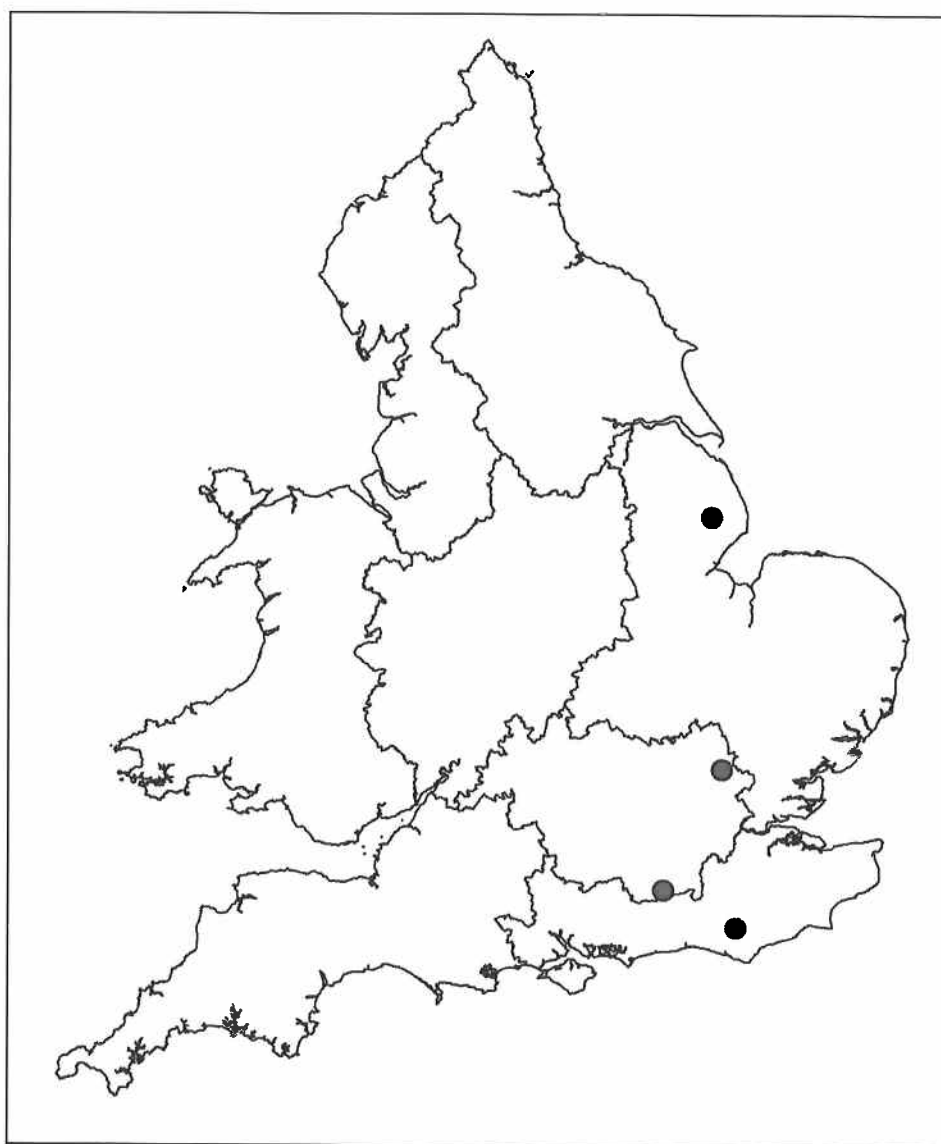


Fig 7.32. Distribution of *P. sanguinea* within fisheries in England and Wales from Environment Agency records 1982 – 2006..

## 7.4 Discussion

**Pathological changes associated with *P. sanguinea***

Nematodes are considered the most economically important helminth parasites of fishes

of the world (Hafsteinsson & Rizvi, 1987). According to literature, the genus

*Philometroides* comprises a number of pathogenic species, including *P. cyprini* (syn. *P.*

*lusiana, P.lusii*) (Vismanis, 1966; Vasilkov, 1967, 1983, Schäperclaus, 1991; 2004a;

Moravec & Cervinka, 2005) *P. sanguinea* (Wierzbicki, 1958; 1960; Vasilkov, 1983;

Vismanis & Nikulina, 1968; Moravec, 1994), *P. fulvidraco* Yu, Wu & Wang, 1993

(Wang, 2002) and *P. huronensis* Uhazy, 1976 (Uhazy, 1978; Vasilkov, 1983; Moravec,

1994). However, information detailing the lesions associated with philometrid

infections in fish is very sparse (Uhazy, 1978). The pathogenicity of parasitic

nematodes has been recognised as one of the most neglected fields in fisheries

parasitology (Williams, 1967; Moravec, 1994). The current study represents the first to

detail the pathological changes caused by *P. sanguinea* within the fins of crucian carp.

Literature indicates that the pathogenesis of parasitic nematodes occurs as a result of

feeding behaviour, attachment mechanisms or migration of both adults and larval stages

within the host (Williams, 1967; Hoffman, 1975; Moravec & Dykova, 1978; Moravec,

1994). Pathological changes associated with gravid *P. sanguinea* were characterised by

distension and distortion of the fins, displacement of fin rays, condensation of

connective tissues around the worms, localised damage to the fin musculature and

varied inflammatory responses. Many of these changes, in particular the inflammatory

responses, were associated with movement into and out of the fins during larval

development and dispersal. These observations suggest that periods of migration are

important events for damage to crucian carp infected with *P. sanguinea*. These changes

were consistent with infections of the related species *P. huronensis* in the white sucker

and may be common host responses to infections of large nematodes (Uhazy, 1978).

The process of larval dispersal, also known as 'functional bursting' (Lewis *et al.*, 1974)

is characteristic of many philometrid nematodes and can be potentially harmful to the

host (Uhazy, 1978; Moravec & Dykova, 1978; Vasilkov, 1983; Moravec, 1994; Wang,

2002). During the present study, this drastic reproductive behaviour was accompanied

by localised necrosis and acute inflammatory responses within the host. These changes

are commonly associated with the migration of nematodes within fish tissues (Dick &

Choudhury, 1995b). Only during larval dispersal was integrity of the skin breached.

These lesions may increase susceptibility of the host to secondary infections

(Schäperclaus, 1991). The absence of severe tissue damage, particularly in large fish

suggests that between the time of establishment within the fins and process of larval

dispersal, parasites remain relatively dormant within the host tissues. The presence of

connective tissue surrounding worms within the fins, were consistent with early stages

of encapsulation. Similar changes have been recorded in *P. huronensis* (Uhazy, 1978).

Consequently, it is likely that the damage caused by female *P. sanguinea* is seasonal,

being most pronounced during periods of movement into and out of the fins. Between

these periods impact may be minimal, although the physiological costs of infection

upon fish are unknown and may prove debilitating during heavy infections.

Haematological and physiological disturbance have been described during infections of

*P. cyprini* in common carp (Sekretaryuk, 1980, 1983).

The damage caused by *P. sanguinea* was influenced strongly by host size, being more pronounced in small crucian carp than larger fish. The occurrence of tissue swellings and fin distortion supports records that disease potential may be greater in juvenile fish. In large crucian carp, pathological changes were limited to minor distortion of host tissues and relatively mild host responses. However, in very small fish, tissue displacement by female worms was accentuated and accompanied by marked distortion of the fins, displacement of fin rays and capillaries, muscle degeneration, localised necrotic changes and marked antigenic responses. This is likely to be reflected by the size of the nematode in relation host tissues. Margolis (1970) highlighted the importance of parasite size in the pathogenicity of parasitic nematodes, suggesting there may be a cut-off size under which fish become noticeably diseased, and over which hosts tolerate infection.

Published literature suggests that *P. sanguinea* causes greatest impact to juvenile hosts that become infected during their first year (Wierzbicki, 1960; Vasilkov, 1983; Moravec, 1994). Experimental observations have also confirmed that crucian carp less than 90mm are unable to survive infections (Wierzbicki, 1958, 1960), although no reference was given to intensity of infection. According to Moravec (1994) infections comprising just 3 *P. cyprini* are known to kill common carp fry, although these parasites exceed 110mm in length. During the current study, the smallest infected host measured 66mm and harboured a single female worm. This fish showed no obvious signs of disease or gross condition loss. However, it is well recognised that crucian carp can vary considerably in size at maturity, depending upon environmental conditions and population density (Maitland & Campbell, 1992; Holopainen *et al.*, 1997; Szczerbowski, *et al.*, 1997). Within populations of Eastern Europe fish may attain

only 20mm in their first year and gain maturity at less than 60mm (Szczerbowski *et al.,*
1997). In the UK, farmed 0+ crucian carp may attain a size of 110mm, although a wild
fish reaching 40mm in its first year is considered exceptional (Henshaw, *pers. comm.*).
The apparent elasticity in morphology of crucian carp populations is therefore likely to
strongly influence the potential impact of *P. sanguinea*. Without understanding of host
size, it is difficult to interpret records of disease in published literature.

It is easy to appreciate how migrations of a parasite, that may exceed the length of its
host could cause serious harm. As current pathological observations were based upon
infections in fish exceeding 66mm, it is possible that these represent a gross
underestimate of the potential damage to very small hosts. It also remains possible that
impacts to juvenile hosts, including mortality may have occurred prior to or after
sampling. The importance of infections in small crucian carp, as well as damage caused
by larval worms during early infections represent important areas of further
investigation. This is likely to require experimental observations following exposure of
juvenile hosts to *P. sanguinea* infection.

Despite these limitations, current observations provide little evidence to suggest *P.
sanguinea* is a serious pathogen of crucian carp at the intensities recorded. In hosts
exceeding 70mm in length harbouring up to 4 worms, *P. sanguinea* did not appear to
have a serious deleterious impact upon the host. The absence of severe tissue damage or
gross pathological changes indicates tolerance within the hosts examined. This
observation may be explained by three possibilities. Firstly, *P. sanguinea* is not as
pathogenic as the literature suggests. Secondly, environmental stressors play an
important role in the characterisation of the disease. In such cases, only heavy

infections may be damaging, whilst light infections lead to minor, sub-clinical debilitation (Moravec, 1994). This is known to occur with nematode burdens in terrestrial animals (Crichton & Burton, 1977; Gulland, 1992). Or thirdly, the parasite is only pathogenic to very small hosts (Wierzbicki, 1958; Vasilkov, 1983; Moravec, 1994), being tolerated in larger fish. The later possibility deserves particular attention as to date, no infected juvenile crucian carp have been recorded within the British Isles. Further studies are necessary to include observations of pathological changes in 0+ hosts.

## Prevalence and intensity of *P. sanguinea* within fisheries

The prevalence and intensity of *P. sanguinea* within the fisheries studied, were lower than those recorded for many other *Philometroides* spp. (Uhazy, 1976; Schäperclaus, 1991; Moravec, 1994, 2006; Wang, 2002; Gollety *et al.,* 2005). Accounts of *Philometroidosis* caused by *P. huronensis* and *P. cyprini* include prevalence of between 70-100% with intensities of up to 90 worms per host (Uhazy, 1976; Schäperclaus, 1991; Moravec, 2006). Infections of *P. hydrocyoni* within the River Nile occur in over 50% of the characin (*Hydrocynus forkahkii* Cuvier) population, with intensities of between 4 and 8 parasites (Fahmy *et al.,* 1976). *P. huronensis* can occur in the fins of *Catastomus* sp. at a prevalence of 94%, with 1-32 parasites per host. According to Moravec (2006), the prevalence of *P. sanguinea* in crucian carp in natural waters is usually between 50 and 60%. Dailey (1966) recorded a similar prevalence (47%) of *P. nodulosus* (Thomas, 1929) in wild *Catastomus* sp. from a Colorado river (Morvaec, 1994). With the exception of *P. dogiel* Vismanis & Yukimenko, 1974, which usually exist in the fins of *Elopichthys bambusa* (Richardson) at a prevalence of 9% (intensity 1-3 worms) (Vismanis & Yukhimenko, 1974), the observed prevalence and intensity of *P.*

*sanguinea* in crucian carp fisheries represent the lowest for any documented species of the genus *Philometroides* (Morvaec, 1994, 2006). The low variability in infections at all of the waters studied suggests that this may be a typical characteristic of *P. sanguinea* within fisheries in England. However, the factors responsible for elevating infections of *Philometroides* spp. to pathogenic levels are poorly understood (Moravec, 2006).

The prevalence of philometrid nematodes in the definitive host may be influenced by many biotic and abiotic factors (Esch, 1977; Chubb, 1980; Schad, 1977; Anderson, 2000). These include the abundance and distribution of the intermediate host, viability of larvae, regulatory mechanisms, distribution and behaviour of crucian carp, feeding characteristics, host reproduction and immunity. Anderson, (2000) proposed that the prevalence of philometrid nematodes is influenced by synchronicity of both host and parasite reproduction. Yashchuk (1974b) revealed that the prevalence of larval *P. sanguinea* within the copepod host ranges between 0.04 and 0.25% depending upon time of year. This worker also confirmed that the majority of copepods contained just a single parasite. Consequently, infections within the definitive host may simply reflect the low prevalence and intensity of larval worms within the copepod population (Yashchuk, 1974b; Moravec, 2006). Despite the ubiquitous occurrence of copepods within many aquatic environments (Fryer, 1957) and massive reproductive potential of female philometrids (Nakajima *et al.*, 1970), it is likely that *P. sanguinea* infections in fish are influenced by many subtle and complex interactions.

Infections of *P. sanguinea* result from the ingestion of infected copepods by the definitive host (Chubb, 1980). Consequently, host diet and feeding behaviour are important in determining dynamics of infection within crucian carp fisheries. In the

current study, all waters examined were actively fished or received supplementary feeding. It is therefore possible that the input of artificial feed, reduced the reliance upon natural food sources and in turn reduced the composition of copepods within the hosts diet. Recent studies at a stillwater fishery in England have confirmed that anglers bait rather than invertebrates make up a large proportion of the ingested food items of small cyprinid fish (Britton, in prep). This diet may therefore limit the disease potential of the parasite and alter the distribution of worms within the definitive host population. If this is a valid assumption, populations from natural waters may harbour higher parasite infections than those influenced by angling activity or fishery management.

Arrested development (hypobiosis) is a well-recorded density-dependant process that regulates infections of many parasitic nematodes (Schad, 1977; Molnar, 1977; Ashworth & Kennedy, 1999; Wang, 2002). Gollety et al., (2005) suggested that this mechanism may explain low intensities of philometrid nematodes. However, according to the definition given by Michel (1974), this phenomenon affects development and maturity of parasites within a host and not the total worm burden. Consequently, this may be used to explain low intensities of mature female worms, but not a complete absence of parasites from a host. Furthermore, Esch et al., (1977) emphasised that infections of all parasite life-stages must be understood before the role of regulatory mechanisms can be discussed. The absence of detailed records of male parasites as well as juvenile life stages therefore limits further assessment of this phenomenon.

The over-dispersed distribution of P. sanguinea within crucian carp fisheries is likely to limit the impact of the parasite at the population level. Such a clumped parasite distribution is considered to be the result of heterogeneity in host behaviour, in host

immune response or in the spatial distribution of infective stages (Anderson, 1993). However, compared with many parasite populations, the observed levels of over-dispersion were very low. It has been proposed that parasite mortality, density-dependant processes and parasite-induced mortality may be responsible for low dispersion in parasite populations (Anderson & Gordon, 1982). There was no evidence of parasite induced mortality during the study period. Similarly, it seems unlikely that density dependant processes would limit infections to a single female worm - the highest intensity recorded at some fisheries. Low variation in parasite intensity within many of the waters examined greatly effected the formation of an over-dispersed pattern of distribution. It is also acknowledged that the focus of the study was on adult female worms due to their size and active migrations within the host. Consequently, poor understanding of intensity, prevalence and distribution of male parasites may result in misleading observations.

**Importance of host size and susceptibility of 0+ crucian carp**

Host size is an important factor influencing the pathogenicity of many parasites, including parasitic nematodes (Tedla & Fernando, 1969; Alston, 1994; Hoole, 1994; Moravec, 1994). It is well known that the developmental stage of the host can affect disease potential and the extent of physiological disruption caused by infection (Johansson *et al.*, 1974; Hoglund *et al.*, 1992; Williams & Jones, 1994).

The greatest intensities of *P. sanguinea* were recorded in crucian carp of approximately 130-150mm in length (age 3+). This is consistent with observations of *P. cyprini* in common carp, where 2+ and 3+ fish harbour the greatest number of parasites (Moravec, 1994). This is likely to reflect the greatest consumption of copepods within the diet of

these fish. It is well recognised that planktonic crustaceans, including copepods, form a large proportion of the diet of juvenile crucian carp (Wheeler, 1978; Giles, 1994). However, in many cyprinids diet becomes more varied and will often shift to include larger food items when fish reach a certain length (Maitland and Campbell, 1992; Couchman, 1997). Intensities of *P. sanguinea* infection may therefore be driven by the ingestion of copepods within the diet up to a host size of approximately 150 mm, after which crucian carp ingest a lower proportion of copepods within the diet in favour of larger invertebrates. This may explain the lower prevalence and intensity of infection in larger fish exceeding 3+ in age. Other factors such as immunity and host behaviour can also influence susceptibility of hosts to infection. However, annual infections of *P. huronensis* in the white sucker indicate that fish do not develop protective immunity against infections (Uhazy, 1977b; Anderson, 2000).

Published literature suggest that the impact of *P. sanguinea* is greatest in small fish and fry (Wierzbicki, 1958). This potential is associated with the large size of gravid female worms and the active migrations undertaken during reproduction. The complete absence of *P. sanguinea* in 0+ hosts during the study is therefore an interesting finding. This observation is consistent with other species of *Philometroides* (de Buron *pers. comm..*) and the philometrid *Margolisianum bulbosum* (Gollety *et al.,* 2005), both of which can be rare in small hosts. Conversely, other species of *Philometroides* are known to infect fish of all age groups, including 0+ fish (Vasilkov, 1967; Uhazy, 1976; Moravec, 1994). Although crucian carp fry were only obtained from two of the fisheries studied, relatively large numbers of fish were examined. It is proposed that there are three possible explanations for the absence of infection in this year class. Firstly, that infected fry were present within these waters, but escaped either detection or sampling.

Secondly, parasite induced mortality of these hosts resulted in complete parasite absence within this year class, or thirdly, these hosts rarely become infected by *P. sanguinea* due to seasonal changes in the reproductive timing of the parasite. These possibilities will be discussed briefly in turn.

Due to the large size of gravid female *P. sanguinea*, it is very unlikely that parasites were present but evaded detection. The large number of 0+ crucian carp examined during the study and care taken with sampling, also makes it unlikely that fish escaped sampling, unless prevalence within these hosts was extremely low. The possibility of parasite induced mortality is difficult to assess without any data on pathological changes within 0+ hosts. The absence of literature detailing disease characteristics or clinical pathology during mortality events provides little to aid this assessment. However, pathogenicity of *P. sanguinea* would need to be extremely high if it were to result in a complete absence of infected juvenile hosts. Current observations of histopathology and an absence of morbidity in any of the hosts examined to date, suggests that parasite induced mortality is unlikely to explain this observation.

The third explanation for the absence of infections in 0+ crucian carp involves life-cycle development and the role of seasonality. The dispersal of larvae from gravid female parasites takes place in spring and coincides with the rising temperatures, increases in the intermediate copepod host population and onset of active feeding by crucian carp. This timing is critical in the susceptibility of crucian carp to *P. sanguinea*. Based upon current seasonal observations and experimental studies of development within the copepod host (Wierzbicki, 1958; Yashchuk & Vasilkov, 1970; Moravec, 1994) it is proposed that the window for parasite recruitment occurs between late May,

peaking in June and early July before declining rapidly in early August. Depending

upon the reproductive timing of the crucian carp populations, it is quite possible that 0+

crucian carp may miss this window of infection, especially if infected copepods are

abundant before fry are present, or at least large enough to ingest them (Fig. 7.33).

Although the effects of parasitism upon copepod fitness are largely unknown (Nakajima

& Egusa, 1977c), the rapid spurt swimming of cyclopoid copepods can make capture by

young fry difficult (Scott & Henshaw, *pers. comm.*).

Crucian carp usually spawn during early summer, when temperatures reach

approximately 18-20°C (Maitland & Campbell, 1972; Maitland, 1972, 2004). This

process is not strictly timed and may differ from year to year. However, based upon the

current seasonality studies, spawning is likely to have occurred during mid June.

Assuming maintenance of 20°C, exogenous feeding of larval crucian carp is known to

begin approximately 14 days after spawning (Laurila *et al.,* 1987; Laurila &

Holopainen, 1990). As such, 0+ crucian carp only enter the transmission window during

July. According to Yaschuk (1974b) this coincides with a period of rapidly declining

infection within the copepod population. It is possible that if this infection window is

missed, fry populations may completely avoid infection until the following year. This is

supported by the fact that crucian carp are known to be small for age within their first

year, but grow rapidly within their second season (Henshaw *pers. comm.*). The absence

of infection in 0+ fish and tolerance of 1+ fish due to their greater size, may therefore

minimise impact within infected populations. Further studies are necessary to

substantiate this hypothesis.
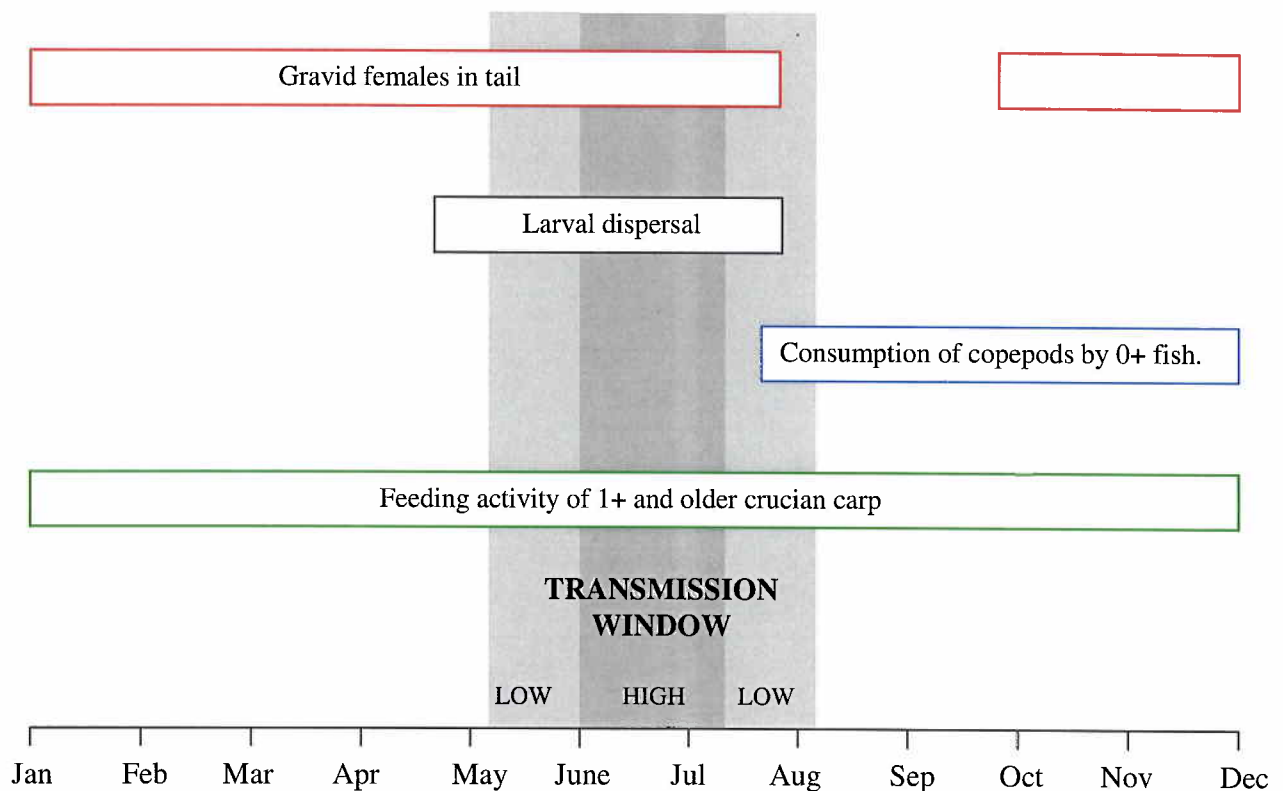
Fig 7.33. Seasonal cycle of *P. sanguinea,* showing differences in the timing of larval dispersal and potential infection of 0+ crucian carp.

Whilst the seasonal development of *P. sanguinea* may reduce the probability of 0+ crucian carp gaining infection, this does not explain the reports of 100% mortality in fry in other parts of the world (Vasilkov, 1983; Moravec, 1994). A possible explanation is that local climatic conditions play an important role in the development of parasite infections. Alternatively, it is possible that the reports of mortality from *P. sanguinea* are based solely upon fish size rather than an accurate assessment of age. In cold climates 1+ crucian carp may attain a length of only a few centimetres, thus may appear like fry. According to the proposed role of seasonality, fish at this age would feed throughout the infection window and would therefore be highly susceptible to infection.

Impact may therefore extend to 1+ fish, if growth were particularly poor. Other potential risk factors might include very warm spring temperatures. This could lead to early spawning behaviour and thus greater chance of 0+ crucian carp attaining a size capable of ingesting copepods throughout early summer (Aho & Holopainen, 2000). However, such environmental conditions are likely to have a similar effect upon parasite reproduction. Consequently, a warm spring may simply shift the timing of transmission and not necessarily increase the risk of infection to 0+ fish. Further studies are necessary to improve understanding in this area and to better assess the potential impact of the parasite to crucian carp fry.

## Life-cycle development and importance of seasonality on host impact.

Whilst experimental studies provide valuable information of the development of *P. sanguinea* (Yaschuk & Vasilkov, 1970; Nakajima & Egusa, 1977a; Moravec, 1994) studies of seasonal development in natural environments are very important in the understanding of dracunculoid infections in fish (Moravec, 2004a). Current studies confirm that *P. sanguinea* follows an annual cycle of maturation, reproduction and development in crucian carp within England (Chubb, 1980; Moravec, 1994). This is consistent with most philometrid nematodes (Anderson, 2000) and supports observations of *P. sanguinea* made in Poland (Wierzbicki, 1960). The only exception to this is the assumption made by Nybelin (1931) who suggested that the life-cycle of *P. sanguinea* is completed in only 6 months (Moravec, 1994). Literature suggests that seasonality holds important implications for the impact of *P. sanguinea* (Vasilkov, 1983; Moravec, 1994). Vasilkov (1983) stated that philometroidosis of carp manifests itself as either an acute disease in 8 week-old fry due the migration of larval stages, or as a chronic form where survivors suffer debilitation and eventual death from the

emergence of the female parasites the following spring. Unfortunately, the inability to gain fish samples during late spring limited an assessment of pathogenicity from larval parasites. Although none of the waters sampled had recorded crucian carp mortalities, it remains feasible that losses of crucian carp fry had occurred, but simply evaded detection due to their tiny size. Despite these limitations, comprehensive sampling during both autumn and early spring allowed evaluation of impact from migrations of adult female worms.

Samples obtained during autumn and spring allowed observations of parasite migrations into and out of the fins, both of which are documented as potentially harmful to infected hosts (Moravec, 1994). However, in the fish examined there were no signs of disease, host debilitation or morbidity. These observations suggest that migrations of juvenile female worms into the fins over the summer period were not damaging to the crucian carp examined. Similarly, the examination of infected hosts in spring prior to larval dispersal did not reveal any evidence for chronic debilitation caused by the development of female parasites over the winter period. This was supported by observations of relatively stable host condition factors. However, it is recognised that these observations were limited by spot sampling and did not include infections within small crucian carp, preventing a complete assessment of impact.

Spring represents an important period for the transmission of *P. sanguinea* to the next host generation. Female worms were recorded within the fins between October and May. Observations of larval dispersal during spring revealed that this process is influenced strongly by water temperature. The release of larvae from female parasites was first seen during mid-April when temperatures reached approximately 10°C. This is

lower than the temperatures recorded for larval dispersal in *P. cyprini* (Moravec, 1994, 2006). Whilst $10^{0}C$ appeared to be the initial trigger for reproduction, larval dispersal was not synchronised and took place between mid-April and the end of July (temperature range 10-25°C). It is therefore possible that other factors influence this behaviour, including photoperiod or a minimum number of degree-day development during spring. As females migrate into the fins over a number of weeks (Wierzbicki, 1960), it seems understandable that their emergence from the fins are equally staggered. These observations confirm that parasite transmission is not strictly timed, or restricted to a sudden and short-lived event. It is therefore feasible that fish may obtain infections of larval parasites before gravid females are shed, allowing simultaneous damage from invading larvae and detaching females (Moravec, 1994). Consequently, early spring poses a potentially harmful period for infected individuals. This may be compounded by the biotic stressors associated with spring, metabolic costs of over-wintering and lowered immunity.

**Detection of *P. sanguinea* within infected fisheries**

Current observations of life-cycle development highlight some important considerations for the detection of *P. sanguinea*. Due to their size, colour and conspicuous location, the presence of gravid, female worms would be easily detected during routine parasitological examinations. Due to their much smaller size and near transparency, the detection of male parasites represents a greater challenge. The parasites require careful examination of the swim-bladder and kidney. More difficulty lies with the detection of pre-gravid female worms, mid-migration between the body cavity and fins. Detection difficulties are compounded by unknown migration routes and the fact that female worms may utilise any of the hosts fins. Furthermore, the fibrous structure and

translucent appearance of skeletal muscle makes the detection of a 2mm transparent nematode very difficult. These limitations are recognised as a limiting factor in the study of many dracunculoid nematodes (Moravec, 2004a).

The tail was the fin most commonly utilised by adult female *P. sanguinea*. This is consistent with historic records of infection that state parasites in the other fins as occasional findings (Wierzbicki, 1960; Moravec, 1971, 1994). The occurrence of parasites with the pectoral fins has not been recorded in previous literature (Wierzbicki, 1958). It is unclear why the caudal fin is favoured by the parasite, although the constant movement of the tail is likely to promote rapid dispersal of larvae into the water. It is also unclear which route migrating parasites take to the fins, or the mechanisms and cues used for fin location. It is possible that the backbone, caudal blood vessels, muscle structure or activity of erector muscles within the fins provide orientation for parasite migrations. In *P. huronensis* the migration routes of larval, pre-adult and adult worms are known to be indirect, inconsistent and complex (Uhazy, 1977a, b; Anderson, 2000). Similarly, studies of other philometrid parasites have focused almost exclusively upon migrations of pre-copulated parasites and not those of inseminated female worms (Adams, 1969; Uhazy, 1976). The mechanisms for these migrations are very poorly understood. The occurrence of female *P. sanguinea* in fins other than the tail may be the result of abnormal parasite migration. However, the ability of parasites to become gravid within all fins, suggests that fin location is not important in the maturation and development of *P. sanguinea*.

Understanding the migrations of *P. sanguinea* represents a challenging, yet important area of further study if the parasite is to effectively detected at all times of the year.

However, it was noted during the study that all female parasites located within the fins were gravid, suggesting that female parasites only migrate following successful copulation. This allows infected crucian carp to be identified through presence of either male or female worms. The presence of a single parasite, whether it is male or female would represent an infected but not infectious individual. Consequently, the inability to detect pre-gravid female worms, may be overcome through the successful detection of males on the swim bladder. Due to the difficulties associated with detection of juvenile parasites, early summer poses the greatest risk for failing to detect *P. sanguinea*. During this period, infections may comprise only larval or pre-copulatory individuals, both of which are very difficult to find during routine parasitological examinations (Moravec, 2006). In view of this, crucian carp movements performed during early summer should be examined with extreme caution. Such risks could be minimised if crucian carp movements were halted as a precautionary measure until this area of understanding has been clarified. However, as this coincides with a busy period for fish movements, such restrictive measures would require justification and greater evidence of pathogenicity.

Records suggest that *P. sanguinea* is the only helminth to infect the fin tissue or swim-bladder of crucian carp in western Europe (Moravec, 1994; Kirk, 2000a; Natural History Museum Host Parasite Database, 2006). This suggests that *P. sanguinea* has invaded an open niche in crucian carp within the British Isles. With respect to invasion potential and impact, it appears that *P. sanguinea* faces no obvious competition from other parasite species. Furthermore, if *P. sanguinea* is truly non-native, then the crucian carp may be considered a naïve host to infection. This finding currently minimises risks of misidentification with other parasitic nematodes found within the fins of crucian carp.

**Host specificity of *P. sanguinea* and distribution records**

Current studies confirm that *P. sanguinea* is primarily a parasite of crucian carp, but may also infect crucian carp x common carp hybrids. This represents a new host record and has important implications for fish movements and parasite dissemination. In the last 5 years, demand for carp hybrids to stock stillwater fisheries has increased significantly in England (LFMD, 2006). Due to the rising popularity of these fish, the potential for parasite dissemination with movements of infected fish is greatly increased.

Despite growing numbers of crucian carp movements to support fishery development, *P. sanguinea* maintains a discrete distribution within England and Wales. This may reflect the relatively sparse distribution of crucian carp or the success of current control measures, which restrict movements of infected fish to all inland waters (Environment Agency, unpublished). According to Kennedy (1994), *P. sanguinea* has many attributes of a successful coloniser. The life-cycle of the parasite utilises a ubiquitous intermediate host and adult female parasites have a high reproductive capacity. Furthermore, approximately 250 legal movements of crucian carp take place each year within England and Wales comprising over 80,000 fish (LFMD, 2003). Although this raises potential for widespread dissemination, the low prevalence and intensity of *P. sanguinea* in all of the fisheries examined may limit this potential. Another explanation for the limited distribution of the parasite concerns detection effort. Many crucian carp movements involve the stocking of fully enclosed stillwater fisheries, where health checks are not a legal requirement. As such, current records may represent an underestimate of the parasites' true distribution. Difficulties associated with detection

of pre-copulatory parasites may also be a factor in the limited number of parasite recordings.

The spread of *P. sanguinea* to many regions of the world have been linked with the importation of infected crucian carp or goldfish (Hoffman, 1970; Hoffman & Schubert, 1984; Moravec, 1994). However, there is little evidence to substantiate these claims with regard to the British Isles. Crucian carp have a far lower economic value than common carp. Current demand in England is met by UK aquaculture, thus crucian carp are rarely imported into the UK for fishery stocking (LFMD, 2006). For this reason, it is unlikely that *P. sanguinea* was introduced with the importation of infected crucian carp. Similarly, *P. sanguinea* has not been recorded in goldfish within the UK, despite the importation of thousandss of fish (Biffar, 1997), the large size of worms, conspicuous location in the tail and containment of many goldfish by hobbyists in clear, easily observed tanks. Consequently, neither fish appear high-risk hosts for the introduction of *P. sanguinea* into the UK. A possible avenue for introduction is through movement of infected carp hybrids with imports of common carp. Common carp are the most frequently imported cold-water fish into the UK (Cefas registry). Fish enter the country from all over the world, including many illegal introductions every year from mainland Europe (Sampson *pers. comm.*). Carp hybrids have not only been imported into the UK, but have been found within consignments of true common carp. Whilst the avenue of introduction of *P. sanguinea* remains unclear, the observations of infections in carp hybrids indicate an additional host for the introduction and dissemination of the parasite within the British Isles.

## 7.5. Summary and risk assessment

Pathological and epidemiological observations made during this study provide little evidence to suggest that *P. sanguinea* is a serious pathogen of crucian carp, at infections of 1-4 parasites. However, it must be emphasised that many factors associated with philometroidosis were either not observed, or were beyond the scope of this work. This included an assessment of impact in 0+ fish and pathological changes associated with migration of newly established parasites. Furthermore, the prevalence and intensity of infection recorded at all of the fisheries studied were extremely low. Whilst this appears to be consistent with infections within England in 2003, literature from other countries suggests that heavier infections are typical of crucian carp in the wild. Due to the limitations of spot sampling, small sample sizes and observations from a single season, further studies are required to assess future disease risks. In the absence of direct observations of infected 0+ fish, information from published literature must be used to support an assessment of disease risk.

Moravec (2006) suggested that small stillwaters, with an abundance of macrophytes and copepods were likely to be high-risk environments for philometrid parasites. These are well-known to be perfect habitats for crucian carp (Maitland, 2004). Due to the vulnerability of crucian carp within England, caution is required when dealing with an introduced parasite that is a specialist of the genus *Carassius* and a known pathogen in other parts of the world. Although current studies provide a foundation of information on *P. sanguinea*, further studies are needed before a comprehensive assessment of pathogenicity can be made. In the mean time, a preliminary risk assessment may be conducted (Fig. 7.34, 7.35) although this involves considerable uncertainty.

Fig. 7.34. Risk analysis process for evaluating the probability of *P. sanguinea* causing undesirable economic and ecological impacts to fisheries. This is based upon available published literature and information gained during the current study.

| Parasite species being assessed – *Philometroides sanguinea* | | | |
|---|---|---|---|
| **Risk analysis based upon parasite understanding** | **Probability** | | |
| | Low | Mod | High |
| **Probability Score** | 0.1 | 0.3 | 0.5 |
| **A Ecological impact**<br><br>1. What is the risk of the parasite having an undesirable effect on ecologically important fish at the host level? | | 0.3 | |
| 2. What is the risk of the parasite having an undesirable ecological effect at the population/fishery level? | | 0.3 | |
| 3. What is the likelihood that the parasite will successfully spread and colonise ecologically important fisheries? | | 0.3 | |
| **B Economic impact**<br><br>1. What is the risk of the parasite having an undesirable effect on economically important fish at the host level? | 0.1 | | |
| 2. What is the risk of the parasite having an undesirable economic effect at the population/fishery level | 0.1 | | |
| 3. What is the likelihood that the parasite will successfully spread and colonise economically important fisheries? | | 0.3 | |
| **A Ecological impact risk analysis**<br>What is the risk of the parasite having an adverse ecological effect on fisheries? | **= 0.3 x 0.3 x 0.3 = 0.027** | | |
| **B Economic impact risk analysis**<br>What is the risk of the parasite having an adverse economic effect on fisheries? | **= 01 x 0.1 x 0.5 = 0.05** | | |

Fig. 7.35. Risk analysis matrix to prioritise the potential impact of *P. sanguinea* upon the ecological and economic development of fisheries. .

| | | Ecological Risk | | |
|---|---|---|---|---|
| | | **Low** (0.001 – 0.005) | **Medium** (0.009 – 0.027) | **High** (0.045 – 0.125) |
| **Economic Risk** | **Low** (0.001 – 0.005) | Low | **Medium** *P. sanguinea* | High |
| | **Medium** (0.009 – 0.027) | Medium | Medium | High |
| | **High** (0.045 – 0.125) | High | High | High |

## Chapter 8. Summary and recommendations

### 8.1 Summary

As bio-diversity of aquatic environments continues to decline throughout many regions of the world (Moyle & Leidy, 1992; Ricciardi & Rasmussen, 1999; Manchester & Bullock, 2000; Williams & Williams, 2004) the management of non-native species becomes increasingly important if ecosystems and economic resources are to be protected (Moyle, 1999; Manchester & Bullock, 2000; Ciruna *et al.*, 2004). With rising global demands for fish, growing numbers of fish movements and a continued drive for free trade, the flow of non-native parasites into the British Isles is unlikely to decline (Kennedy, 1976; Hill, 1991; Ganzhorn *et al.*, 1992; Howarth & McGillivray, 1994; Ruesink *et al.*, 1995; Hedrick, 1996; Lester & Evans, 1999). Although the potential for disease may not be realised for every introduced parasite (Kennedy, 1994), it is extremely difficult to predict the dangers of future invasions. The movement of fish without serious considerations for disease transfer may be considered ecological roulette (Carlton & Geller, 1993). Effective management of such invasions therefore relies upon the use of risk-based approaches to identify the greatest disease threats to fisheries and focus efforts upon these to minimise subsequent impacts.

The most desirable scenario for dealing with any non-native organism is to prevent introduction (Ruesink *et al.*, 1995; Ciruna *et al.*, 2004). This not only protects native resources, but reduces the need for prolonged and expensive monitoring, control measures and management strategies. The second most desirable option is to ensure rapid detection and prevent establishment through eradication (CBD, 2001; UK Defra, 2003). If such measures are not achievable then a more problematic situation arises, for which there are two contrasting courses of action. The first is to do nothing, based upon

the belief that once a non-native species has successfully established it is already too late and dissemination is inevitable, even if slowed through attempted containment. In this case, it has been argued that limited resources could be diverted to less questionable projects (Rejmenek, 2001). The second option is to manage these invasions to minimise impacts despite establishment (Environment Agency, 1999; UK Defra, 2003; CBD, 2004; Booth & Wood, 2004). In view of the ecological and socio-economic value of fisheries and the massive impacts non-native species can exert upon native resources, a 'do nothing' approach is a potentially damaging and irresponsible option (UK, Defra, 2003; Environment Agency, 2004).

Prior to this study, the management of non-native fish parasites raised several problems for the Environment Agency – the statutory authority for freshwater fishery development and environmental protection in England and Wales (Chare *et al.*, 2002). These surround two primary issues, namely poor understanding of impacts caused by some established parasites and the absence of a process for dealing with future invasions. Initial efforts were focused upon developing a foundation of information on the Category 2 parasites. Literature reviews and histopathological investigations provided an understanding of disease potential and observations of host level changes. In efforts to progress understanding further, a structured process for impact assessment was developed. This provided a framework for highlighting information of host, population and fishery level changes. From this, further studies were identified and prioritised. Four prioritised studies were undertaken to improve understanding of the effects of *P. longidigitus, E. briani, P. sanguinea* and *A. huronensis* on fisheries. At time of writing, the status of *P. longidigitus* and *A. huronensis* is under review by from

the Category 2 Parasite Review Group based upon the risk assessment and findings of this project.

The management of environmental risk is high on the political agenda and considered an important component of modernising government in the UK (Pollard, 2001). Risk assessment is therefore a vital component of sustainable fishery management (Lane & Stevenson, 1998). The risk assessment process presented in this project represents the first attempt to specifically address the management of non-native parasite introductions to fisheries in England and Wales. This provides a formal structure for highlighting risks, reviewing current understanding and directing future research efforts. It is hoped that this will facilitate better use of available resources, minimise undesirable impacts to fisheries and provide a scientifically robust foundation on which to base sustainable policies and proportionate control measures (Kluge *et al.*, 1986; Munro, 1986; Environment Agency, 1997, 2000; Moyle, 1999; Dehnen-Schmutz *et al.*, 2004). This process also ensures that the management of non-native parasites does not, as it has in the past, become a static process, leading to a growing list of non-native species with unknown impacts (Environment Agency, 1999). This represents a significant change in the management of the Category 2 parasites from that of risk-avoidance to that of a risk-based decision-making.

Although the current risk-assessment provides a step forward in the management of non-native fish parasites, a number of limitations are recognised. The process requires refinement, validation and further development to ensure it is workable, reliable and defendable (Pollard, 2001; UK Defra, 2005; Copp *et al.*, 2005b; Peeler, 2006a, 2006b). The current system also provides a relatively basic approach to tackle a complex

problem. This may represent an oversimplification of the different ways in which an exotic pathogen may affect fisheries. Whilst it is relatively straightforward to list the broad factors that determine the impact of a parasite, measuring and then evaluating the importance of these interacting components is not (Parker *et al.,* 1999). Impacts of non-native pathogens can be extremely varied and range from large-scale mortality, to relatively benign and almost undetectable changes (FAO, 1975; Hedrick, 1998; Hall & Mills, 2000; Welcomme, 2001). Consequently, non-native species are not simply 'harmful' or 'harmless' but usually lead to a varied and broad sliding scale of effects (Carlton, 2002). The difficulty lies with not only identifying these changes, but also assessing their severity and ecological or economic importance.

It is also recognised that current epidemiological studies were based upon spot-sampling, observations from a relatively small number of fisheries and were often limited by small sample sizes. Furthermore, for many of the parasites studied, time and available resources allowed only superficial investigations. However, understanding every impact that a parasite may have in a time frame that allows informed management actions represents an unrealistic and unattainable challenge. Consequently, a risk assessment for any parasite will always involve a level of uncertainty that must be managed accordingly. It is therefore important to ensure that the most serious risks have been identified and are carefully considered within any decision-making process. In such cases, measures of impact can afford to be wrong for minor changes, but must be sure to capture major effects (Parker *et al.,* 1999).

In view of such challenges, it is easy to become daunted by the enormity and complexity of parasite impact assessment (Bartley, 2004). From the outset of this work,

the scale of the problem required a compromise between what could be realistically achieved and the depth of understanding gained. The number of parasites in need of attention, magnitude of 'impact assessment' and difficulties involved with measuring changes of parasites in wild fish populations posed many problems. It is acknowledged that the scale of this subject falls far beyond the scope of one person. Whilst efforts were made to collaborate and seek expert opinion, these must be actively pursued in the future if understanding of these parasites is to progress. The involvement of experts in the fields of parasitology, pathology, epidemiology, fisheries management, genetics and risk assessment, amongst others, is essential in overcoming these challenges, maintaining scientific credibility of decision-making and ensuring communication of future disease threats.

It is relatively straightforward to highlight the problems surrounding national disease controls, produce an endless wish-list of further studies or devise a perfect scenario for preventing future impacts (e.g. the cessation of fish movements). It is not simple to develop a process that is consistent, achievable, workable and effective in the current climate of de-regulation, free-trade, limiting resources and within current legislative constraints. It is hoped that this study will provide a basis on which to further understanding and identify future disease risks. This information, combined with rapid detection, structured impact assessment, stringent targeted control measures and clear communication will afford better protection of the environment within the context of social and economic progress (Pollard, 2001).

## 8.2 Recommendations for further studies

This study has highlighted a number of areas in need of further attention. For clarity these are divided as per the previous chapters and then prioritised accordingly.

### 8.2.1 Management and awareness of future parasite invasions

**Continued development of non-native parasite risk assessment** – the proposed risk assessment requires further development and validation to ensure it is workable, effective and applicable as a fishery management tool.

**Health checks on all fish imports destined for fisheries** - Most fish parasites are introduced to new localities with infected hosts. Health screening, including parasitological examinations should be conducted as routine on all fish imported to stock fisheries (this recommendation has already been implemented).

**Horizon scanning for potential parasite invasions** – the flow of non-native parasites into England and Wales is unlikely to slow. Awareness of emerging disease threats within other regions of the world is essential for the early detection of future introductions.

**Protocols to restrict dissemination of new findings or high-risk parasite introductions** - Defined protocols are required to promote consistency of actions following the detection of any non-native parasite. Co-ordination with national enforcement teams are needed to prevent initial spread. Efforts to progress understanding of any newly detected parasite must be an immediate and dynamic

process. Decision-making should take in consideration information from literature reviews, pathological investigations, risk assessments, eradication proposals and involve clear communications to all interested parties.

**Better communication of new findings and future disease risks** - Efforts are necessary to develop better links with fish health professionals and the fisheries industry to improve communication of disease threats and management strategies.

**Greater efforts to attempt eradication of non-native introductions** – although many factors influence the success and feasibility of eradication, a more pro-active approach to attempt eradication of non-native parasites would minimise risks to fisheries and reduce resources associated with long term controls.

**Development of management strategies to reduce the impacts of a parasite within infected fisheries** – where eradication is not a feasible option, efforts are needed to develop management strategies to minimise impacts of introduced diseases to fisheries. Such education is important for the continued maintenance of fishery performance.

**Awareness of effects of climate change upon parasite impacts** – it is well recognised that climate change could have important influences upon host, parasite environment interactions. Awareness of these changes represents an important consideration for future risk assessments.

## 8.2.2 Studies specific to *A. huronensis*

**Confirmation of taxonomic status of British parasites** – Despite considerable taxonomic confusion and morphological inconsistencies, it is important that British parasites are confirmed as either *A. sagittatus* (syn. *M. sagitatta*) or *A. huronensis*. This will clarify the relevance of current literature and route of parasite introduction (this is work is ongoing in collaboration with Dr Ruth Kirk) .

**Effect/pathological changes in very small hosts** – Host size is an important factor influencing effects of intestinal tapeworms. Efforts are needed to confirm histopathological changes in juvenile carp.

**Role of parasite seasonality** - the occurrence of caryophyllaied cestodes in the definitive host is influenced strongly by seasonality. Seasonal changes in parasite populations effect impact as well as detection.

**Role of parasite secretions** – current studies provide evidence of secretions from the neck of *A. huronensis*. Studies involving transmission electron microscopy (TEM) are needed to confirm this and determine the importance of these to host tissues damage.

**Importance of physiological changes** – the role of *A. huronensis* upon disruption to gut function requires elucidation. Although current studies provided a preliminary assessment of this, efforts involving larger sample sizes are necessary.

## 8.2.3 Studies specific to *E. briani*

**Disease potential at different fisheries and times of year** – further studies are needed to study the effects of *E. briani* in different types of fishery (e.g. different water types and host species compositions) and at different times of year. This should take into account different environmental conditions e.g. a very warm summer, a long cold winter.

**Role of *E. briani* upon fishery performance and populations structure within infected cyprinid fisheries** – Due to the difficulties with the capture, handling and examination of fry, impacts may also be measured by improved understanding of effects on fishery performance and population structure (through ageing analysis).

**Threshold for disease in a range of fish species (experimental)** – literature detailing experimental studies provide valuable understanding of the infection threshold for condition loss and mortality (Alston, 1994). It would be very useful to extend such observations to different host species (in particular common bream) as well as 0+ hosts. Such observations may then be applied to observations of parasite prevalence and intensity in wild fish populations.

**Pathology associated with parasite feeding behaviour** – the feeding behaviour of ergasilid parasites can influence the type and severity of pathological changes. Whilst current studies established a number of pathological changes consistent with parasite feeding, these were not conclusive. Further studies with use of scanning electron microscopy are needed to clarify this area of parasite impact.

**8.2.4 Studies specific to** *P. longidigitus*

**Base-line responses of cyprinids to pheromones** – base-line information of normal physiological responses of cyprinids to pheromones is needed to confirm current experimental findings and assist future studies on common carp olfaction.

**Fishery level studies to determine role of** *P. longidigitus* **upon fishery performance** – Anecdotal evidence suggests that recruitment of cyprinid fish is not adversely affected by the presence of *P. longidigitus*. A number of fisheries have reported successful spawning behaviour of cyprinid fish and large numbers of juvenile fish being produced annually. This requires confirmation.

**Confirmation of seasonality** – current studies indicate marked seasonal changes in *P. longidgiitus* populations. Further studies are required to confirm these observations, define environmental parameters that determine these changes and correlate these changes with the reproductive timing of fish species. These observations may be applied to the management of other ergasilid species.

**Measure of seasonal changes in epithelial re-generation** - the damage caused by *P. longidigitus* is balanced against the regenerative capabilities of the nasal epithelium. However, it is unclear at what rate this occurs, intensity of infections necessary to overcome this capacity and the importance of host impacts during spring.

**Presence of** *P. longidigitus* **within estuarine and marine environments** - literature suggests that *P. longidigitus* is capable of infecting fish within both estuarine and

marine environments. This requires confirmation due to the implications for further spread, control and susceptibility of different fishery types.

**Impact of *P. longidigitus* upon the morphology of olfactory rosette, with specific reference to damage and loss of sensory receptors** -it is difficult to corrrelate the damage caused by *P. longidigitus* to loss of sensory function. It is unclear what damage is caused by *P.longidigitus* the abundance and distribution of receptor cells. Histochemistry or scanning electron microcrosopy studies would provide valuable understanding of the specific characteristics of pathological changes.

**Use of electro-olfactograms (EOG)** – The olfactory function of fish may be measure by means of an electro-olfactogram (a measure of electrical responses of olfactory neurons to stimuli). This may represent a more reliable means of testing the olfactory function of fish infected with nare-dwelling parasites.

**Development of parasite populations under controlled conditions** – the study of parasite infections in wild fish populations raises a number of practical and financial difficulties. The ability to artificially infect fish with *P. longidigitus* would ensure availability of infected material and would allow studies to include fish species that have a well documented olfactory pheromone system.

### 8.2.5 Studies specific to *P. sanguinea*

**Translation of foreign literature** – A large proportion of information pertaining to *P. sanguinea* is confined to Asian and Russian literature. Whilst every effort was made to access this information, much of it remained unobtainable. Further efforts including translation of foreign texts are needed to improve understanding of impact and disease characteristics.

**Effect of adult female parasites upon 0+ crucian carp** – Records of disease caused by *P. sanguinea* focus upon infections within fry. Further efforts are needed to assess the susceptibility and impact of parasites upon 0+ hosts. This may be achieved through either field or experimental studies.

**Methods to improve detection of migrating parasites** - the detection of juvenile *P. sanguinea* as well as migrating adult parasites represents a considerable challenge to the study of drancunculoid nematodes. Novel ways of detection (e.g. staining, radio-labelling) would confirm clarify migration routes, aid detection and facilitate pathological investigations.

**Pathological changes associated with parasite migrations** – there is a need to extend observations of pathology caused by migration of parasites within host tissues. These include fertilised females and recently established parasites. Literature suggests that these can be the cause of disease within infected juvenile hosts.

**Seasonality studies** - It is proposed that seasonality is an important influence upon infections of *P. sanguinea* within crucian carp fry. However, further studies are

required to test the hypothesis and to confirm whether the low prevalence and intensity of infection is typical of *P. sanguinea* within fisheries in England and Wales.

**Distribution studies** – current records suggest that *P. sanguinea* has a localised distribution within south-east England. However, this is likely to be limited by issues surrounding detection. Studies are needed to examine the number and distribution of infected fisheries.

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