Larval gryporhynchid tapeworms (Cestoda: Cyclophyllidea) of British freshwater fish, with a description of the pathology caused by *Paradilepis scolecina*

C.F. Williams[1]*, A.J. Reading[1], T. Scholz[2] and A.P. Shinn[3]

[1]Environment Agency, Bromholme Lane, Brampton, Cambridgeshire PE28 4NE, England, UK: [2]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budejovice, Czech Republic: [3]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

Abstract

Larvae of the cyclophyllidean tapeworms *Paradilepis scolecina* (Rudolphi, 1819), *Neogryporhynchus cheilancristrotus* (Wedl, 1855) and *Valipora campylancristrota* (Wedl, 1855), are described from British freshwater fish. The morphometrics of the rostellar hooks, infection characteristics and host ranges of these parasites from fisheries in England and Wales are presented. Difficulties in the detection, handling and identification of these tapeworms are highlighted, and may in part explain the paucity of records from Britain. Tissue digestion was shown to be a useful technique for the examination of these parasites, providing clear and consistent preparations of the rostellar hooks for measurement. The pathological changes caused by *P. scolecina* to the liver of wild tench, *Tinca tinca*, are detailed for the first time. Tapeworms located in the hepatic parenchyma and pancreatic tissues caused little pathological damage and invoked only mild inflammatory responses. The small size of these tapeworms and their encapsulation within host tissues appear to limit the severity of pathology, compared with parasites that insert their rostellum during attachment.

Introduction

Larval cestodes of the family Gryporhynchidae (Cestoda: Cyclophyllidea) infect the internal organs of both brackish and freshwater fish and mature in piscivorous birds (Jarecka, 1970a, b; Kozicka, 1971; Murai *et al.*, 1997). Although these parasites have been recorded from much of Europe, Asia, Africa and America, published information from many geographical localities, including Britain, remains sparse (Khalil & Thurston, 1973; Hoffman, 1999; Natural History Museum Host Parasite Database (http://www.nhm.ac.uk/research-curation/

research/projects/host-parasites/database/); Scholz *et al.*, 2002, 2004, 2008). A recent review of larval gryporhynchid cestodes by Scholz *et al.* (2004), which included data on 21 species worldwide, confirmed a paucity of information on these parasites from the British Isles. This work highlighted the difficulties associated with the detection and identification of these tapeworms and the need to improve understanding of their geographical distribution, host ranges and pathological effects. Molnár (2005) supported this, suggesting that these larval tapeworms can be pathologically important parasites warranting further study.

Compared with other tapeworms of British freshwater fish, members of the Gryporhynchidae have received very little attention. Khatun (1973) provided light micrographs of *Paradilepis scolecina* (Rudolphi, 1819) in

*Fax: + 44 01480 433873
E-mail: chris.williams@environment-agency.gov.uk

a PhD thesis on stickleback parasites, suggesting that this might represent a new species record for Britain. Only *Valipora campylancristrota* (Wedl, 1855), however, was detailed in a subsequent key to the tapeworms of British freshwater fish (Chubb *et al.*, 1987). Kirk (2000) included three species of gryporhynchid tapeworm in a checklist of British fish parasites, but highlighted that these records lacked detail and required confirmation. This scarce and fragmented information has led to uncertainty over current species records and confusion with identification of these tapeworms during parasitological examinations. Chubb *et al.* (1987) emphasized the need for up-to-date species descriptions to promote the rapid and accurate identification of fish parasites and to raise awareness of those species not yet encountered.

This paper provides the first detailed account of larval gryporhynchid cestodes from British freshwater fish. Tissue digestion methodologies are explored as a technique to aid parasite identification. Pathological changes associated with infections of *P. scolecina* in the liver of wild tench, *Tinca tinca* (L.), are described for the first time.

Materials and methods

Sample collection

Records of larval gryporhynchid tapeworms from freshwater fisheries in Britain were collated from both published and grey literature, including unpublished parasitological surveys. Archived material held by the lead author was used to support species identification. A sample of *T. tinca* and rudd, *Scardinius erythrophthalmus* (L.), was obtained from a stillwater fishery in Berkshire, southern England to provide additional material for parasite identification and histopathological study. Fish were killed by an overdose of the anaesthetic benzocaine (Sigma-Aldrich, Poole, Dorset, UK). Parasitological examinations included low-power examinations of the liver, gall bladder and intestinal tract and organ squashes using a compound microscope at 100–400× magnification.

Morphology and morphometry

Morphometrics of the rostellar hooks were recorded according to Bona (1975). Measurements of the hook handle and blade were made using the web-based software 'ImageJ' (Abramoff *et al.*, 2004). Parasites were either examined live, or fixed in hot saline and transferred to 80% ethanol for long-term storage. Berlese fluid and glycerine–ammonium picrate were used to aid clarification of fixed specimens, as described by Ergens (1969) and Baccarani *et al.* (1998). Additional ethanol-fixed specimens of *P. scolecina* were subjected to complete proteolytic digestion in efforts to gain clear rostellar hook preparations. Parasites were rinsed for 20 min in nanopure water and digested with 4.5 µl of a proteinase K-based solution (100 µg/ml proteinase K (Sigma–Aldrich®); 50 mM Tris-HCl; 7 mM EDTA; 3.5% SDS). The state of digestion was monitored by visual observations of specimens at ×20 magnification. Digestion was arrested by the addition of 3.5 µl of a 50:50 formaldehyde:glycerine solution. Preparations were cover slipped (18 × 18 '0' thickness coverslip (VWR International, Lutterworth, Leicestershire, UK)) and sealed in nail varnish. Specimens of *P. scolecina* and *V. campylancristrota* were deposited in the Natural History Museum, London (accession numbers 2009.9.29.1 and 2009.9.29.2, respectively).

Histopathology

Samples of liver from *T. tinca* infected with *P. scolecina* were fixed in Bouin's fixative and processed for histopathological examination. Five-micrometre-thick sections were stained using Mayer's haematoxylin and eosin (H&E) and examined microscopically for pathological changes.

Results

Occurrence and host records of parasites from British freshwater fish

Three species of larval gryporhynchid tapeworm have been recorded from freshwater fish in Britain, namely *P. scolecina*, *Neogryporhynchus cheilancristrotus* (Wedl, 1855) and *V. campylancristrota*. The morphology (fig. 1a) and measurements of the large and small rostellar hooks (table 1) are summarized from specimens recovered from freshwater fisheries in England.

Records of gryporhynchid tapeworms from unpublished parasitological examinations were sparse and included a number of unclear and unconfirmed species identifications. Despite this confusion, records confirm that all three species have long been established in Britain, but differ markedly in distribution.

Adults of *P. scolecina* were first recorded in North Wales from the intestines of cormorants in 1960 (J. Chubb, UK, pers. comm.). Larval parasites were first reported in Britain in 1973, following the examination of three-spine sticklebacks, *Gasterosteus aculeatus* (L.), from a brook in Surrey, south-east England (Khatun, 1973). In the past decade, *P. scolecina* has been confirmed from 16 fisheries in England and Wales, with ten of these observations made in the past 5 years. These records suggest that *P. scolecina* is the most common and widely distributed species of gryporhynchid tapeworm in England and Wales. This parasite has so far been recovered from *G. aculeatus*, *S. erythrophthalmus*, *T. tinca* and crucian carp, *Carassius carassius* (L.).

Neogryporhynchus cheilancristrotus was first recorded from a stillwater fishery in south-eastern England in 1997 (Environment Agency, unpublished). In contrast to *P. scolecina*, this tapeworm has been recorded on only one occasion since, also from a stillwater fishery in southern England. To date, only very small numbers of this parasite (<5) have been recovered from the intestinal tract of common carp, *Cyprinus carpio* L., and *C. carassius*.

Valipora campylancristrota was first reported in England in 1979 (Dearsley *et al.*, 1982), later published in Chubb *et al.* (1987). The parasite has since been confirmed from approximately 14 freshwater fisheries in England and
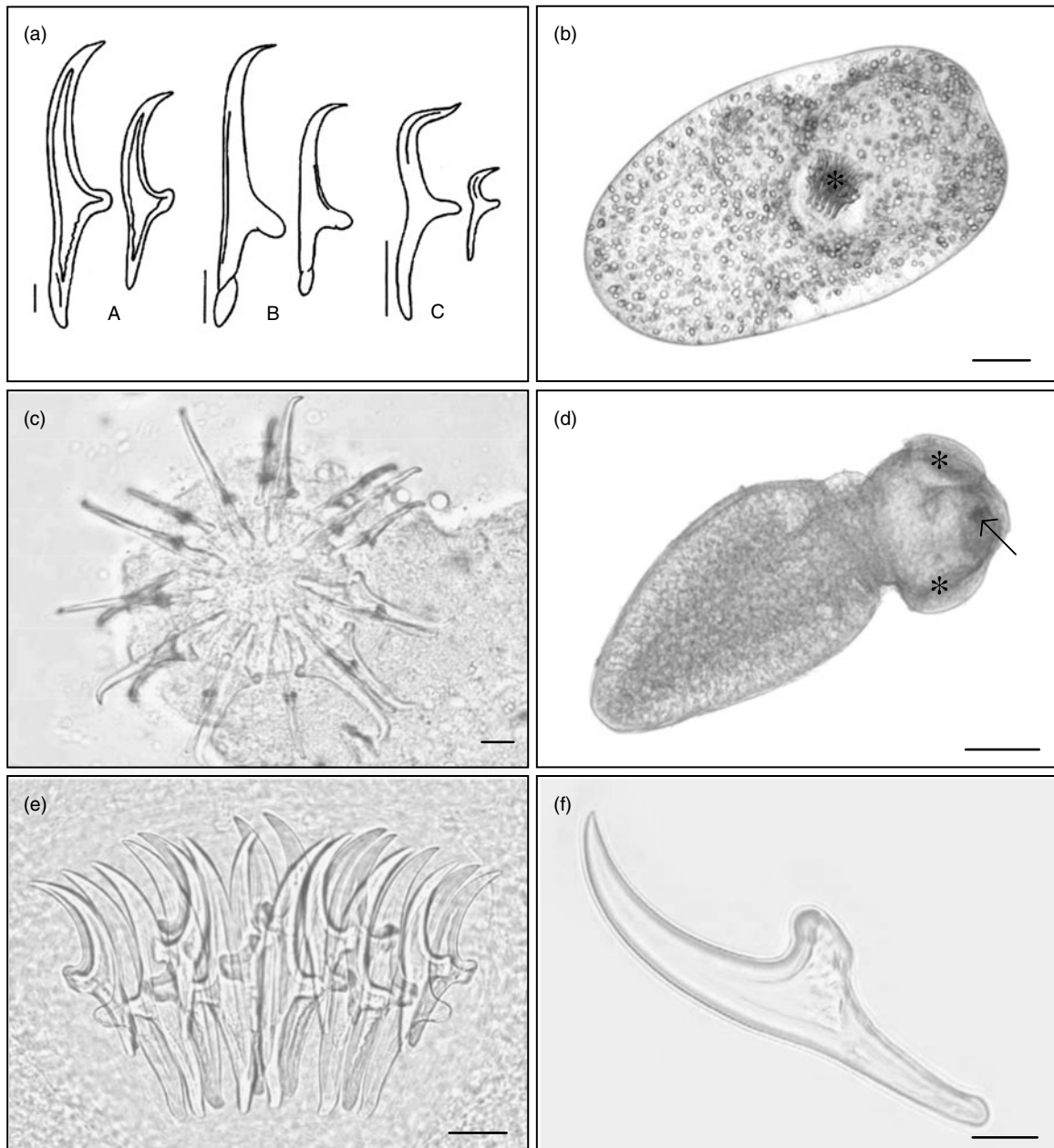
Fig. 1. (a) Morphology of rostellar hooks of *Paradilepis scolecina* (A), *Neogryporhynchus cheilancristrotus* (B) and *Valipora campylancristrota* (C) from freshwater fish in England (scale bar 10 μm). (b) *Paradilepis scolecina* following removal from its thin-walled cyst, showing the fully inverted rostellum (*) (scale bar 100 μm). (c) Extended rostellum of *N. cheilancristrotus* (apical view) following removal from the intestinal tract of *Carassius carassius*, showing concentric rings of ten large and ten small rostellar hooks. (scale bar 10 μm). (d) *Valipora campylancristrota* from the gall bladder of *Cyprinus carpio*, showing suckers (*) and rostellum (arrow) emerging from the body of the parasite (scale bar 100 μm). (e) Squash preparation of *P. scolecina,* showing the overlying hooks of the inverted rostellum (specimen cleared with Berlese fluid) (scale bar 20 μm). (f) Individual small hook of *P. scolecina* following proteolytic digestion (scale bar 10 μm). (A colour version of this figure can be found online at journals.cambridge.org/jhl)

Wales. Host records of this parasite in Britain include *T. tinca*, *C. carassius*, *S. erythrophthalmus*, *C. carpio* and roach, *Rutilus rutilus* (L.). Historical records of this parasite from the liver and intestinal tract of *T. tinca* and *C. carassius* require confirmation as these represent atypical locations for this species, and possible misidentifications of other species cannot be ruled out. There are currently no published records of gryporhynchid cestodes from Scotland or Northern Ireland (D. Evans, UK, pers. comm.).

Table 1. Measurements of rostellar hooks of larval gryporhynchid tapeworms from British freshwater fish (measurements taken from a minimum of three large and three small hooks of each specimen examined).

Species (specimens examined)	Large hook (μm)				Small hook (μm)			
	Length	Blade	Handle	B/H ratio	Length	Blade	Handle	B/H ratio
Paradilepis scolecina [a] (*n* = 8)	103–107	56–60	48–52	1.09–1.23	72–77	38.5–42	34–37	1.11–1.18
Neogryporhynchus cheilancristrotus [b] (*n* = 1)	56–59	36–40	21.5–24	1.60–1.69	34–39	21–24.5	15–17.5	1.21–1.42
Valipora campylancristrota [c] (*n* = 6)	24–28	10–13	14–17	0.69–0.86	11–12.5	3.5–4.5	7.5–9	0.45–0.49

Hook measurements were based on specimens obtained from [a]*Scardinius erythrophthalmus*, [b]*Cyprinus carpio* and [c]*Tinca tinca*.
B/H ratio, blade/handle ratio.

Detection and attachment characteristics

Paradilepis scolecina were found exclusively on the external surfaces of the liver and gall bladder of the tench and rudd. Heavy infections, comprising up to 200 parasites per host, were conspicuous during low-power microscopic examinations. Light infections dispersed throughout the liver and connective tissues surrounding the gall bladder were less obvious and required thorough microscopic examinations. *Paradilepis scolecina* were recorded in oval cysts, measuring approximately 1 mm in length. The thickness of the cyst wall varied considerably between parasites, presumably related to longevity of infection. Parasites within these cysts varied between 400 and 900 μm in length and usually possessed an invaginated rostellum (fig. 1b). Activity of these tapeworms often increased markedly following removal from the host. For parasites in thin-walled cysts, this movement occasionally led to their unaided emergence, with elongation of the body and either partial or full extension of the suckers and hooks. However, parasites in thicker cysts, maintaining an inverted rostellum, were also recovered. Detailed examinations of heavy *P. scolecina* infections in *T. tinca* revealed both live and degenerate parasites, the latter characterized by greater opacity and thickness of the cyst wall. Microscopic examination of these cysts revealed only remnants of recognizable hooks.

Detection of *N. cheilancristrotus* within the intestine required microscopic examination due to the near transparency of these very small tapeworms and their attachment between the intestinal folds. Detection was aided by the slow movement of these parasites and the presence of small, raised nodules which surrounded them. Specimens of *N. cheilancristrotus* recovered from the intestine of *C. carassius* measured approximately 350 μm in length. Most of the parasites found within the intestine possessed an inverted rostellum, although a small number of specimens with extended hooks (fig. 1c) were also recovered.

Detection of *V. campylancristrota* was relatively straightforward due to the localization of tapeworms within the gall bladder. Parasites were usually found attached by their suckers to the internal wall of the gall bladder and bile duct. Specimens removed from the gall bladder measured between 450 and 900 μm in length, depending on extension or retraction of the scolex. Parasites usually became increasingly active following removal from the host, with gradual emergence of suckers and occasionally the rostellum (fig. 1d).

A number of inactive parasites with permanently inverted hooks were also recovered.

Parasite handling and identification

Gaining clear preparations of the rostellar hooks for measurement was dependent upon the size and position of the rostellum. The circular arrangement of these overlaying structures occasionally impeded clear and consistent hook preparations. The addition of Berlese fluid aided clarity of these structures (fig. 1e) and was considered essential for the examination of fixed parasites, especially those with inverted hooks.

Tissue digestion provided clear and consistent hook preparations (fig. 1f) irrespective of parasite condition and rostellum position. Full parasite digestion took approximately 2–3 min and resulted in the disconnection of hooks from their normal circular arrangement. This reduced the risk of hook damage through excessive flattening, producing clear hook preparations in a single plane. Maintaining parasites on a microscope slide during the process of digestion eliminated risks of hook damage or loss.

Histopathological changes associated with *P. scolecina*

Paradilepis scolecina was recorded in all of the *T. tinca* examined for histopathological study (*n* = 30), with a maximum intensity of approximately 120 parasites per host. Tapeworms were recorded primarily on the surface of the liver, but also throughout the hepatic parenchyma and occasionally within the exocrine pancreatic tissues. Live parasites were encapsulated within a thin wall of connective tissue (fig. 2a). A small number of lymphocytes were recorded in the immediate proximity of encysted *P. scolecina.* The inflammatory changes associated with these parasites, however, were generally mild. The use of Bouin's fixative resulted in the uptake of picric acid by the rostellar hooks, promoting detection of these tapeworms within tissue sections. Depending upon the orientation of sectioning, both morphology and rostellar hook number (fig. 2b) were occasionally distinguishable.

Heavy infections of *P. scolecina* within the liver of infected *T. tinca* comprised both live tapeworms as well as parasites in varying states of degeneration. This included aggregations of degenerate tapeworms encapsulated within thickened layers of connective tissue (fig. 2c). Hook fragments discharged from a small number of degenerate parasites were occasionally found lodged
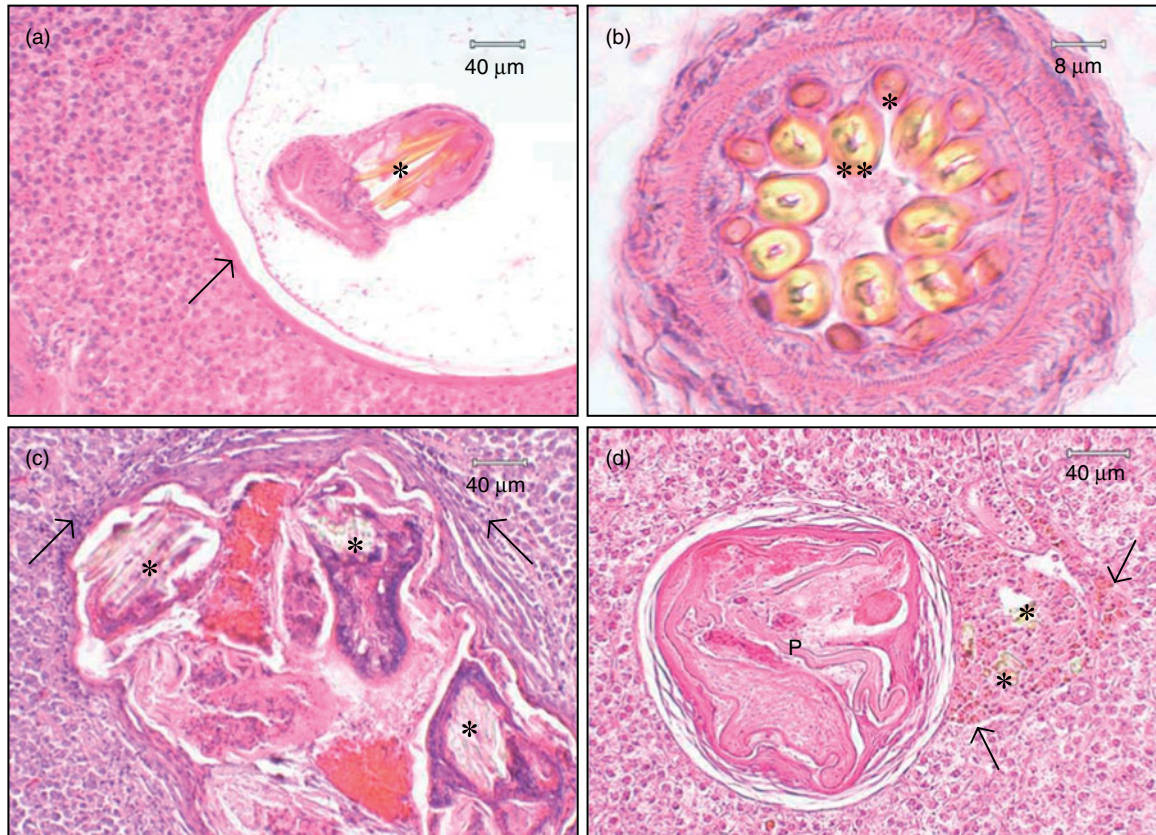
Fig. 2. (a) Histopathological section of *Paradilepis scolecina* encysted within the liver of *Tinca tinca*. The parasite, exhibiting stained hooks (*), is separated from the hepatic parenchyma by a thin wall of connective tissue (arrow). (b) Transverse section through the rostellum of *P. scolecina* showing concentric rings of ten small (*) and ten large (**), hollow rostellar hooks. (c) Aggregation of four degenerate *P. scolecina* encapsulated in a fibrous cyst within the liver of *T. tinca*. Remnants of the rostellar hooks (*) can be seen along with lymphocytes (arrows) within the surrounding hepatic tissue. (d) Hook fragments (*) discharged from an encysted, degenerate *P. scolecina* (P) located within the hepatic parenchyma of *T. tinca*. These structures are surrounded by a focus of macrophages engorged with light brown pigment (arrows).

within the hepatic parenchyma. These were surrounded by a focus of macrophages engorged with light brown pigment (fig. 2d). In all of the tench examined, the hepatic cells throughout the liver were considered normal, even during infections comprising over 100 parasites.

Discussion

This paper provides the first descriptions of *P. scolecina*, *V. campylancristrota* and *N. cheilancristrotus* from British freshwater fish. The rostellar hooks of these tapeworms conform to previous morphological descriptions (Priemer & Scholz, 1989; Scholz, 1989a, b; Baccarani *et al.*, 1998; Scholz & Salgado-Maldonado, 2001; Scholz *et al.*, 2004). It is hoped that this paper will complement existing keys of fish tapeworms, aiding the accurate identification of these small and inconspicuous parasites (Chubb *et al.*, 1987).

Records suggest that gryporhynchid tapeworms are infrequently encountered during parasitological examinations and that *N. cheilancristrotus* may have a particularly sparse distribution in England. This is perhaps surprising in view of the wide fish-host ranges of these parasites, the role of common piscivorous birds as definitive hosts,

the number and frequency of fish movements in England and Wales further facilitating spread, and the large numbers of parasitological examinations conducted annually on susceptible fish species from both wild and farmed fish populations (Jarecka, 1970a, b; Kennedy, 1974; Kirk, 2000; Scholz *et al.*, 2004; Environment Agency and Cefas Live Fish Movement Database 2009). In contrast, the larval tapeworms *Ligula intestinalis* (L.), *Schistocephalus solidus* (Müller, 1776), *Triaenophorus nodulosus* (Pallas, 1781) and *Diphyllobothrium* spp. have been documented throughout Britain and have received considerable attention (Fraser, 1960; Arme & Kennedy, 1968; Kennedy, 1974; Chubb *et al.*, 1987; Hoole, 1994; Barber *et al.*, 2000; Dezfuli *et al.*, 2007). This interest has undoubtedly been stimulated by the pronounced clinical abnormalities caused by these large plerocercoid infections and the pathogenic importance of these parasites to fish populations. In contrast, the small size and relatively benign appearance of gryporhynchid cestode infections, as well as the lack of comprehensive literature detailing their identification and impact, may have limited awareness, understanding and interest in these parasites in Britain (Kennedy, 1974).

The detection of tapeworms in fish is generally a straightforward process (Chubb *et al.*, 1987; Hoole *et al.*, 2001). Although the detection and identification of larval gryporhynchid tapeworms is not necessarily difficult, it is more problematic than for most fish tapeworms. Protocols that omit organ squashes and gall bladder examinations (Hoole *et al.*, 2001) risk failed detection. Even when encountered, the tiny size and appearance of these parasites differ markedly from that of other adult and larval cestodes, providing the potential for taxonomic uncertainty. This is particularly evident for species that encyst within host tissues or possess an inverted scolex. The absence of *N. cheilancristrotus* and *P. scolecina* from existing keys of British tapeworms (Chubb *et al.*, 1987) and limitations of alternative texts prior to the comprehensive review of Scholz *et al.* (2004) may have impeded accurate identification of these tapeworms in Britain.

Records of tapeworms from historical parasitological examinations highlight two notable observations, namely a scarcity of records of the intestinal parasite *N. cheilancristrotus* and, in comparison with other species, increasing reports of *P. scolecina* within the past decade. It is proposed that this may reflect differences in the detection of these parasites, but also the abundance of their definitive hosts.

The primary definitive hosts of *V. campylancristrota* and *N. cheilancristrotus* are herons, *Ardea* spp., whereas *P. scolecina* matures in the cormorant, *Phalacrocorax carbo* (L.) (Jarecka, 1970a, b; Pietrock & Scholz, 2000; Scholz *et al.*, 2004). Although these birds are common residents of freshwater fisheries, cormorant populations have undergone a rapid increase in the past decade (Mitchell *et al.*, 2004; Mavor *et al.*, 2006; Newson *et al.*, 2007). Until the 1980s, the cormorant was almost exclusively a coastal breeding bird, but has since established inland colonies throughout England and Wales. Although adult *P. scolecina* have been recorded in cormorants in Britain since 1960, it is likely to be the rapid increase in abundance of these birds that has led to more frequent findings of their larvae in freshwater fish. The potential for very large numbers of cormorants to frequent individual fisheries may, in turn, increase infections within resident fish populations, thus increasing the likelihood of successful detection. In view of the continued rise in the number of cormorants breeding inland, it is likely that *P. scolecina* will become an increasingly common parasite of freshwater fisheries.

The scarcity of records for *N. cheilancristrotus* is not so easily explained. Herons are common piscivorous birds in Britain (Sterry, 1997) and the intestines of cyprinid fish have been subjected to large numbers of parasitological examinations spanning many decades. Pietrock & Scholz (2000) detailed a number of factors that may dictate the prevalence of gryporhynchid larvae in fisheries, including seasonality, copepod abundance, and the distribution and feeding behaviour of both intermediate and definitive hosts. Current observations, however, suggest that the scarcity of records for *N. cheilancristrotus* may also be due to the inconspicuous appearance of this parasite within the fish host.

The relatively large size of encysted *P. scolecina* and their attachment to the external surfaces of the liver aid detection during parasitological examinations. Although slightly smaller in size, the mobility of *V. campylancristrota* and their localized attachment within the gall bladder also makes detection a relatively straightforward process during low-power microscopic examinations.

In contrast, *N. cheilancristrotus* recovered from the intestines of cyprinid fish were much smaller and harder to detect. The literature highlights considerable variation in the size of *N. cheilancristrotus* from fish, with specimens measuring as little as 180 μm reported from the intestines of cyprinid fish in Europe (Scholz, 1989a; Pietrock & Scholz, 2000). This factor, combined with the large surface area of the intestinal tract, near transparency of these parasites and attachment between the intestinal folds, can make detection problematic, especially during light infections. Records of *V. campylancristrota* from the intestines of cyprinid fish (Environment Agency, unpublished) require confirmation as it is feasible that these represent misidentifications for *N. cheilancristrotus* (Scholz *et al.*, 2004).

Despite these difficulties, it is clear that *P. scolecina*, *N. cheilancristrotus* and *V. campylancristrota* exhibit different site preferences within the fish host and possess hooks of markedly different sizes. This simplifies the identification of the species present in Britain, even allowing tentative species identification from straightforward post-mortem observations. However, due to the morphological similarity of other gryporhynchid species (Scholz *et al.*, 2004, 2008) and potential for the introduction of tapeworms to new localities with fish movements or bird migrations (Chubb & Yeomans, 1995), it is vital that the identification of these tapeworms is based on clear rostellar hook measurements (Bona, 1975; Scholz *et al.*, 2008).

The fastest and most simple approach for the identification of these parasites is to flatten live specimens on a microscope slide, followed by the subsequent measurement of hooks. The examination of fixed specimens benefits from clearing techniques that clarify individual hooks in squash preparations. This may be achieved through use of glycerine–ammonium picrate, which provides semi-permanent mounts for medium-term storage (Ergens, 1969). However, this process can be time-consuming and preparations are susceptible to crystallization, requiring re-mounting after 2–3 years (Ergens, 1969). Clarification of parasites with Berlese fluid was found to be very quick and overcomes many of these problems. This is considered the most desirable method for the routine examination of these larval tapeworms, providing clear preparations that can be sealed if permanent mounts are required.

Tissue digestion methodologies, identical to those employed for the identification of monogenean parasites (Shinn *et al.*, 2001), provided an alternative approach to aid tapeworm identification. Although not always necessary, this technique was simple and rapid, providing a consistent and reliable way of obtaining very clear hook preparations, irrespective of rostellum position, hook size or quality of specimen fixation. This makes it a suitable technique if there is limited material available for study, or if subsequent examinations of the rostellar hooks are needed (e.g. scanning electron microscopy). Tissue digestion is also considered to be a valuable and reliable method for the differentiation of morphologically

similar species and deposition of reference material. This is important in view of the lack of good-quality reference material that exists for these parasites, a factor that has contributed to considerable taxonomic confusion and persistent nomenclatural problems (Bona, 1975; Scholz, 1989a; Scholz et al., 2004).

Literature on the pathology of gryporhynchid cestodes is largely confined to the species N. cheilancristrotus and V. campylancristrota (Bauer et al., 1969; Körting, 1984; Molnár, 2005). Reports suggest that these parasites can be pathogenic to both fish and bird hosts (Karstad et al., 1982; Molnár, 2005). Valipora campylancristrota, the cause of valiporosis, is considered of pathogenic importance to common carp, C. carpio (Bauer et al., 1969; Sapozhnikov et al., 1974; Schäperclaus et al., 1991). According to these latter authors, carp fry lost condition and even died as a consequence of heavy parasite infections. Jara & Orech (1964) described marked congestion and mechanical damage to intestinal blood vessels as a result of V. campylancristrota infections in common carp (recorded as Cysticercus dilepis-campylancistrotae Aubert), although no adverse influences on the weight gain of infected fry were found.

Körting (1984) described focal necrosis, haemorrhage and signs of enteritis resulting from the attachment of N. cheilancristrotus in the intestines of carp. Molnár (2005) supported these findings with detailed observations of intestinal pathology caused by N. cheilancristrotus. This latter author proposed that this species, as well as other gryporhynchid cestodes, may be considered pathogenic parasites of fish. It is not known, however, whether this statement is consistent for other gryporhynchid species, in particular P. scolecina (Scholz, 1989b; Molnár, 2005). Karstad et al. (1982) detailed the pathology of adult P. scolecina in the intestines of wild cormorants, P. carbo, from Kenya. Parasite attachment involved near complete penetration of the scolex through the gut, accompanied by fibrosis and pronounced inflammatory responses. Murai et al. (1997) described the component layers of the cysts surrounding P. scolecina metacestodes on the gall bladder of common bream. However, these authors highlighted that the pathological significance of larval P. scolecina in the fish host remains unclear.

During the current study, T. tinca heavily infected with P. scolecina exhibited no gross abnormalities or clinical signs of disease. The pathological changes caused by these infections to the liver, pancreas and surface of the gall bladder were generally localized and mild. The hepatocytes of heavily infected individuals showed no obvious signs of abnormality and were interspersed with only mild inflammatory responses. These changes were not considered of pathological importance to the fish examined. In contrast, Cone & Anderson (1977) provided some evidence that heavy infections of larval gryporhynchid parasites in the livers of pumpkinseed, Lepomis gibbosus L., were capable of mortality. Furthermore, it is well documented that heavy infections of other larval tapeworms can cause significant liver pathology, leading to host debilitation and even death (Williams & Jones, 1994).

Heavy infections of Triaenophorus nodulosus plerocercoids in the livers of perch, Perca fluviatilis L., can cause serious pathological alterations leading to reduced growth (Brinker & Hamers, 2007). Plerocercoids of Diphyllobothrium ditremum (Creplin, 1825) are also known to cause severe pathological lesions in the livers of salmonid fish, resulting in pronounced liver dysfunction and mortality during heavy infections (Weiland & Meyers, 1989). Current observations suggest that this is an unlikely consequence of P. scolecina infection. This is consistent with observations of Saraiva et al. (2009), who detailed chronic inflammatory reactions in common carp from infections of Parvitaenia samfyia (Mettrick, 1967) and an unidentified species of Cyclustera, but no obvious detrimental impacts on heavily infected hosts. It is proposed that the very small size of these tapeworms and their encapsulation within host tissues limit damage to infected fish. These observations contrast with species that use their rostellum to penetrate host tissues during attachment (Karstad et al., 1982; Molnár, 2005). It is likely that both depth of attachment and insertion of hooks into host tissues are responsible for the greatest organ damage and host responses to infection. Observations during the current study of inflammatory reactions, including macrophage accumulations around fragments of rostellar hooks discharged from degenerate P. scolecina, provide support for this.

Acknowledgements

References

Abramoff, M.D., Magelhaes, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International* **11**, 36–42.

Arme, C. & Kennedy, C.R. (1968) Occurrence and pathology of *Ligula intestinalis* infections in British fishes. *Journal of Parasitology* **54**, 272–280.

Baccarani, E.M., Bona, F.V., Buriola, E. & Canestri-Trotti, G. (1998) Larval cestode infections in tench (*Tinca tinca*). *Parassitologia* **40**, 5.

Barber, I., Hoare, D. & Krause, J. (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries* **10**, 131–165.

Bauer, O.N., Musselius, V.A. & Strelkov, Yu.A. (1969) *Diseases of pond fishes*. 220 pp. Moscow, Izdatel'stvo

Kolos (in Russian; English translation: Israel Program for Scientific Translations, Jerusalem).

Bona, F.V. (1975) *Etude critique et taxonomique des Dilepididae Fuhrman, 1907 (Cestoda) parasites des Ciconiiformes.* 750 pp. Rome, Consigilio Nazionale delle Ricerche.

Brinker, A. & Hamers, R. (2007) Evidence for negative impact of plerocercoid infection of *Triaenophorus nodulosus* on *Perca fluviatilis* L. stock in upper Lake Constance, a water body undergoing rapid reoligotrophication. *Journal of Fish Biology* **71**, 129–147.

Chubb, J.C. & Yeomans, W.E. (1995) *Khawia sinensis* Hsü, 1935 (Cestoda: Caryophyllidea) a tapeworm new to the British Isles: a threat to carp fisheries? *Fisheries Management and Ecology* **2**, 263–277.

Chubb, J.C., Pool, D.W. & Veltkamp, C.J. (1987) A key to the species of cestodes (tapeworms) parasitic in British and Irish freshwater fishes. *Journal of Fish Biology* **31**, 517–545.

Cone, D.K. & Anderson, R.C. (1977) Parasites of pumpkinseed (*Lepomis gibbosus* L.) from Ryan Lake, Algonquin, Ontario. *Canadian Journal of Zoology* **55**, 1410–1423.

Dearsley, A.F., Moore, J.F. & Thomas, R.M. (1982) *A checklist of fish parasites encountered in Lea Division between 1976 and 1981 with maps showing their distribution.* 97 pp. Thames Water, Lea Division.

Dezfuli, B.S., Pironi, F., Simoni, E., Shinn, A.P. & Giari, L. (2007) Selected pathological, immunohistochemical and ultrastructural changes associated with an infection by *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Cestoda) plerocercoids in *Coregonus lavaretus* (L.) (Coregonidae). *Journal of Fish Diseases* **30**, 471–482.

Ergens, R. (1969) The suitability of ammonium picrate–glycerin in preparing slides of lower Monogenoidea. *Folia Parasitologica* **16**, 320.

Fraser, P.G. (1960) The occurrence of *Diphyllobothrium* in trout, with special reference to an outbreak in the west of England. *Journal of Helminthology* **34**, 59–72.

Hoffman, G.L. (1999) *Parasites of North American freshwater fishes.* 2nd edn. 539 pp. London, Comstock Publishing.

Hoole, D. (1994) Tapeworm infections in fish; past and future problems. pp. 119–141 *in* Pike, A.W. & Lewis, J.W. (*Eds*) *Parasitic diseases of fish.* UK, Samara Press.

Hoole, D., Bucke, D., Burgess, P. & Wellby, I. (2001) *Diseases of carp and other cyprinid fishes.* 364 pp. London, Blackwell.

Jara, Z. & Orech, W. (1964) Zmiany w jelicie karpi (*Cyprinus carpio* L.) spowodowane obecnością *Cysticercus dilepidis campylancristrotae* (Aubert). *Wiadomozci Parazytologyczne* **10**, 521–522.

Jarecka, L. (1970a) On the life cycles of *Paradilepis scolecina* (Rud., 1819) Hsü, 1935, and *Neogryporhynchus cheilancristrotus* (Wedl, 1855) Baer and Bona, 1958–1960 (Cestoda – Dilepididae). *Bulletin de l'Academie Polonaise des Sciences (Classe II)* **15**, 159–163.

Jarecka, L. (1970b) Life cycle of *Valipora campylancristrota* (Wedl, 1855) Baer and Bona 1958–1960 (Cestoda – Dilepididae) and the description of cercoscolex – a new type of cestode larva. *Bulletin de l'Academie Polonaise des Sciences (Classe II)* **28**, 99–102.

Karstad, L., Sileo, L., Okech, G. & Khalil, L.F. (1982) Pathology of *Paradilepis scolecina* (Cestoda: Dilepididae) in the white-necked cormorant (*Phalacrocorax carbo*). *Journal of Wildlife Diseases* **18**, 507–509.

Kennedy, C.R. (1974) A checklist of British and Irish freshwater fish parasites with notes on their distribution. *Journal of Fish Biology* **6**, 613–644.

Khalil, L.F. & Thurston, J.P. (1973) Studies on the helminth parasites of Uganda including the descriptions of two new species of digeneans. *Revue de Zoologie et de Botanique Africaines* **87**, 209–248.

Khatun, A. (1973) Ecological studies on the parasites of the three-spined stickleback *Gasterosteus aculeatus* L. Unpublished PhD thesis, University of London.

Kirk, R.S. (2000) *Checklist of parasites from British freshwater fish.* 100 pp. Bristol, Environment Agency.

Körting, W. (1984) Larval cyclophyllidean cestodes in carp and tench. *Bulletin of the European Association of Fish Pathologists* **4**, 40–41.

Kozicka, J. (1971) Cestode larvae of the family Dilepididae Fuhrmann, 1907 parasitizing fresh-water fish in Poland. *Acta Parasitologica Polonica* **19**, 85–93.

Mavor, R.A., Parsons, M., Heubeck, M. & Schmitt, S. (2006) *Seabird numbers and breeding success in Britain and Ireland, 2005.* 107 pp. UK Nature Conservation no. 30. Peterborough, Joint Nature Conservancy Council.

Mitchell, P.I., Newton, S.F., Ratcliffe, N. & Dunn, T.E. (2004) *Seabird populations of Britain and Ireland.* London, Poyser, T. & Poyser, A.D.

Molnár, K. (2005) Histopathological changes caused by the metacestodes of *Neogryporhynchus cheilancristrotus* (Wedl, 1855) in the gut of the gibel carp, *Carassius gibelio*. *Acta Veterinaria Hungarica* **53**, 45–52.

Murai, É., Molnár, K. & Gubanyi, A. (1997) Occurrence of the adult and plerocercus forms of *Paradilepis scolecina* (Rudolphi, 1819) (Cestoda: Dilepididae) in Lake Balaton, Hungary. *Biologica Hungarica* **29–30**, 33–38.

Newson, S.E., Marchant, J.H., Ekins, G.R. & Sellers, R.M. (2007) The status of inland-breeding great cormorants in England. *British Birds* **100**, 289–299.

Pietrock, M. & Scholz, T. (2000) Morphometry and seasonal occurrence of metacestodes of *Neogryporhynchus cheilancristrotus* (Cyclophyllidea: Dilepididae) in the blue bream (*Abramis ballerus*) from the Oder River (Germany/Poland). *Folia Parasitologica* **47**, 181–185.

Priemer, J. & Scholz, T. (1989) Larvae of Dilepididae (Cestoda) in *Cyprinus carpio* from the DDR. *Angewandte Parasitologie* **30**, 27–30.

Sapozhnikov, G.I., Skvortsova, R.K. & Ladukhin, A.I. (1974) Dilepidosis of carp. *Veterinaria* **6**, 73–74 (in Russian).

Saraiva, A., Boane, C. & Cruz, C. (2009) Effects of gryporhynchid metacestodes (Cestoda: Cyclophyllidea) in common carp (*Cyprinus carpio*) from Mozambique. *Bulletin of the European Association of Fish Pathologists* **29**, 139–143.

Schäperclaus, W., Kulow, H. & Schreckenbach, K. (*Eds*) (1991) *Fish diseases.* 5th edn. 1398 pp. New Delhi, Oxion Press.

Scholz, T. (1989a) Amphilinida and Cestoda, parasites of fish in Czechoslovakia. *Acta Scientiarum Naturalium Brno* **23**, 1–56.

Scholz, T. (1989b) First record of the larva of *Paradilepis scolecina* (Rudolphi, 1819) (Cyclophyllidea: Dilepididae) in fishes from Czechoslovakia. *Folia Parasitologica* **36**, 65–66.

Scholz, T. & Salgado-Maldonado, G. (2001) Metacestodes of the family Dilepididae (Cestoda: Cyclophyllidae) parasitizing fishes in Mexico. *Systematic Parasitology* **49**, 23–40.

Scholz, T., Steele, E., Beckham, M. & Bray, R. (2002) Larval tapeworms (Cestoda: Dilepididae) from the mummichog *Fundulus heteroclitus* (Linnaeus, 1766) and striped killifish *Fundulus majalis* (Walbaum, 1792) from South Carolina, U.S.A. *Comparative Parasitology* **69**, 104–108.

Scholz, T., Bray, R.A., Kuchta, R. & Řepová, R. (2004) Larvae of gryporhynchid cestodes (Cyclophyllidea) from fish: a review. *Folia Parasitologica* **51**, 131–152.

Scholz, T., Boane, C. & Saraiva, A. (2008) New metacestodes of gryporhynchid tapeworms (Cestoda: Cyclophyllidea) from carp (*Cyprinus carpio*) from Mozambique, Africa. *Comparative Parasitology* **75**, 315–320.

Shinn, A.P., Gibson, D.I. & Sommerville, C. (2001) Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* **24**, 83–97.

Sterry, P. (1997) *Complete British wildlife*. 384 pp. London, Harper Collins.

Weiland, K.A. & Meyers, T.R. (1989) Histopathology of *Diphyllobothrium ditremum* plerocercoids in coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* **6**, 175–178.

Williams, H. & Jones, A. (1994) *Parasitic worms of fishes*. 593 pp. London, Taylor & Francis Ltd.