CHAPTER ONE

The European eel *Anguilla anguilla*

1. Introduction

Eels are important long-lived bony fish, with a complex life cycle, belonging to the family Anguillidae. The morphology of anguillid eels is quite similar, however; the colour, body size, position of the dorsal fin and maxillary bands of the teeth may vary (Proman & Reynolds, 2000; Teng *et al.*, 2009; Watanabe *et al.*, 2009; Leander *et al.*, 2012). In general, anguillids are categorized as either temperate or tropical and these vary in their distribution and spawning seasons (Arai, 2014; Miller *et al.*, 2014; Watanabe *et al.*, 2014).

1.1 The genus *Anguilla* (Schrank, 1798)

The genus *Anguilla* consists of 20 species of eel that are widely distributed throughout the world (Lintas *et al.*, 1998; Lecomte-Finiger, 2003). Morphological characteristics of the species often overlap, including those of the two species found in the Atlantic; *A. anguilla* and *A. rostrata* (Lintas *et al.*, 1998). Both species spawn in the Sargasso Sea, but they differ in morphometric and genetic traits and are considered as two separate species with a relatively recent evolutionary divergence (Lecomte-Finiger, 2003; Jacobsen *et al.*, 2014).

The European eel, *Anguilla anguilla*, contributes to the ecosystems of the sea, rivers and lakes across Europe. Indeed, the eel ensures a balanced ecology both as a predator and a prey species (Musumeci *et al.*, 2014). The eel diet changes with age (Musumeci *et al.*, 2014). Eel larvae are thought to feed on plankton, or organic detritus (Fitzsimons *et al.*, 2013) and elvers on aquatic insects and small crustaceans (Jellyman, 1989). The yellow eel diet mostly comprises of insect larvae, fish, crab, worms and clams (Wenner & Musick, 1975), whilst larger yellow eels and immature silver eels have a diet that is predominantly piscivorous (Dörner & Benndorf, 2003). Upon migration, adult eels do not feed (van Ginneken *et al.*,

2005). With respect to certain features of the eel life-cycle, including aspects of their migration (Tsukamoto *et al.*, 2002; Watanabe *et al.*, 2014), there remains a paucity of knowledge and hence the European eel is considered an enigmatic animal that is worthy of further detailed study (Miller *et al.*, 2014).

Eels are catadromous; adults spawn in marine environments and the hatched larvae, known as lepotocephali, are transported back to continental areas by oceanic currents (Kimura *et al.*, 1994). Metamorphosis occurs to form the glass eel and this developmental stage enters estuarine waters (Tsukamoto *et al.*, 2002). Within freshwater, further growth and development occurs, resulting in formation of the yellow eel. After a period of at least five years, developmental changes generate a silver eel which then migrates to the spawning grounds. Following spawning, all adult eels die. The precise location of the spawning grounds remains unknown; however, Japanese eel eggs have been observed near the West Mariana Ridge (Tsukamoto *et al.*, 2002). In contrast to the long migrations made by temperate eels, tropical eel species have much shorter migrations and spawn closer to their freshwater habitats (Aoyama *et al.*, 2003; Arai, 2014).

The larvae of temperate eel species usually enter estuaries between early winter and late spring, which is indicative of seasonal spawning (Friedland *et al.*, 2007; Arai, 2014). Indeed, the mass migration of temperate eels to spawning grounds primarily occurs throughout autumn and winter months, with some slight variation between the species (Wang & Tzeng, 2000; Aoyama *et al.*, 2003). In contrast, tropical eel larvae are found within estuaries throughout the year, suggesting that they are potentially spawning all year round (Tabeta *et al.*, 1976; Arai *et al.*, 2000). The precise factors that cause initiation of migration in sufficiently mature eels remain unknown; however, rainfall/water levels, water temperature and weather conditions are hypothesized as important (Lowe, 1952; Acou *et al.*, 2008; Tsukamoto, 2009).

The sexual differentiation of eels into male and female is not fully understood, though it is a process that occurs in older, silver eels, and population density is thought to have a role (Beullens *et al.*, 1997; Tzeng *et al.*, 2000). Males are likely to be produced in population dense environments that are typically downstream, with increased temperature and saline conditions, whereas females are most likely generated in more upstream, lower density environments (Wenner & Musick, 1974; Davey & Jellyman, 2005).

1.1.1 Biology and life-cycle of *Anguilla anguilla*

Throughout much of their life cycle the European eel, *A. anguilla*, is distributed across estuarine and freshwater habitats of Northern and Western Europe and the Mediterranean Sea (Wheeler, 1977; Maitland & Lyle, 1991; Dekker, 2003b). It is a long-lived fish, with reproductive ages ranging between 6 and 60 years (Casselman, 2003). Adults undergo a long migration across the Atlantic Ocean to reach remote spawning grounds in the eastern Sargasso Sea (Aoyama, 2009) (Figure 1.1). After spawning, the adults die and fertilized eggs undergo embryo development, resulting in leptocephali larvae hatching 47-60 hours post-fertilization (Pedersen, 2003, 2004). Leptocephali are carried by the Gulf Stream and also, via countercurrents that facilitate their transport northeastward (Miller *et al.*, 2014). Much remains unknown about the leptocephali during transport across the Atlantic Ocean, not least the duration of the journey, which is estimated to vary between 1.5 to 3 years (Kleckner & McCleave, 1982; Knights, 2003; Miller *et al.*, 2014). Upon reaching the continental shelf, the larvae metamorphose into glass eels. Glass eels enter coastal waters and rivers whereupon they darken in colour to yellow-brown elvers, which migrate further into rivers and then develop into yellow eels. The yellow eel may inhabit freshwater for extended periods of between 5 and 50 years (Melià *et al.*, 2006). Once a yellow eel has stored sufficient body fat, it becomes a partially mature silver eel. Eventually, further development results in formation of the silver eel and this developmental stage reduces feeding and the gonads begin to

develop (Han *et al.*, 2001; Durif *et al.*, 2006). Unknown factors trigger the complete cessation of feeding and the silver eel then initiates migration to the natal spawning grounds in the Sargasso Sea; a process that is thought to take between 3.5 to 6 months (Pelster, 2015).

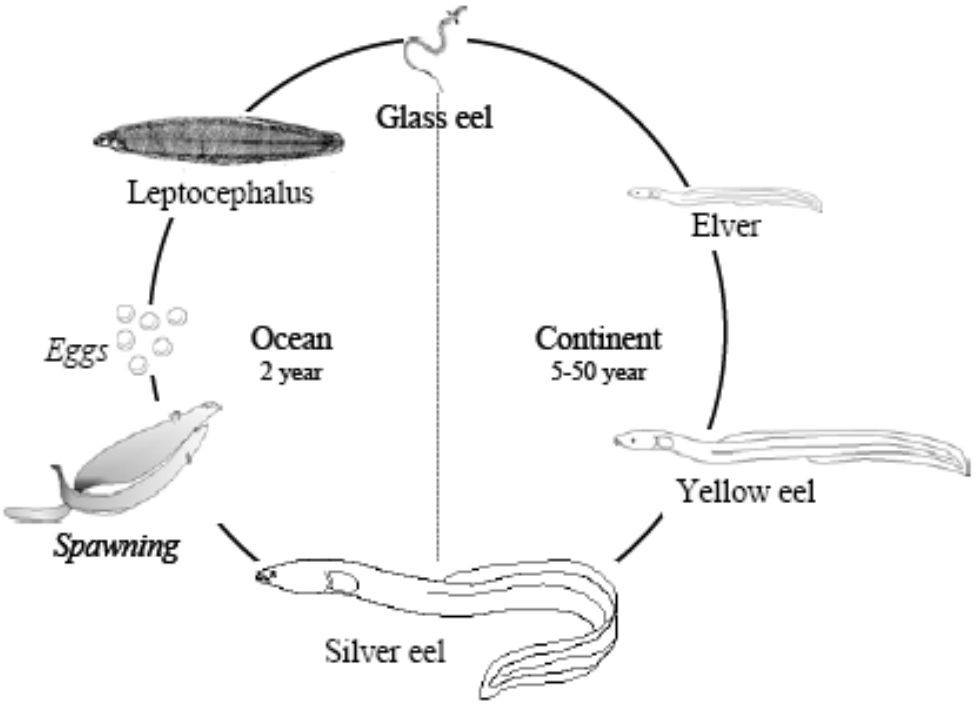

Figure 1.1: The life-cycle of the European eel *A. anguilla* (Dekker, 2000).

1.1.2 The European eel as an economically important species

A. anguilla is widely distributed throughout European freshwaters and stocks represent an important economic resource for fisheries and aquaculture facilities throughout Europe. In the UK, the main glass eel fisheries are linked to rivers that drain into the Bristol Channel (Peirson *et al.*, 2001); these fish are primarily exported to other European countries for re-stocking and aquaculture and generate an economic return of between £1 million and £3 million (Environment Agency UK, 2014). UK yellow and silver eel fisheries are mostly in areas of southern and eastern England and captures of 20-30 tonnes occur annually, generating between £100,000 to £150,000 (Environment Agency UK, 2014).

1.1.3 Status of the European eel population

European eel stocks are in decline across their geographical distribution and their status is now considered below safe biological limits (Geeraerts & Belpaire, 2010). Since the early 1980s, a 95% decline in recruitment of *A. anguilla* has occurred and one important contributory factor is the insufficient biomass of the spawning stock (Dekker, 2003a; Aprahamian *et al.*, 2007). A similar trend has also been noted for the Japanese eel and more recently, the American eel (Figure 1.2).

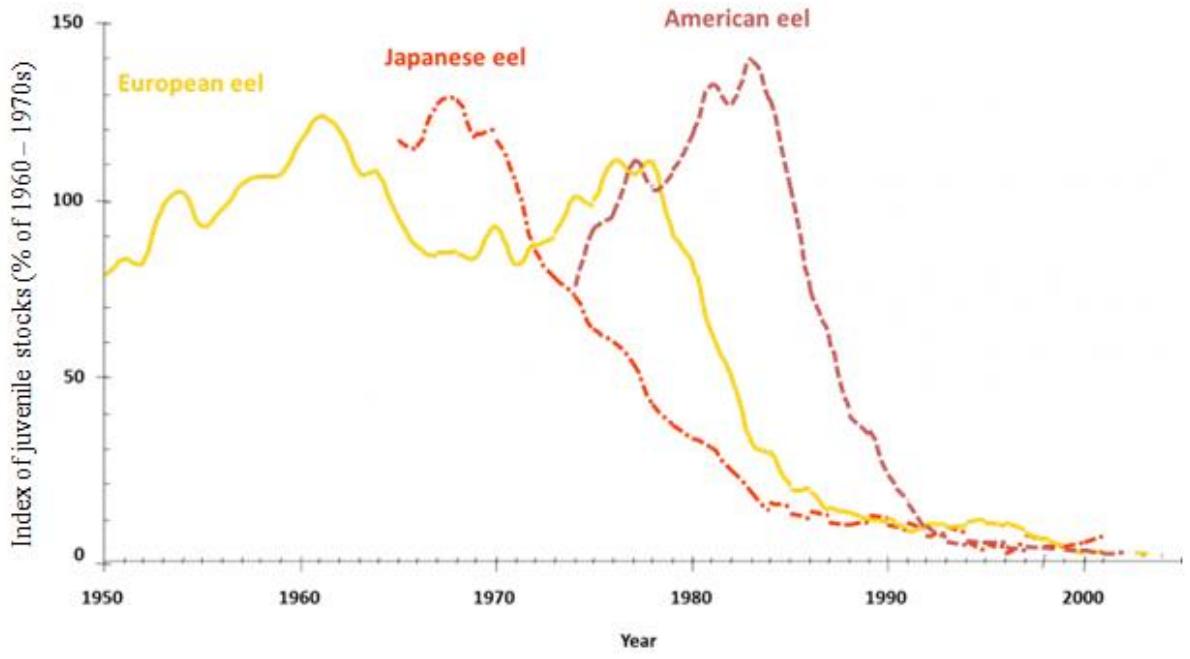

Figure 1.2 Trends in glass eel recruitment for the European eel (*A. anguilla*), Japanese eel (*A. japonica*) and American eel (*A. rostrata*) (modified from Dekker, 2003).

In the UK, the European eel is widely distributed throughout rivers and other freshwater habitats of England, Wales, Northern Ireland and Scotland (Naismith & Knights, 1993; Starkie, 2003; Bark *et al.*, 2007; Lobon-Cervil & Iglesias, 2008). Data on eel stocks in most rivers in the UK are derived from multispecies surveys which tend to record presence/absence, or relative abundance. Unfortunately, the data shows that numbers have

suffered a major decline over a 20-30 year period and now *A. anguilla* is considered a threatened species (Feunteun, 2002; Starkie, 2003; Durif *et al.*, 2010). Moreover, *A. anguilla* is the fastest declining UK vertebrate and not surprisingly, it is listed as a priority species in the recently revised UK Biodiversity Action Plan (UKBAP) (Laycock *et al.*, 2009; Morgan *et al.*, 2011). In England and Wales, eel recruitment peaked between 1972-1982 and 1983-1999 and then was reported to have declined by about 55% (Knights *et al.*, 2001; Bark *et al.*, 2007). Recruitment of glass eels has now fallen to approximately 5% of the levels of the late 1970s and shows no sign of recovery (Aprahamian *et al.*, 2007; Bark *et al.*, 2007; Dekker *et al.*, 2007; Heinen & Mangino, 2007).

1.1.4 Eel management plans

In 2007, *A. anguilla* was listed in appendix II by CITES (The Convention on International Trade in Endangered Species of wild fauna and flora). This listing demands that the exporting state has an export permit, which is only issued after scientists have confirmed that the export will not be detrimental to survival of the species. This CITES measure is designed to assist establishment of a sustainable fishery for the European eel (Rosen & Smith, 2010).

The European Commission has also initiated an Eel Recovery Plan to protect European eel stocks at sustainable levels (Council Regulation No 1100/2007) (ICES, 2011). Each European Member State is now required to establish a national Eel Management Plan to allow at least 40% of adult eels to escape from inland waters to the sea for spawning. Specifics include limiting eel fishing and further improving river management to assist eel migration and restocking.

Increased concern about reduced eel numbers has led the UK government, through DEFRA (Marine, Fisheries and Biodiversity), to legislate for the sustainable exploitation of eel fisheries throughout England and Wales. Since the above legislation and measures have only recently been implemented, the status of the species remains as critically endangered and *A.*

anguilla has been included on the IUCN red list of threatened species (ICES, 2006; Freyhof & Kottelat, 2008).

1.1.5 Factors contributing to the European eel population decline

The European eel population decline is no doubt a consequence of multiple factors that together, impact upon eel health and elver recruitment (Bonhommeau *et al.*, 2008; Jacoby *et al.*, 2015). A declining eel stock in Europe causes a reduction in the eel spawning stock and hence a reduced elver recruitment (Durif *et al.*, 2010).

1.1.5.1 Environmental factors

Environmental factors, including climatic changes in ocean conditions, are considered an important contributor to the European eel population decline (Castonguay *et al.*, 1994; Dekker, 2003a). Such factors are likely to impact migration of the silver eel to the spawning grounds and also, to increase larval mortality during transport to the continental areas (Hanel *et al.*, 2014).

1.1.5.2 Habitat loss

Eel habitat loss due to drainage of wetland habitats, construction of hydropower stations and associated dams and other river barriers, are considered major causes of eel population decline (Dekker, 2003a; Liermann *et al.*, 2012; Piper *et al.*, 2013; Davidson, 2014).

1.1.5.3 Pollution

Reduced water quality due to organic pollution undoubtedly reduces elver recruitment and silver eel migration success to the Sargasso Sea for breeding (Palstra *et al.*, 2006; Geeraerts & Belpaire, 2010). The impact of various pollutants on the behaviour and physiology, and subsequently the mortality of European eels, has been widely documented (Guimaraes *et al.*, 2009; Privitera *et al.*, 2014; Belpaire *et al.*, 2015).

1.1.5.4 Over fishing

Over fishing is another important contributing factor in eel population decline since glass and yellow eels are captured for export for human consumption and also aquaculture (Biró, 1977; Carder *et al.*, 2007) (see section 1.1.2).

1.1.5.5 Infectious agents

The European eel is susceptible to different infectious agents such as parasites, bacteria and viruses. Jacob and colleagues compiled a total of 161 parasitic species, including helminths, protozoa, arthropods, annelids and molluscs from European eels surveyed from 30 European/North African countries (Jakob, Walter, *et al.*, 2009). Representative groups of helminths have been reported in Atlantic and Pacific eels; however, only a very small number of helminth species have been identified as actual, or potential pathogens (Kennedy, 2007b). Some eel parasites are reported to be pathogenic and responsible for organ damage, or even death. These pathogenic parasites include the gill monogeneans *Pseudodactylogyrus anguillae* and *P. bini* (Buchmann, Mellergaard, *et al.*, 1987) (see Chapter 2) and the swim bladder nematode *Anguillicoloides* (=*Anguillicola*) *crassus* which causes impairment of swim bladder function (Abdelmonem *et al.*, 2010) and is considered a significant threat to the migratory success of silver eels (Pelster, 2015).

1.1.5.5.1 Helminth parasites

The Natural History Museum Parasite Database in London catalogues 118 species of helminth parasites in the European eel (Gibson *et al.*, 2005) and the parasitic worm phyla are documented as follows: trematodes (42 species), nematodes (39 species), cestodes (16 species), acanthocephala (16 species) and monogenea (5 species). With respect to European eel from the UK, the Natural History Museum Parasite Database includes information on 30 parasite species as follows: nematodes (10 species), trematodes (9 species), acanthocephala (6 species), monogenean (3 species) and cestodes (2 species) (Table 1.1).

Table 1.1 Helminth parasites reported in *A. anguilla* surveyed from the UK (modified from the Natural History Museum Database in London UK) (Gibson *et al.*, 2005).

Parasitic group	Parasite species	Reference
Nematodes	*Streptocara* sp.	(Kennedy, 1993a)
	Goezia inermis (Zeder, 1800)	(Norton *et al.*, 2003)
	Raphidascaris acus (Bloch, 1779)	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Kennedy, 1997b; Kennedy, 2001a; Norton *et al.*, 2003)
	Camallanus lacustris (Zoega, 1776)	(Nie & Kennedy, 1991a; Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003)
	Anguillicola (*Anguillicoloides*) *crassus* (Kuwahara, Niimi & Itagaki, 1974)	(Kennedy & Fitch, 1990; Ashworth, 1993; Kennedy, 1993b; Pilcher & Moore, 1993; Kennedy, 1998; Ashworth & Kennedy, 1999; Kelly *et al.*, 2000; Kennedy *et al.*, 2000; Kirk, Kennedy, *et al.*, 2000; Kirk, Lewis, *et al.*, 2000; Kennedy, 2001a; Kirk *et al.*, 2002)
	Spinitectus inermis (Zeder, 1800)	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1997a; Kennedy *et al.*, 2000; Kennedy, 2001b)
	Cucullanus truttae (Fabricius, 1794)	(Kennedy, 2001b; Norton *et al.*, 2003)
	Paraquimperia tenerrima (Linstow, 1878)	(Esch *et al.*, 1988; Nie & Kennedy, 1991c; Kennedy, 1993a; Kennedy, 1997b; Kennedy *et al.*, 2000; Kennedy, 2001a; Norton *et al.*, 2003)
	Capillaria spp.	(Kennedy, 1993a)
	Pseudocapillaria spp	(Kennedy, 1997b; Kennedy, 2001a)
Trematodes	*Deropristis inflata* (Molin, 1859)	(Kennedy *et al.*, 2000)
	Telogaster opisthorchis (Macfarlane, 1945)	(Hine, 1978)
	Crepidostomum farionis (Muller, 1780)	(Kennedy, 1993a)
	Diplostomum spathaceum (Rudolphi, 1819)	(Esch *et al.*, 1988)
	Phyllodistomum folium (Olfers, 1816)	(Nie & Kennedy, 1992a)
	*Stegodexamene anguill*ae (Macfarlane, 1951)	(Hine, 1978)
	Nicolla gallica (Dollfus, 1941)	(Norton *et al.*, 2003)
	Podocotyle atomon (Rudolphi, 1802)	(Kennedy, Nie, Kaspers, *et al.*, 1992)
	*Sphaerostomum bra*mae (Mueller, 1776)	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Kennedy, 1997a; Kennedy, 2001b)
Acanthocephalans	*Acanthocephalus anguillae* (Muller, 1780)	(Bates & Kennedy, 1991; Kennedy, 1992; Norton *et al.*, 2003)
	Acanthocephalus clavula Dujardin, 1845	(Esch *et al.*, 1988; Kennedy *et al.*, 2000; Lyndon & Kennedy, 2001)
	Acanthocephalus lucii (Muller, 1777)	(Kennedy, 1992; Norton *et al.*, 2003)
	*Echinorhynchus trut*tae Schrank, 1788	(Kennedy, 2001a; Norton *et al.*, 2003)
	Neoechinorhynchus rutili (Muller, 1780)	(Kennedy, 1993a, 2001a)
	Pomphorhynchus laevis (Zoega in Muller, 1776)	(Brown, 1989; Kennedy, 1996)
Monogeneans	*Gyrodactylus anguillae* Ergens, 1960	(Kennedy & Di Cave, 1998)
	*Pseudodactylogyrus angu*illae (Yin & Sproston, 1948)	(Nie & Kennedy, 1991d; Kennedy, Nie, Kaspers, *et al.*, 1992; Gibson, 1993; Kennedy, 1993b; Kennedy *et al.*, 2000)
	Pseudodactylogyrus bini (Kikuchi, 1929)	(Gibson, 1993; Kennedy, 1993b)
Cestodes	*Bothriocephalus claviceps* (Goeze, 1782)	(Esch *et al.*, 1988; Kennedy, Nie, Kaspers, *et al.*, 1992; Nie & Kennedy, 1992b; Kennedy, 1993a; Nie & Kennedy, 1993; Kennedy, 1997b; Kennedy, 2001a; Norton *et al.*, 2003)
	Proteocephalus macrocephalus (Creplin, 1825)	(Nie & Kennedy, 1991b; Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, Nie, & Rostron, 1992; Kennedy, 1993a; Kennedy, 1997b; Kennedy *et al.*, 2000; Kennedy, 2001a; Norton *et al.*, 2003)

Some helminth parasites are known to cause severe symptoms that may result in eventual death of the host; including the monogeneans, *Pseudodactylogyrus anguillae* and *P. bini* (Buchmann et al., 1987; Nie and Kennedy, 1991; Rodrigue et al., 1996; Buchmann, 1997) and the swim bladder nematode, *Anguillicoloides crassus* (Wurtz and Taraschewski, 2000; Knopf and Manhke, 2004; Abdelmonem et al., 2010).

Indeed, these parasitic helminths are considered a threat to both wild and farmed eels (Pelster, 2014; Newbold *et al.*, 2015; Pelster, 2015; Terech-Majewska *et al.*, 2015). Given that *A. anguilla* production in aquaculture has grown significantly and the culture conditions are intensive, eel diseases are particularly problematic within aquaculture facilities (Denmark, 1987). Moreover, eel farming is currently dependent on the capture of wild glass eels and this facilitates the introduction of potential pathogens into the aquaculture environment. The most important eel farm pathogen is the gill monogenean *Pseudodactylogyrus* spp.(Buchmann, Mellergaard, *et al.*, 1987; Mellergaard & Dalsgaard, 1987; Buchmann, 1988a). Recently, in some countries such as the Netherlands, farmed yellow eels have been restocked into the wild to restore natural eel populations; when performed without prior health checks, this represents a risk for wild eels (Haenen *et al.*, 2012).

A. crassus has become one of the most important threats to wild populations of the European eel (Muñoz *et al.*, 2015). Kirk (2003) described the spread of the parasitic nematode *A. crassus* to UK eel stocks and suggested that the serious pathology inflicted on the swim bladder, and the associated physiological effects, are likely to impair capacity of European eels to complete their migration to the spawning grounds and hence impact recruitment levels (Kirk, 2003).

1.1.5.5.1.1 *Anguillicoloides crassus* (Kuwahara, Niimi and Itagaki 1974)

Anguillicoloidae Yamaguti, 1935 is a family of parasitic nematodes infecting eels of the genus *Anguilla* and comprising of two genera: *Anguillicola* (a single species, *A. globiceps*) and *Anguillicoloides* (four species: *A. crassus*, *A. papernai*, *A. australiensis*, and *A. novaezelandiae*) (Moravec, 2006; Laetsch *et al.*, 2012). All species have similar life cycles that involve intermediate (eg. copepod) and paratenic (eg. small fish) hosts and the adult nematodes inhabit the eel swim bladder and feed upon host blood from vessels within the wall of the swim bladder (Moravec, 2006).

A. crassus is indigenous to East Asia and parasitizes the native Japanese eel *A. japonica* as well as introduced and cultured *A. anguilla* and *A. rostrata* (Nagasawa *et al.*, 1994; Han *et al.*, 2008). In its native host *A. japonica* the nematode causes little pathogenicity (Nagasawa *et al.*, 1994).

As a consequence of the worldwide eel trade, *A. crassus* was accidentally introduced into Europe in the early 1980s, possibly with importation of infected Japanese eels from Taiwan into Germany (Koops & Hartmann, 1989). As such, *A. crassus* is considered to be an invading parasite of the European eel and it is now also documented in the American eel, *A. rostrata* (Johnson *et al.*, 1995; Kirk, 2003). The spread of the parasite has been facilitated by a number of factors, though the primary cause is human movement of infected eels for trade purpose (Koops & Hartmann, 1989; Kennedy & Fitch, 1990). The success of *A. crassus* colonization outside of Asia has been attributed to its adaptability to different water salinities, and to the wide range of intermediate and paratenic hosts (Kirk, Kennedy, *et al.*, 2000; Kirk *et al.*, 2002).

Introduction of *A. crassus* into UK rivers is hypothesized to have occurred via water changes carried out during transport of infected eels (Kennedy & Fitch, 1990; Kirk, 2003). The parasite was first documented in UK eels in 1987 following examination of specimens from

the River Welland and River Trent in eastern England, and also from the River Thames (Kennedy & Fitch, 1990).

1.1.5.5.1.2 Life cycle of *A. crassus*

Eels are infected by *A. crassus* following ingestion of third-stage larvae present in crustacean intermediate hosts and/or by ingestion of juveniles in fish paratenic hosts (Figure1.3). Moreover, it is most likely that crustacean intermediate hosts serve as the source of infection for smaller eels (<20 cm), whilst larger eels mainly acquire infection by preying on paratenic hosts (Kirk, 2003; Emde *et al.*, 2014).

Adult male and female nematodes copulate in the lumen of the eel swim bladder. Females release eggs passively which leave the swim bladder via the pneumatic duct, pass down the intestine and hatch in the water, though some eggs hatch in the swim bladder (Kirk, Kennedy, *et al.*, 2000). Mature eggs contain a motile second stage larva that hatches in a range of salinities and is infective to intermediate and paratenic hosts (Kirk, Kennedy, *et al.*, 2000). Many species of copepod and cyclopoid can serve as an intermediate host (Kennedy & Fitch, 1990; Kirk, Kennedy, *et al.*, 2000; Kirk, Lewis, *et al.*, 2000). When consumed by the intermediate host the second-stage larvae of *A. crassus* penetrate the digestive tract, enter the body cavity and moult into third-stage larvae. If intermediate hosts are consumed by eels, third stage larvae penetrate the digestive tract, migrate across the body cavity and enter the swim bladder wall. These parasites moult to fourth-stage juveniles that then develop into pre-adults and finally mature adults that feed on host blood and become sexually active. The duration of the life cycle is temperature-dependent and in laboratory investigations is reported as 3–4 months at 20–22 ^{o}C (Moravec & Konecny, 1994). If intermediate hosts are consumed by paratenic hosts such as small fish, the third-stage larvae may remain, or they may moult into fourth-stage larvae or pre-adults. Paratenic hosts are important since they can accumulate large numbers of juvenile *A. crassus* and hence they have contributed to the rapid increase in

prevalence of the parasite that has been recorded in Europe (Moravec & Konecny, 1994; Kirk, 2003).

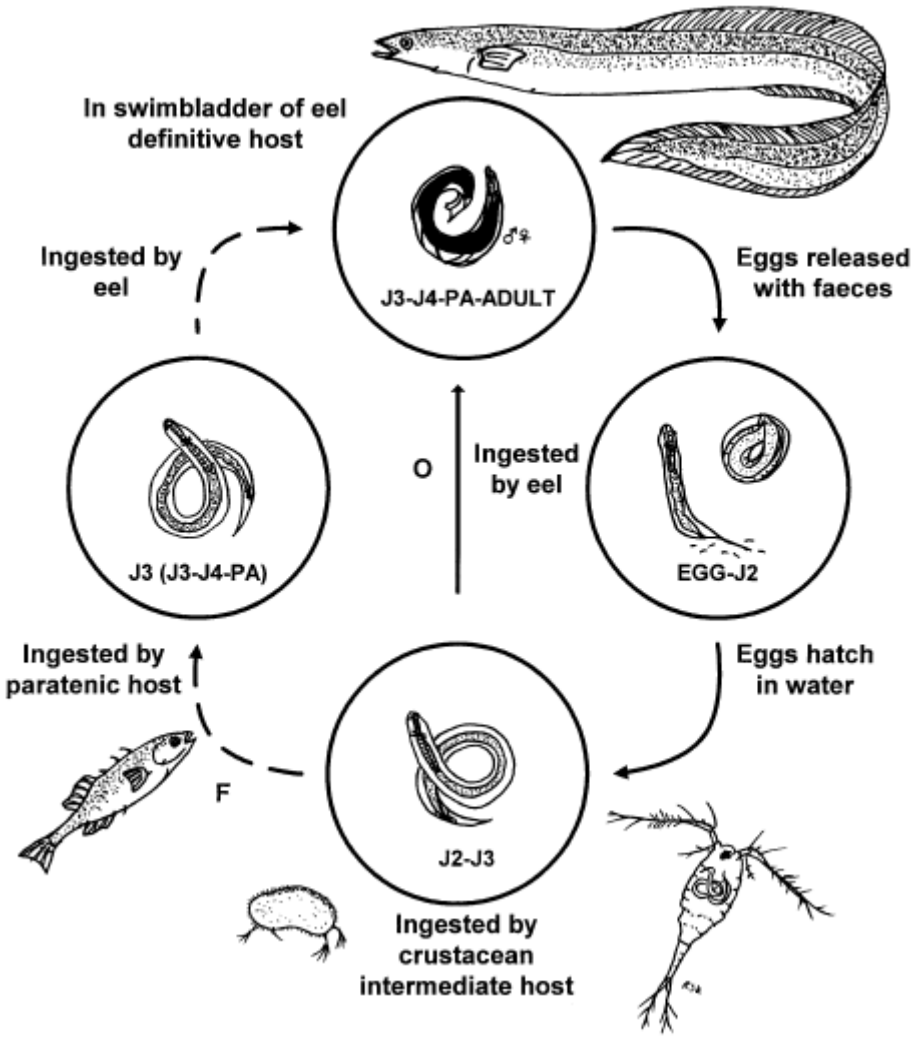

Figure 1.3 The life cycle of *A. crassus* (Kirk, 2003).

1.1.5.5.1.3 Pathological impact of *A. crassus*

In the native Japanese eel *A. japonica* swim bladder nematodes tend to occur at low intensities and hence cause little pathology (Han *et al.*, 2008; Heitlinger *et al.*, 2009; Keppel *et al.*, 2014). In contrast, the European eel *A. anguilla* has very limited resistance to *A. crassus* and the parasite is considered a major pathogen and threat to European eel populations (Newbold *et al.*, 2015; Pelster, 2015). Indeed, *A. crassus* can severely impair swim bladder function and is responsible for mortality in both farmed and wild populations,

and hence severely limits the capacity of European eels to complete the spawning migration (Kirk, 2003; Kennedy, 2007b; Muñoz *et al.*, 2015; Pelster, 2015).

The swim bladder wall is damaged by L3 penetration and by feeding of L4 and adult nematodes upon the blood supply (Kirk, 2003; Keppel *et al.*, 2014). Moreover, repeated larval invasion of the swim bladder results in haemorrhage and injury to the connective tissue and the blood feeding activity of adults causes mechanical injury to the epithelium (Han *et al.*, 2008). As a result, the wall of the swim bladder markedly thickens and displays degenerative inflammatory and proliferative changes (Van Banning *et al.*, 1990; Abdelmonem *et al.*, 2010). Acute processes are characterized by epithelial hyperplasia and hyperaemia of the swim bladder wall (Molnár *et al.*, 1993). Chronic swim bladder inflammation is characterised by oedema and hyperplasia of the tissues of the tunica propria submucosa, granulomatoid infiltration by mononuclear cells and fibrinoid degeneration around the larvae (Molnár *et al.*, 1993; Knopf, 2006). The abdomen of heavily infected eels is often swollen due to enlargement of the swim bladder which becomes filled with adults, and a black mass of decaying worms and a cloudy fluid of eggs and second-stage juveniles (Kirk, 2003).

In addition to the thickening of the swim bladder wall, the host response includes a macrophage reaction against larvae. Epithelioid macrophages aggregate around the larvae whilst it is in the gut, initiating the formation of a connective capsule around it (Würtz & Taraschewski, 2000). *A. anguilla* antibodies against the L3 larval stage have also been detected, but they seem insufficient to control the nematode infection (Knopf, 2006).

On examining the response of *A. crassus* upon cultured Japanese eels, it was evident that a large percentage of L3 larvae in the gut are eliminated by the host immune response (Heitlinger *et al.*, 2009). Furthermore, examination of the swim bladder of these eels showed that they contained a high amount of dead encapsulated larvae (Keppel *et al.*, 2014). This

encapsulation was also associated with a distinct thickening of the swim bladder wall (Keppel *et al.*, 2014). Indeed, this process was apparent to a much greater extent in Japanese eels compared to European eels and as a result, *A. japonica* displays only minor changes to the swim bladder wall (Keppel *et al.*, 2014).

1.1.5.5.2 Viral infections of *A. anguilla*

Viral infections are a threat to both cultured and wild *A. anguilla* and are considered a contributory factor in the decline of European eel stocks (Haenen *et al.*, 2012; Bandin *et al.*, 2014). Three viruses of particular concern have been noted; *Herpesvirus anguillae* (HVA or Anguillid Herpesvirus 1 (AngHV-1), an *Aquabirnavirus* (Eel Virus European (EVE)) and a Rhabdovirus (Eel Virus European X (EVEX)) (Haenen *et al.*, 2009). In the UK, HVA has recently been reported to cause mortality to wild eel stocks (Armitage *et al.*, 2014).

1.1.5.5.3 Bacterial infections of *A. anguilla*

Pathogenic bacteria, including *Vibrio vulnificus, V. anguillarum, Pseudomonas anguilliseptica* and *Edwardsiella tarda*, may cause disease to both wild and cultured *A. anguilla*; particularly when a stress factor, such as injury, is involved (Amaro *et al.*, 1995; Amaro & Biosca, 1996; Marco-Noales *et al.*, 1999; Marco-Noales *et al.*, 2001). Edwardsiellosis, a lethal bacterial septicaemia caused by the Gram-negative bacterium *E. tarda*, is a common epizootic disease of cultured and wild European eel; indeed, the rates at which *E. tarda* have been isolated from wild populations are reported to range of 9 to 23% (Alcaide *et al.*, 2006). Another important bacterial-induced disease, particularly responsible for mortality in farmed environments, is Vibriosis, caused by the pathogenic bacterium *V. vulnificus* serovar E (biotype 2) (Collado *et al.*, 2000).

1.1.6 The European eel immune system

Both innate and adaptive immune responses are mounted by fish to control infections (Alvarez-Pellitero, 2008) and their molecular study is provoking increasing interest towards further understanding of fish immunology (Zhu *et al.*, 2013). Similar to other fish species, the innate immune system in eels comprises a large number of physical, cellular, and humoral factors that act as the first line of defence against viruses, bacteria and parasites (Gollock *et al.*, 2005; Knopf *et al.*, 2008). Moreover, macrophages seem to play an important role in the immune responses of fish towards helminth parasites (Whyte *et al.*, 1989; Secombes & Chappell, 1996). Active phagocytes, complement components and enzyme activities, including lysozyme and cathepsins, are present early in eel development, before, or soon after hatching, to control infection (Magnadóttir, 2006).

External factors can influence the activity of innate immune responses, including water temperature and pH, oxygen concentration and pollution and also, handling and crowding stress, food additives and immunostimulants within the aquaculture environment(Sures *et al.*, 2001). Internal factors, such as nematode infection, result in increased stress and young fish are especially sensitive to such stress due to infestation by the L3 stage of *A. crassus* (Sures *et al.*, 2001). Indeed, this resulting increased stress leads to a diminished macrophage response that ultimately may cause higher susceptibility to other pathogens (Sures *et al.*, 2001). Experimental results suggest that *A. crassus* infection may be responsible for a decreased macrophage phagocytic response and hence higher susceptibility to other pathogenic conditions. This effect on the macrophage response seems directly linked to the number of adult parasites present in the swim bladder (Terech-Majewska *et al.*, 2015).

An extensive non-specific eel immune response has been found in response to juvenile parasites entering the swim bladder wall from the body cavity and also, to adults and juvenile

nematodes present in the swim bladder lumen (Kirk, 2003; Knopf, 2006). It is believed that this cellular response plays an essential role in the development of immunity against *A. crassus* as it results in fibrosis that is thought to inhibit subsequent invasion by further juvenile nematodes (Kirk, 2003). Molnar (1994) suggested that phagocytes and cytokines were crucial to this response. However, other work has demonstrated that macrophages and granulocytes around *A. crassus* larvae in the swim bladder wall do not seem to attack the L3 and L4 stage nematodes (Knopf, 2006); albeit, L3 larvae appear to have a positive effect on the migration of these cells to this location (Knopf *et al.*, 2008).

Eels heavily infected with *A. crassus* need at least 20% more energy reserves to manage migration due to the damaged swim bladder and this increased energetic requirement results in less energy available for other physiological functions, including immune responses (Palstra *et al.*, 2007; Sjöberg *et al.*, 2009). Importantly, total protein level and total immunoglobulin levels in serum were found to be significantly declined in *A. crassus* infected eels compared with non-infected animals (Muñoz *et al.*, 2015). Furthermore, immunological parameters such as the spleen phagocyte respiratory burst activity and potential killing activity were found to be significantly reduced in *A. crassus* infected eel compared to controls (Terech-Majewska *et al.*, 2015).

Genes of the major histocompatibility complex (MHC) code for key functions in the adaptive immune response of vertebrates and most of them show exceptionally high polymorphism. This polymorphism is associated with selection by diverse and changing parasite communities (Lenz *et al.*, 2009). Recently, it has been reported that relatively high diversity exists at the MHC class IIA and class IIB loci of the European eel; however, there appeared to be a lack of positive selection on the MHC class IIB gene and this may have contributed to the lack of adaptive potential for the species (Bracamonte *et al.*, 2015).

1.2 Objectives

As discussed earlier (sections 1.1.3 and 1.1.5), the European eel has suffered major population decline and multi-factorial efforts are underway to understand further the reasons for the species being listed as threatened. Some eel parasite infections are associated with host morbidity and mortality and hence have contributed to the species decline. In particular, *A. crassus* and *Pseudodactylogyrus* spp. are considered major eel pathogens. However, much less is known about some of the other eel helminths, including nematodes, cestodes and acanthocephalans. To this end, the overall aim of this PhD study is to provide more detailed knowledge on helminth infections in wild European eels sampled from multiple locations across England and Wales. It is an extension of an earlier study that examined swim bladder nematode infections, and also, trypanosome and herpes viral infections, in *A. anguilla* from English and Welsh rivers (Ab-Aziz, 2012).

The objective of the current chapter is to provide details about the individual host animals. Subsequent chapters are then focused upon describing infection data for gill monogeneans (Chapter 2), gastrointestinal nematodes (Chapter 3), acanthocephalans and cestodes (Chapter 4) and finally, the helminth community structures (Chapter 5).

1.3 Materials and Methods

1.3.1 Sampling

One hundred and forty European eels, *A. anguilla*, were acquired from 14 sampling sites (10 eel per site) across England and Wales between August 2009 and October 2012. The eel sampling was carried out during routine surveys conducted by the Environment Agency (contacts: Dr Chris Williams and Dr Miram Aprahaimen) at the following sites; North Wales: River Mawddach-eden, River Clwyd-Meirchion, River Dee-Eitha and Clwyd-Elwy; South Wales: River Rhymney, River Cadoxton and River Taff; North West England: River Leven, River Gowy, River Bela, River Hether Burn and River Petteril; South East England: River Crouch (Figure 1.1). In addition, samples were received from the River Crane (South East England) following a severe raw sewerage pollution event in October 2011 (The Guardian, 2011). Specimens were frozen and shipped on ice to The University of Salford for parasite analyses. All specimens were then stored in the -20 ^{o}C freezer and thawed prior to processing.

1.3.2 Classical Morphological Examination

Eel samples were labelled according to the catchment location and date of collection. Prior to processing, specimens were thawed out overnight. Body length and weight of each eel was determined and recorded. These measurements were utilized to calculate the eel condition factor, K (Fulton, 1904).

$$K = \frac{W(100)}{L^3}$$

W = weight (g) and L = length (cm)

1.3.3 Statistical analyses

Statistical analysis of data was carried out using Minitab 16 (licensed to The University of Salford).

1.4 Results

1.4.1 Eel morphometric data

Eel body length, weight and condition factor were determined for the 140 specimens from the 14 river catchment sites (Figure 1.4) across England and Wales (Appendix I and Table 1.2). The body length of the examined eels was non-parametrically distributed and ranged from 10 to 86 cm (mean body length = 30.7 ±14.9 cm) and the body weights ranged from 1.2 g to 1380 g (mean body weight = 94.0 ±212.2 g).

The greatest mean length and mean weight were recorded in eels collected from the River Crane in England (mean length = 75.7±6.1 cm; mean weight = 810.7±241.1 g); indeed, the largest eel specimen (CN3) (Figure 1.5) had a body length of 86 cm and weighed 1380 g. The lowest mean length and weight were recorded in eels sampled from the River Dee-Eitha in Wales (mean length = 21.7±7.5 cm; mean weight = 19.8±22.1 g). A closer inspection of the eels morphometric data indicated that all ten specimens from the River Crane appeared significantly larger (Mann-Whitney test: $p < 0.0001$ for both length and weight) than the eel from the other 13 catchment sites (Figure 1.6 and Figure 1.7). Indeed, mean eel length and weight data from each of these remaining 13 rivers showed positive skewness (Figure 1.7) (Anderson-Darling normality test: $p = 0.027$) and there were no significant differences in these morphometrics compared to the population (excluding the River Crane) (Mann-Whitney test: $p > 0.05$).

Overall, the condition factor of the eels ranged from 0.04-0.29 (mean = 0.15±0.04) and the distribution was non-parametric. The greatest mean condition factor was recorded in eels from the River Crane in England (0.19±0.03), whereas the lowest mean condition factor was noted in specimens from the River Dee-Eitha in Wales (0.11±0.04). There were significant differences between the condition factors of the eels sampled from the Rivers Crane, Petteril, Dee-Eitha and Hether Burn relative to the population (Mann-Whitney test: $p < 0.05$).

Figure 1.4 Map of England and Wales showing the 14 eel sampling sites. (http://WWW.euwfd.com/html/a quick guide to rbmaps in the uk.html) 1: Mawddach-eden; 2: Clwyd-Meirchion; 3: Dee-Eitha; 4: Rhymney; 5: Cadoxton; 6: Leven; 7: Gowy; 8: Bela; 9: Hether Burn; 10: Petteril; 11: Clwyd-Elwy; 12: Taff; 13: Crouch; 14: Crane.

Table 1.2 Mean length (ML), weight (MW) and mean condition factor (MK) of eel examined at different catchment sites from England and Wales. Ten eels were examined at each site (sd = standard deviation)

Eel Catchment Site	Code	ML(cm) (±sd)	MW(g) (±sd)	MK (±sd)	Date of acquisition
Crouch: England	C	35.4±8.8	50.2±51.0	0.15±03	02/08/2009
Leven: England	RL	30.4±8.3	50.3±48.4	0.15±0.02	29/09/2009
Crane: England	CN	75.7±6.1	810.7±241.1	0.19±0.03	08/10/2012
Hether Burn: England	HB	21.4±3.6	13.0±5.6	0.13±0.04	29/09/2009
Petteril: England	RP	29.4±8.4	58.4±39.6	0.18±0.06	17/09/2009
Bela: England	B	29.8±10.6	43.6±59.5	0.15±0.02	18/08/2009
Gowy: England	RG	34.1±4.7	54.9±17.5	0.13±0.02	03/06/2010
Mawddach-eden: Wales	M	26.1±10.2	38.4±48.1	0.14±0.01	03/03/2010
Clwyd-Meirchion: Wales	MC	23.9± 8.7	28.6±31.2265	0.16±0.05	03/03/2010
Dee-Eitha: Wales	D	21.7±7.5	19.8±22.1	0.11±0.04	03/03/2010
Cadoxton: Wales	CD	24.9±4.3	22.7±16.1	0.11±0.03	30/10/2009
Rhymney: Wales	R	28.8±3.1	40.5±16.4	0.16±0.04	30/10/2009
Clwyd-Elwy: Wales	CE	24.9±8.4	29.4±26.1	0.14±0.02	03/03/2010
Taff: Wales	TB	23.8±2.9	22.4±9.4	0.16±0.02	30/10/2009
TOTAL		30.7±14.9	94.0±212.2	0.15±0.04	

Figure 1.5 Representative images of (A): a small eel acquired from the River Cadoxton and (B): a large eel acquired from the River Crane.

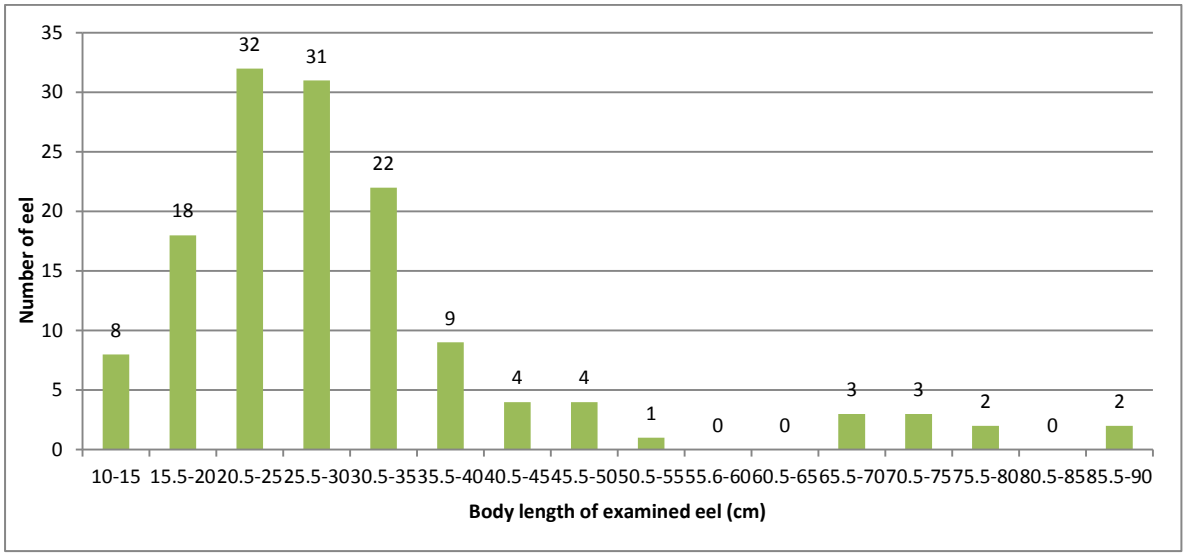

Figure 1.6 Distribution of the eel samples based upon body length category.

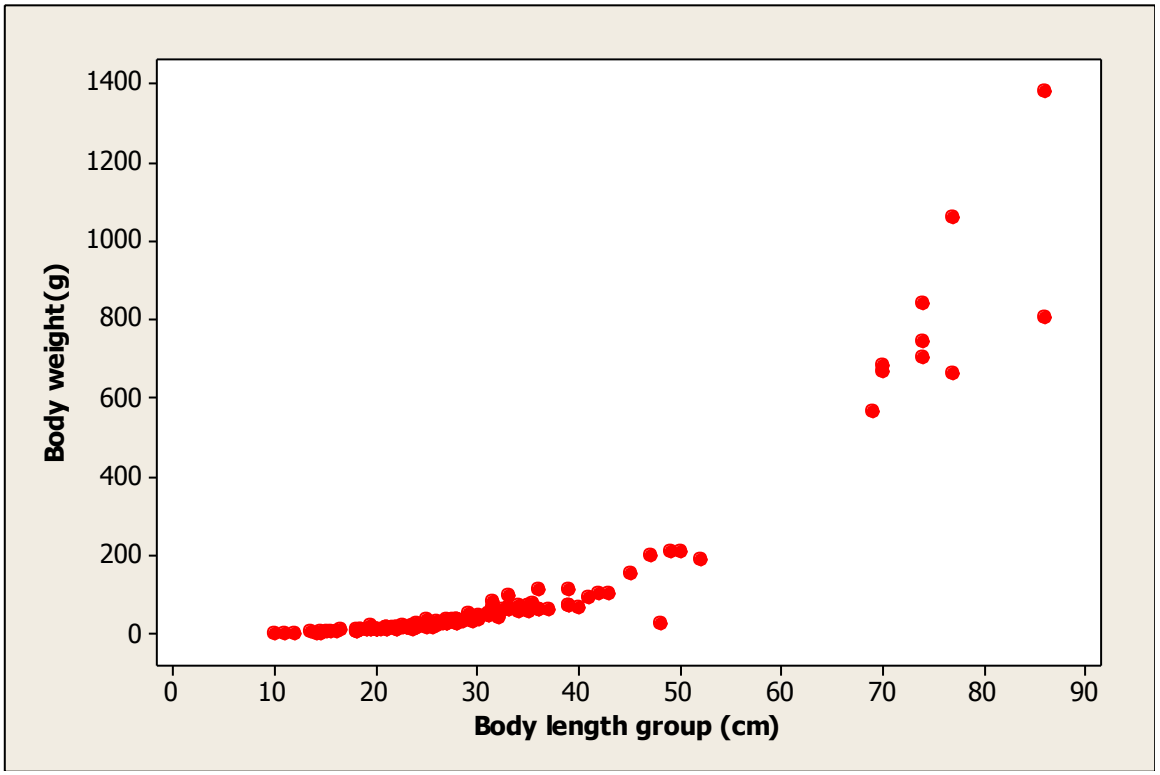

Figure 1.7 A scatterplot of eel length against eel weight for the 140 eel specimens surveyed across England and Wales.

1.5 Discussion

The eels studied in this thesis were obtained from the Environment Agency as detailed in Table 1.1. River Crane specimens were acquired following a serious pollution event involving raw sewerage (The Guardian, 2011) whereas all remaining specimens were obtained as a consequence of routine eel surveys conducted as part of the legislation covered by European Eel Management Plans. As such, there was limited input into this aspect of the study design and hence the precise sampling dates and locations, along with other biological and chemical data associated with these sites are unfortunately unknown. Nonetheless, some general characteristics of the sites are known. For example, the River Bela is associated with an area of limestone geology in Cumbria and hence the water is reported to be reasonably alkaline (pH 8.5) (South Cumbria Rivers Trust, www.scrt.co.uk). Moreover, it is generally considered that the water quality of UK rivers is improving. In England, the biological and chemical quality of the water is reported as 'good' across 73 % and 80% of the rivers. In Wales, these characteristics are enhanced since 87% and 95% of Welsh rivers are considered biologically and chemically 'good' (Brown *et al.*, 2011).

The most recent Biodiversity Action Plan (BAP) indeed corroborates the biological and chemical analyses of UK river water (Morgan *et al.*, 2011). In England, 4748 river sites were surveyed and 1755 qualified as having at least one category of special interest (37%) associated with biodiversity. In Wales, 989 river sites were surveyed and 491 had at least one category of special interest (49.6%) associated with biodiversity. With respect to the catchment sites from which eels were sampled in this thesis, the BAP data is summarised below (Table 1.3). The River Petteril had the highest number of qualifying criteria (n=5) given that it is a shingle river that is a site of special scientific interest within a conservation area and contains at least three CB vegetation components. Overall though, 6 out of the 7

surveyed Welsh rivers had qualifying criteria considered important for the BAP and for England, this was 5 out of the 7 rivers.

Table 1.3 Summary of the data on the 14 catchment sites surveyed in this study utilised for the Biodiversity Action Plan (Morgan *et al.*, 2011). # Contains 3 or more of the following CB vegetation components: crowfoots, starworts, pondweeds, milfoils, bryophytes, emergent and other aquatics as defined by the EC Habitats Directive, Annex 1, Habitat H3260.

River site	Shingle river	Number of qualifying BAP criteria	Special Area of Conservation	Site of Special Scientific Interest	Species list: category A (all) and category B (fish only)
Crouch: England	-	0	-	-	*A. anguilla* (B)
Leven: England	-	2	-	-	*Austropotamobius pallipes* (A) and 6 category B species including *A, anguilla* and *Salmo trutta* and *S. salar*
Crane: England	-	1	-	-	*Osmerus eperlanus* (A) and 3 category B species: *A. anguilla, S. salar* and *Cottus gobio*.
Hether Burn: England	-	1	-	-	6 category B species including *A. anguilla, S. trutta, S. salar* and *C. gobio*.
#Petteril: England	Yes	5	Yes	Yes	7 category B species including *A. anguilla, S. trutta, S. salar, C. gobio, Lampetra fluviatilis,* and *Petromyzon marinus.*
Bela: England	-	1	-	-	6 category B species including *A. anguilla, S. trutta, S. salar, C. gobio* and *Lampetra sp.*
Gowy: England	-	0	-	-	5 category B species: *A. anguilla, S. trutta, C. gobio, L. fluviatilis, L. planeri* and Petromyzontidae (B).
Mawddach-eden: Wales	Yes	3	Yes	Yes	2 category A species: *Margaritifera margaritifera* and *Rhytidiadelphus* and 4 category B species including *A. anguilla, S. trutta* and *S. salar*.
Clwyd-Meirchion: Wales	-	1	-	-	3 category B species including: *A. anguilla, C. gobio*.
Dee-Eitha: Wales	-	2	Yes	Yes	2 category B species: *A. anguilla* and *C. gobio.*
Cadoxton: Wales	-	0	-	-	4 category B species including *A. anguilla, C. gobio* and *L. planeri.*
Rhymney: Wales	Yes	2	-	-	6 category B species including *A. anguilla, S. trutta, S. salar, C. gobio*.
Clwyd-Elwy: Wales	Yes	2	-	-	6 category B species including *A. anguilla, S. salar, S. trutta, C. gobio, L. planeri* and Petromyzontidae (B).
Taff: Wales	-	1	-	-	*Meotica anglica* (A) and 5 category B species including *A. anguilla, C. gobio* and *S. trutta*.

The eels sampled from all the Welsh rivers and 6 of the English rivers have morphometric parameters indicative of them being at the yellow eel developmental stage (Simon, 2007; Lasne & Laffaille, 2008). In contrast, the size of the eels sampled from the River Crane is significantly larger than the other 130 specimens and is consistent with at least some of them being at the silver eel stage (Holmgren *et al.*, 1997; Melià *et al.*, 2006). Although freezing results in a reduction in eel size and weight (Wickstrom, 1986), no correction was applied to

the morphometric data since the length of freezing and number of freeze-thaw cycles was unknown upon acquisition of the samples.

Eel length can be used as a pseudomeasure of age (Tzeng *et al.*, 1994; Hedger *et al.*, 2008) and hence it is highly likely that all the specimens from the River Crane were significantly older than the remainder of the surveyed fish. Indeed, an eel ageing study performed on specimens sampled from different lakes in Germany concluded that the average eel growth rate was 4.5 cm per year and that individuals of approximately 45 cm in length were between 7 and 10 years old (Naismith & Knights, 1993; Simon, 2007). Exact ageing by otolith examination was not used in this study because the mode of sample acquisition and potential differences in sample handling and storage between the sites may impact upon the viability of the otoliths as a means of eel ageing (Proctor & Thresher, 1998).

The mean condition factor data reported in this study are consistent with other published reports (Kangur & Kangur, 1998; Simon, 2007). The eels sampled from the Rivers Crane and Petteril had significantly higher K values than the population and this could be interpreted as these animals being in a healthier condition. However, since the eel from the Crane are significantly longer than the rest of the population, it is likely that the high K value is at least in part due to the specimens being at a later developmental stage. Consequently, the condition factor of the Crane eel may add support to the interpretation that these animals are mostly at the silver stage and preparing to migrate by depositing fat stores. Since eel sampled from the river Petteril are of average length, the significantly greater condition factor most likely indicates that these specimens were sampled from an environment that was providing a plentiful supply of prey and hence was ecologically good for *A. anguilla*. This is certainly corroborated by the data from the BAP (Morgan *et al.*, 2011) (Table 1.2). The eel sampled from the Rivers Dee-Eitha and Hether Burn were the smallest specimens surveyed and they had significantly lower K values than the population. Interpretation of this requires caution

since there are multiple factors that could account for the difference in condition factor, including a paucity of prey, the developmental stage and the time of year. As such, it is not possible to state with certainty that the eel from the Dee-Eitha and Hether Burn were of poorer overall health than the rest of the population. Indeed, given that these eel were sampled from rivers assessed as having criteria important for the BAP (Morgan *et al.*, 2011) (Table 1.2) then the river quality of the Dee-Eitha and Hether Burn could be considered as being ecologically good.

In summary, the eel specimens studied in this thesis have been acquired from diverse sites across England and Wales. The population has a range of body size that is indicative of considerable variation of age and also includes some specimens most likely to be at the silver eel stage. As such, this population should form a good representative sample set for analysis of helminth infections in UK eel.

CHAPTER TWO

Gill monogeneans

2.1 Introduction

The Monogenea is a class of Platyhelminths that includes more than 53 families, mostly parasitic on the external surfaces and gills of freshwater and marine fishes. Monogeneans are usually well adapted to their fish host and due to their short and direct life cycle they often reach epizootic levels relatively quickly in confined habitats such as an aquaculture facility (Rohde & Watson, 1985; Boeger & Kritsky, 1993, 1997; Whittington, 2004). These parasites feed on the superficial layers of fish skin and gills (Buchmann, Køie, *et al.*, 1987; Buchmann & Lindenstrøm, 2002). Despite their high host specificity in the wild (Sasal *et al.*, 1999) some monogeneans are also capable of infecting other hosts when several species are confined together (Thoney & Hargis, 1991). The health of the host is usually impaired by heavy monogenean infection and death is not uncommon (Thoney & Hargis, 1991).

2.1.2 *Pseudodactylogyrus spp*.

2.1.3 Morphology

Species of *Pseudodactylogyrus* are very similar in appearance and can be very difficult to distinguish. Generally, *P. bini* is longer and narrower than *P. anguillae* (Ogawa & Egusa, 1976; Chan & Wu, 1984). For example, under fixed conditions the maximum length of *P. bini*, is 1960 µm and the maximum and minimum width is reported as 336 µm and 120 µm. In contrast, the maximum length of *P. anguillae* is 1659 µm and the maximum and minimum width is recorded as 364 µm and 138 µm (Ogawa & Egusa, 1976; Chan & Wu, 1984). However, species differentiation is based on the sclerotized structures because different treatments during preparation of the parasites can affect the size of unsclerotized structures. Consequently, the size and shape of the hamuli are characteristics on which a taxonomic

separation of the 2 species is based (Arafa & Reda, 2012). The hamuli of *P. bini* are shorter and stouter than these of *P. anguillae* (Ogawa & Egusa, 1976; Chan & Wu, 1984; Ogawa *et al.*, 1985). Ogawa *et al.* (1985) stated that the hamuli of *P. bini* were up to 70 µm in length, whereas the hamuli are at least 80 µm long in *P. anguillae*. The length of the bar connecting the hamuli has also been noted as generally longer in *P. anguillae* than in *P. bini*, although an overlap exists (Table 2.1).

Table 2.1 Reported dimensions of the gill monogeneans *P. bini* and *P. anguillae* and their associated hamuli and connective bars.

Measurements	*P. bini* (µm)	*P. anguillae* (µm)
Maximum body length #	1960	1659
Maximum body width #	336	364
Minimum body width #	120	138
Hamuli length #	$\leq$ 70	$\geq$ 80
length of the connective bar *	9-13	9-16
Width of the connective bar *	42-54	50-64

(Buchmann, Mellergaard, *et al.*, 1987); * (Gelnar *et al.*, 1996).

2.1.4 Origin and distribution

Species of the genus *Pseudodactylogyrus* (Gusev, 1965) are specific monogenean gill parasites of anguillid eels. The two known species, *Pseudodactylogyrus anguillae* (Yin & Sproston, 1949) and *P bini* (Kikuchi, 1929), were originally described from the Japanese eel *Anguilla japonica* in China and Japan, respectively. Kikuchi (1929) described *Dactylogyrus bini* on the gills of *A. japonica* and also reported another parasite with larger hooks; however, no morphological description of the latter specimen was given. Yin & Sproston (1948) found the same two dactylogyrids on *A. japonica* from China and re-assigned *D. bini* as *Neodactylogyrus bini* (Kikuchi, 1929) and the other species as *Neodactylogyrus anguillae* Yin & Sproston, 1948.

Gussev (1965) studied gill monogeneans on *A. reinhardti* Steindachean, 1867 from Australia and proposed the genus *Pseudodactylogyrus* by highlighting that the haptor and the two hamuli were directed ventrally, instead of dorsally, as occurs for the genus *Dactylogyrus*. As such, the names of these two species were proposed as *Pseudodactylogyrus bini* (Kikuchi, 1929; Gussev, 1965) and *P. anguillae* (Yin & Sproston, 1949; Gussev, 1965).

P. anguillae infections of *A. anguilla* have been recorded from Japan, China and Taiwan (Kikuchi, 1929; Yin & Sproston, 1949; Ogawa & Egusa, 1976; Chan & Wu, 1984; Chung *et al.*, 1984), as well as from localities throughout Europe (Buchmann, Mellergaard, *et al.*, 1987; Nie & Kennedy, 1991d; Gelnar *et al.*, 1996; Škoríková *et al.*, 1996; Sures, Knopf, Würtz, *et al.*, 1999; Aguilar, Álvarez, *et al.*, 2005; Gerard *et al.*, 2013). In addition, the discovery of *P. anguillae* infections in the African longfin eel, *A. mossambica* (Peters, 1852), represents the first record of a pseudodactylid occurring in the Indian Ocean (Christison & Baker, 2007). A subsequent study on the island of Reunion confirmed that pseudodactylids may reach high prevalence in *A. mossambica* and also, that *P. bini* is present, though at greater reduced intensity relative to *P. anguillae* (Sasal *et al.*, 2008).

P. anguillae infection in the American eel, *A. rostrata*, was first noted in Canada (Cone & Marcogliese, 1995). Subsequently, *P. bini* and also *P. anguillae*, were reported in *A. rostrata* for the first time in the United States (Hayward *et al.*, 2001) and pseudodactylid infections continue to be problematic in the American eel (Larrat 2012).

The first report of *Pseudodactylogyrus bini* and *P anguillae* in Europe was from an eel production plant in the Kalinin region of the western Soviet Union (Golovin, 1977) and the parasites were subsequently noted to cause disease problems at eel farms (Buchmann, 1988a). It was assumed that both species of gill monogenean were introduced into Europe with imports of *Anguilla japonica*, with a resulting host switch to *A. anguilla* and hence *P.*

anguillae and *P. bini* are considered invasive parasites (Buchmann, 1987). However, Nie & Kennedy (1991) have suggested that *P. anguillae* may not be an invasive parasite since it could potentially be considered as an overlooked natural parasite of *A. anguilla*.

Indeed, the report of the presence of *P. anguillae* from North America may support the idea that this monogenean is a natural parasite of the American eel and by extension, the European eel, and hence it is not an introduced Asian species (Hayward *et al.*, 2001). Moreover, *P. anguillae* has been documented infrequently at some localities; for example, it was reported in only 8 of 1066 *A. rostrata* eels sampled from 29 sites across Nova-Scotia, Canada. (Cone & Marcogliese, 1995).

The first UK report of the occurrence of *P. anguillae* in the European eel, *A. anguilla* was from three localities in Devon, southwest England (Nie & Kennedy, 1991d). The study investigated the seasonal dynamics of *P. anguillae* in fresh and estuarine waters throughout a complete year. The data confirmed that the prevalence and abundance of *P. anguillae* correlated with the season and was highest in late summer/early autumn and low throughout the winter. Consequently, the study (Nie & Kennedy, 1991d) confirmed that populations of *P. anguillae* are able to reproduce and maintain themselves in eel in English waters and that the parasites overwinter on eel at low levels of infection. *P. bini* was not reported in this study and absence of this parasite was hypothesized to be due to the enhanced salinity of the water at the locations examined (Nie & Kennedy, 1991d).

2.1.5 Life-cycle

The life cycles of both species of *Pseudodactylogyrus* are similar; they are direct and typical of most monogenean parasites (Figure 2.1). *P. bini* and *P. anguillae* differ slightly in their ecology; *P. bini* is less able to tolerate salinity than *P. anguillae* which can reproduce in waters of up to 20% salinity, though not in sea water (Nie & Kennedy, 1991d). In addition, *P.*

bini requires higher temperatures for reproduction; it is unable to develop at 10^{o}C, though at 25-34^{o}C it produces more eggs than *A. anguillae* (Buchmann, 1988d).

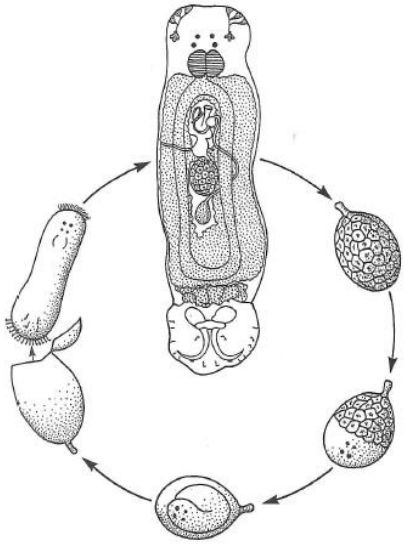

Figure 2.1 The life cycle of *Pseudodactylogyrus* spp. involves an egg which embryonates and hatches to release a free swimming ciliated larva (oncomirasidium). This larval stage attaches to the host gill, sheds its ciliated cells and develops into the adult parasite from which eggs are produced by self-fertilisation (Buchmann, 2012).

Eggs of both species are oval and have a 7 to 14 µm long pedicule that possesses a distal extension with an adhesive function. Eggs of *P. bini* are 56-112 x 49-63 µm (Chan & Wu, 1984), whilst those of *P. anguillae* are slightly smaller at 60-80 x 50-60 µm (Golovin & Shukhgalter, 1979). The egg release rate of *P .anguillae* is temperature dependent; at 10^{o}C an average of 1.2 eggs were released per worm per day, whilst at 20^{o}C and 28^{o}C the mean eggs released per worm per day was reported as 9.6 and 7.7 respectively (Imada & Muroga, 1978). There is no comparable data published for *P. bini*.

Development time for the egg is dependent on water temperature. Eggs fail to hatch at temperatures below 10^{o}C and the optimal temperature for development and hatching is around 25 - 29^{o}C, which allows development to occur in 10-12 days (Buchmann, 1988d,

1990). Indeed, this is the usual temperature for eel aquaculture (Koie, 1991; Buchmann, 1993a). In rivers, *P. bini* is reported to overwinter as eggs whereas *P. anguillae* may overwinter on gills (Koie, 1991). Shed eggs hatch to produce oncomiracidia, which are short lived and need to find an eel before they can develop further into adults. Oncomiracidia of *Pseudodactylogyrus* spp. have 2 pairs of eye spots, a spherical pharynx, 14 marginal hooks and 4 ciliated areas. Oncomiracidia of *P. bini* measure 162-193 x 42-70 µm and display a fast swimming movement, both in straight and curved lines for 30 min at 19 - 26^{o}C. Following attachment to the host, the oncomiracidia of *P. anguillae* develop into mature parasites in 6 - 7 days at 28 ^{o}C (Golovin & Shukhgalter, 1979).

P. bini and P. anguillae parasitize the gills of eel and can co-occur on the same eel (Buchmann, 1988b). The distribution of the two species on the gills, although partially overlapping, is reported to differ; *P. bini* prefers the anterior gill arches and *P. anguillae* the posterior ones on smaller eels, whilst larger eels do not show the same distribution (Buchmann, 1988c, 1989a; Matejusova *et al.*, 2003; Fang *et al.*, 2008b). There is a positive correlation between the body length of an eel and the parasite intensity; small eels from aquaculture may harbour up to 200+ parasites, whereas infection levels in larger eels may exceed 1000+ (Buchmann, 1989b).

2.1.6 Pathology

It is thought that *Pseudodactylogyrus* species are normally not pathogenic to their natural eel host *Anguilla japonica* in the wild and that in aquaculture, they generally only cause gill damage when present in large numbers (Kennedy, 2007b; Fang *et al.*, 2008a; Buchmann, 2012). However, the European eel *A. anguilla* is highly susceptible to *Pseudodactylogyrus* infections (Koie, 1991; Knopf & Mahnke, 2004; Kania *et al.*, 2010) and in experimental

studies it has been shown that infection prevalence and parasite intensities are significantly higher in European eels compared to *A. japonica* (Jianping et al, 2008).

A. anguilla of all stages from glass eels to adults, appear susceptible to infection (Buchmann, 1988a, 1988d). Generally, infections in wild eels are reported to initiate a stress response and also, to cause some localised gill tissue damage (Koie, 1991), though this is not thought to impact upon the ability of the eel to migrate (Kennedy, 2007b). However, *Pseudodactylogyrus* infection in eel farms is considered a serious problem that results in significant economic losses (Buchmann, 1993b; Kennedy, 2007b). The pseudodactylids are introduced to aquaculture via infected elvers which are the basis of commercial eel production. The parasites are able to live and reproduce rapidly under the favourable farm conditions. In moderate to heavily infected eels, the parasites are widely distributed over the gill filaments, and the hamuli perforate the gill tissue and contact gill cartilage resulting in increased mucus secretion and destruction of gill structures (Chan & Wu, 1984). Histologically, gill filaments appear congested with erosions and ulcers and they are surrounded by mucous desquamated epithelial cells and leukocytes. The gill filaments show edema, congestion and hyperplasia of the mucus secreting cells (Abdelmonem *et al.*, 2010).

2.2 Objectives

There is very limited information on the extent of *Pseudodactylogyrus* spp. in wild eel populations in the United Kingdom. The main objective of this chapter therefore is to screen the European eel, *A. anguilla,* from English and Welsh river systems in order to report primary infection data for pseudodactylids within UK water systems. In addressing this objective the project will (i) determine the primary infection data for *Pseudodactylogyrus* spp. within European eel populations from fourteen river systems across England and Wales and (ii) develop molecular diagnostic tools to assist species identification between *P. anguillae* and *P. bini*.

2.3 Materials and Methods

2.3.1 Classical Morphological Examination

2.3.1.1 Sampling

Eel samples were acquired according to Chapter 1 (1.3).

2.3.1.2 Processing

On the day of examination, eels were thawed and necropsies performed using a dissection kit and the gill tissue removed. Necropsy included the microscopic examination of the gill surfaces and any exudate for the presence of gill monogenean parasites. For each animal, gills were dissected in a Petri dish containing distilled water under x 100 and x 160 magnification using a Wild Heerbrugg, M3B (Switzerland) dissecting microscope.

2.3.2 Prevalence and intensity

Pseudodactylogyrus spp. were collected from the examined infected eels and placed in a Petri dish containing distilled water, The total number of parasites was determined for each animal allowing a description of the primary infection data. Representative parasites were examined further by microscopy as described below and the remaining was stored at -20^{o}C in 70% (v/v) ethanol.

2.3.3 Imaging

Representative gill parasites were fixed in 10% (v/v) formalin and mounted unstained onto microscope slides. A Leica Dulb M26 microscope was utilized in conjunction with the Leica Application suite, version 3.5 and digital camera DFC31FX to capture images of the parasites to assist in identification.

2.3.4 Molecular parasitological analysis

Given the difficulty in morphological discrimination between the pseudodactylids caused by repeated freeze/thawing of animals a molecular identification was carried out based on differences in the 18S rRNA gene sequences of *P. anguillae* (gi: 13810563) and *P. bini* (gi: 14717824).

2.3.4.1 DNA extraction from pseudodactylids

DNA was extracted from parasites using the PureLink Genomic DNA Kit (Invitrogen by Life Technologies) as described by the manufacturer. Briefly, a pseudodactylid was placed in a 1.5 ml eppendorf tube and 180 µl PureLink Genomic Digestion Buffer and 20 µl Proteinase K (20 mg / ml) was added for each sample. The sample was incubated overnight in a water bath at 55^{o}C with occasional vortexing to ensure complete sample lysis. To remove any particulates the lysate was centrifuged at 13,000 g for 3 minutes. The supernatant was transferred to a sterile microcentrifuge tube and 20 µl RNase (20 mg / ml) was added. The sample was briefly vortexed and 200 µl genomic lysis/binding buffer was added. The sample was briefly vortexed and 200 µl 96-100% ethanol was added and then transferred to the spin column placed inside a collection tube. The column was centrifuged at 13,000 g for 1 minute. The collection tube was discarded and the spin column placed inside a clean collection tube. 500 µl wash buffer 1 was added to the spin column and centrifuged at 13,000 g for 1 minute. The spin column placed inside a new collection tube and 500 µl wash buffer 2 was added and the column was centrifuged at 13,000 g for 3 minutes. Finally, the spin column was placed inside a sterile 1.5 ml microcentrifuge tube and 100 µl genomic elution buffer was added. After one minute incubation at room temperature the column was centrifuged at 10,000 g for 1 minute. The eluted DNA was removed from the collection tube and placed in a labelled Eppendorf tube for storage at -20^{o}C prior to further molecular analysis.

2.3.4.2 DNA quality control

The concentration and purity of extracted DNA samples were determined spectophotometerically (A_{260}/A_{280}) using a NanoDrop Lite Instrument (ThermoFisher Scientific). Briefly, 1 µl of distilled water was added to the centre of the surface of the NanoDrop instrument to allow instrument calibration at 260 nm and 280 nm. The surface was then cleansed using tissue to remove excess water, and the above procedure was repeated. The surface was cleaned again and 1µl of the DNA sample was added to the instrument. Readings at 260nm and 280 nm were recorded, allowing DNA concentration and purity to be determined.

2.3.5 Polymerase chain reaction (PCR)

Inspection of the NCBI GenBank database revealed limited molecular information for *Pseudodactylogyrus* spp. as only 18S rRNA gene sequences were available. Upon alignment, it was apparent that a few nucleotide changes were present in the DNA sequences encoding the 18S rRNA genes of *P. anguillae* (gi: 13810563) and *P. bini* (gi: 14717824). PCR primers were therefore designed to amplify a 514 bp fragment of the 18S rRNA gene of both *P. anguillae* and *P. bini* that included a region of variation between these species. The primer sequences were as follows, PseudoF primer: 5'-TGGGAGGATTGACAGAATGA-3' and PseudoR primer: 5'-GGCCTTGCTAAACCATTCAA-3'. Oligonucleotides were synthesized by Eurofins MWG Operon and re-suspended in PCR-grade H_2O to a stock concentration of 10 pM and stored at -20^{o}C until required.

2.3.5.1 PCR Profile

The PCR was carried out in a total volume of 25 µl as follows: 2 µl of pseudodactylid genomic DNA template, 0.5 µl Taq polymerase (10 units/µl, BioTaq DNA polymerase, Bioline), 0.5µl each of forward and reverse primers (5 pmol/µl), 2.5 µl of 10x PCR buffer, 2.5 µl of deoxynucleotide triphosphate mix (dNTPs; 1.25 mM each dNTP), 2.5 µl of $MgCl_2$ (25 mM stock) and 14 µl PCR-grade H_2O. For negative control purposes, distilled water was utilized instead of DNA template. Throughout PCR set-up, all aliquots were carried out using barrier tips to prevent potential aerosol contamination of samples. The PCR cycling profile consisted of an initial denaturation step at 94^{o}C for 5 minutes, followed by denaturation of 94^{o}C for 30 seconds, annealing at 64^{o}C for 30s and extension at 72^{o}C for 30s. This cycle profile was repeated a total of 36 times, followed by a final extension of 10 minutes at 72^{o}C. All PCR reactions were performed on a MultiGene machine (Labnet International. Inc.)

2.3.5.2 Optimization of PCR conditions

PCR reaction conditions were optimized using a temperature gradient and a dilution series of $MgCl_2$. The optimisation was assessed by determination of PCR product recovery and specificity using agarose gel electrophoresis (2.3.6).

2.3.6 Agarose gel electrophoresis

PCR products were visualized on a 1% (w/v) agarose gel. The gel was prepared by dissolving 1g of agarose powder (Sigma) in 100ml of 1 X TAE buffer (Tris-Acetate-EDTA, Severn Biotech Ltd) by gentle heating in a microwave oven. When the solution had cooled to approximately 50^{o}C, 5µl of Gelred dye (Biotium) was added to the mixture and then the molten agarose was poured into a gel casting tray and a multi-well comb inserted. The solution was allowed to set for at least 30 min and then the comb removed and the gel transferred to an electrophoresis tank containing sufficient 1 X TAE buffer to cover the gel surface.

12µl of PCR product was mixed with 3µl 5X gel loading buffer (Fermentas) and then carefully aliquoted into the wells of the agarose gel. In an adjacent well, 4 µl of the 1 kb Hyperladder (Bioline) was also aliquoted to allow subsequent size determination of the PCR products. The DNA was electrophoresed through the agarose gel at 110 volts (100mA) for approximately 60 minutes. PCR products were then visualized using a short-wave UV Transillaminator Box and imaged with Gene Snap software (SynGen). The gel image was captured using a Polaroid camera (Sony UPP-110 HG) and the resulting image was saved.

2.3.7 PCR product purification

The PCR product was purified using the Isolate II PCR and Gel purification Kit (BioLine) as described by the manufacturer. Briefly, 2 volumes of binding buffer were added to 1 volume of PCR product and mixed well. The mixture was added onto the surface of the purification column, placed within a 2 ml collection tube and centrifuged at 13,000 g for 30 seconds. The flow-throw was discarded and 700 µl wash buffer was added onto the column and centrifuged at 13,000 g for 30 seconds. The flow-throw was again discarded and the centrifugation was repeated as above to remove any residual ethanol. Finally, the column was placed in a sterile 1.5ml microcentrifuge tube and 30 µl elution buffer was added to the column. Following 1 minute incubation at room temperature the column was centrifuged at 13,000 g for one minute to elute the purified PCR product. The recovery of purified PCR product was verified by subjecting a 1µl aliquot to spectrophotometric analysis using the Nana-Drop (2.3.4.2).

2.3.8 DNA sequencing

The PCR product was purified (2.3.7) and the concentration was determined as described previously (2.3.4.2). A sufficient quantity of the PCR product at the recommended DNA concentration (1 ng/µl per 100bp) was then subjected to DNA sequencing (Source Bioscience) with the required primers, PseudoF and PseudoR (3.2 pmol/µl). The resulting sequences were analysed using FinchTV (v.1.4.0) (Geospiza Inc) and subsequently compared with other reference sequences in the GenBank DNA database using BLAST (Altschul *et al.*, 1990). Nucleotide sequences were aligned with reference sequences available in GenBank, using the multiple sequence alignment program ClustalW2 (Larkin *et al.*, 2007).

2.3.9 Restriction digestion of PCR products

Purified PCR products were digested using the restriction endonuclease Xag1, (EcoN1) (Fermentas, Life Sciences) following the recommended protocol of the manufacturer. Briefly, 8 µl of the purified PCR product was added to 9.6µl PCR-grade water, 2µl 10x Buffer R and 0.4 µl Xag1 (20 units/ µl). A control digest comprising of 0.5 µl (Concentration 491µg/ml) unmethylanated λ DNA (Promega, Madison, WI USA) was also digested with Xag1 in a total volume of 20 µl. Reactions were mixed gently and incubated at 37^{o}C for 3 hours. The digests were then mixed with 4 µl of 5X gel loading buffer (Fermentas) and analysed by agarose gel electrophoresis as described above (2.3.6).

2.3.10 Statistical analyses

The Fisher's exact test was used to compare differences in prevalence between geographic regions and also between host factors (length, weight and condition factor). The Mann-Whitney test was used to assess the significance of differences in intensity of infection between the different geographic regions and also between host factors (length, weight and condition factor). All tests were conducted using Minitab 16 (licensed to the University of Salford).

2.4 Results

2.4.1 Parasitological Examination

Morphological examination of eel gills confirms that *Pseudodactylogyrus* spp. parasites were present in 50 of the 140 (35.7%) European eel, *Anguilla anguilla,* that were surveyed from 6 locations in England and only 4 River sites in Wales (Figure 2.2).

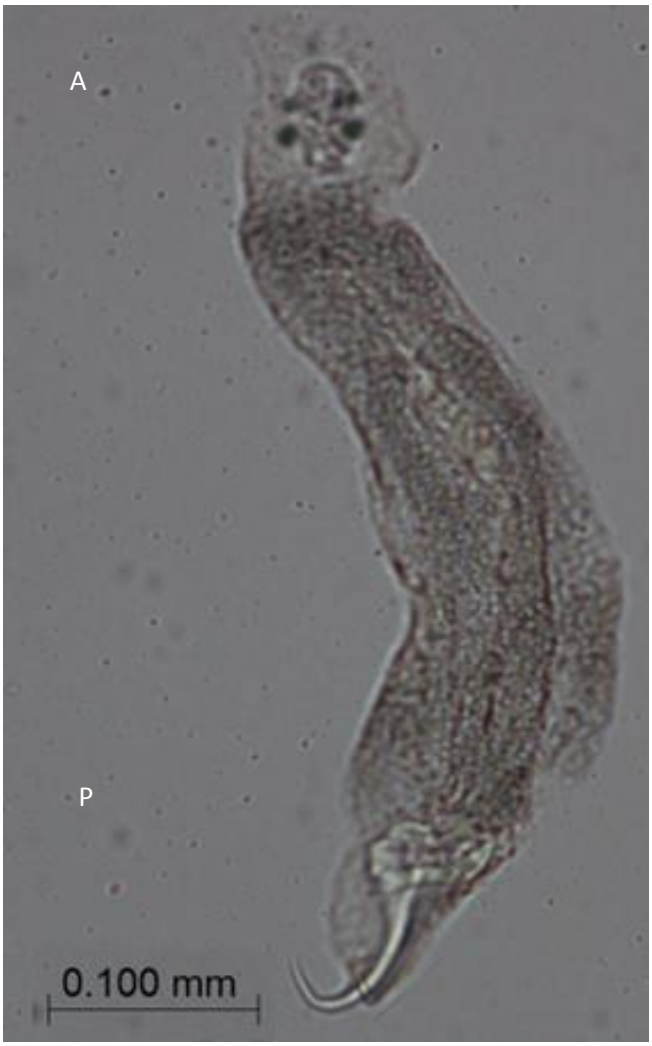

Figure 2.2 Representative light micrograph showing a *Pseudodactylogyrus spp.* extracted from an infected eel sampled from the River Leven. A: Anterior end showing eye spots. P: Posterior end showing the free hook (X100).

2.4.1.1 Primary infection data: the environment

In total, 50 of the 140 (35.7%) European eel examined were infected with pseudodactylids. The infected eel were sampled from 10 out of the 14 (71.4%) river systems surveyed across England and Wales (Table 2.2). The rivers Clwyd-Meirchion, Maddach-Eden and Dee-Eitha from North Wales, and the River Gowy from Northwest England, appear from the sampling and analysis carried out in this study, to be uninfected with the gill monogenean parasites. At sites positive for pseudodactylids, the prevalence ranged from 10 to 100%. The River Crouch showed the highest prevalence of infection (100%), while the lowest prevalence (10%) was observed in the Rivers Bela in England and Taff in Wales.

Table 2.2 Summary of pseudodactylid infection in wild eel sampled from 14 catchment sites across England and Wales. Ten eels were examined from each site.

Catchment location	Site label	Pseudodactylid infected eel (%)
Mawddach-eden: Wales	M	0%
Clwyd-Meirchion: Wales	MC	0%
Dee-Eitha: Wales	D	0%
Taff: Wales	TB	10%
Clwyd-Elwy: Wales	CE	40%
Rhymney: Wales	R	60%
Cadoxton: Wales	CD	70%
Gowy: England	RG	0%
Bela: England	B	10%
Petteril: England	RP	20%
Hether Burn: England	HB	40%
Crane: England	CN	70%
Leven: England	RL	80%
Crouch: England	C	100%

Overall, 619 pseudodactylids were collected from the eel (mean abundance = 4.42 sd±13.94; mean intensity =12.38 sd±21.12). The number of pseudactylids extracted from individual eel ranged between 1 and 125; the latter being isolated from a fish sampled from the river Leven (specimen RL2). A single pseudodactylid was extracted from 16% of infected animals (8/50). Moreover, the majority 70% (35/50) of the infected hosts carried less than 10 pseudodactylids and hence only 22% (11/50) of the infected eels harboured 10+ gill monogeneans (Tables 2.3 and 2.4). With respect to the sampling sites, the River Leven yielded most pseudodactylids (n = 232) and the lowest number was recovered from the River Taff (n = 1).

Table 2.3 Eel morphometric data for animals with gill monogenean infections sampled from sites across Wales. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of pseudodactylids
River Cadoxton: CD	CD1	33	59.9	0.17	14
	CD2	27	30.4	0.15	5
	CD3	24	21.4	0.06	4
	CD4	28	28.3	0.13	2
	CD5	28.5	32.6	0.05	4
	CD6	22	11.0	0.10	2
	CD9	21	8.8	0.09	29
River Rhymney: R	R1	32	48.9	0.15	5
	R3	30	42.3	0.16	22
	R4	31.5	67.2	0.22	9
	R5	27	29.3	0.15	11
	R6	30	39.7	0.15	3
	R9	23.5	12.7	0.10	3
River Clwyd-Elwy: CE	CE1	39	72.9	0.12	4
	CE4	34	70.1	0.18	11
	CE5	29	39.3	0.16	1
	CE8	22	16.8	0.16	1
River Taff: TB	TB4	22	15.6	0.15	1

Table 2.4 Eel morphometric data for animals with gill monogenean infections sampled from sites across England. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of pseudodactylids
River Crouch: C	C157	33	65.8	0.18	56
	C107	32	47	0.14	19
	C117	28	27.5	0.13	34
	C59	32	50.1	0.15	12
	C74	50	209.2	0.17	2
	C116	29	36.4	0.15	3
	C71	47	198.2	0.19	7
	C131	39	112	0.19	2
	C40	32	43.9	0.13	5
	C48	31	44.7	0.15	1
River Leven: RL	RL1	21	13.9	0.16	5
	RL2	52	187.0	0.13	125
	RL3	27	35.8	0.18	51
	RL5	34	55.9	0.14	10
	RL6	35	71.3	0.17	2
	RL8	29.5	30.3	0.11	5
	RL9	30	38.1	0.14	1
	RL10	26	22.3	0.13	33
River Crane: CN	CN3	86	1380.3	0.22	12
	CN5	70	668.6	0.19	2
	CN6	74	839.7	0.21	1
	CN7	86	805.7	0.13	2
	CN8	77	661.9	0.14	3
	CN9	69	563.5	0.17	2
	CN10	74	743.4	0.18	52
Hether Burn: HB	HB3	23	14.7	0.12	3
	HB4	24	16	0.12	2
	HB5	25	18.6	0.12	27
	HB7	20	7.8	0.09	1
River Petteril: RP	RP5	35.5	74.9	0.17	2
	RP6	27.5	38.2	0.18	1
River Bela: B	B2	32	49.1	0.15	5

The mean intensity of pseudodactylid infection in eels examined from different catchment sites ranged from 1 to 29±42.7 parasites per eel; the former from the river Taff in Wales and the latter from the river Leven in England. The mean parasitic abundance in infected eels from different river sites surveyed ranged from 0.1±0.31 to 23.2±39.6 pseudodactylids; the former from the river Taff and the latter from the river Leven (Table 2.5). Overall, the parasites were over-dispersed (dispersion index = 44) (Figure 2.3).

Table 2.5 Summary of the primary infection data for gill monogeneans isolated from European eel sampled across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence rate (%)	Total number of parasite	Mean parasitic abundance (±sd)	Mean Infection Intensity (±sd)
Crouch: England	100	141	14.1±17.9	14.1±17.9
Leven: England	80	232	23.2±39.6	29±42.7
Crane: England	70	74	7.4±15.2	10.6±17.3
Hether Burn: England	40	33	3.3± 7.9	1.4±0.8
Petteril: England	20	3	0.4±0.7	1.5±0.5
Bela: England	10	5	0.5±1.6	5
Cadoxton: Wales	70	60	6 ±8.6	8.6± 9.1
Rhymney: Wales	60	53	5.3 ±6.7	8.8 ±6.6
Clwyd-Elwy: Wales	40	17	1.7±3.5	4.3±4.7
Taff: Wales	10	1	0.1±0.3	1
	35.7	619	4.42 ±13.94	12.38 ±21.12

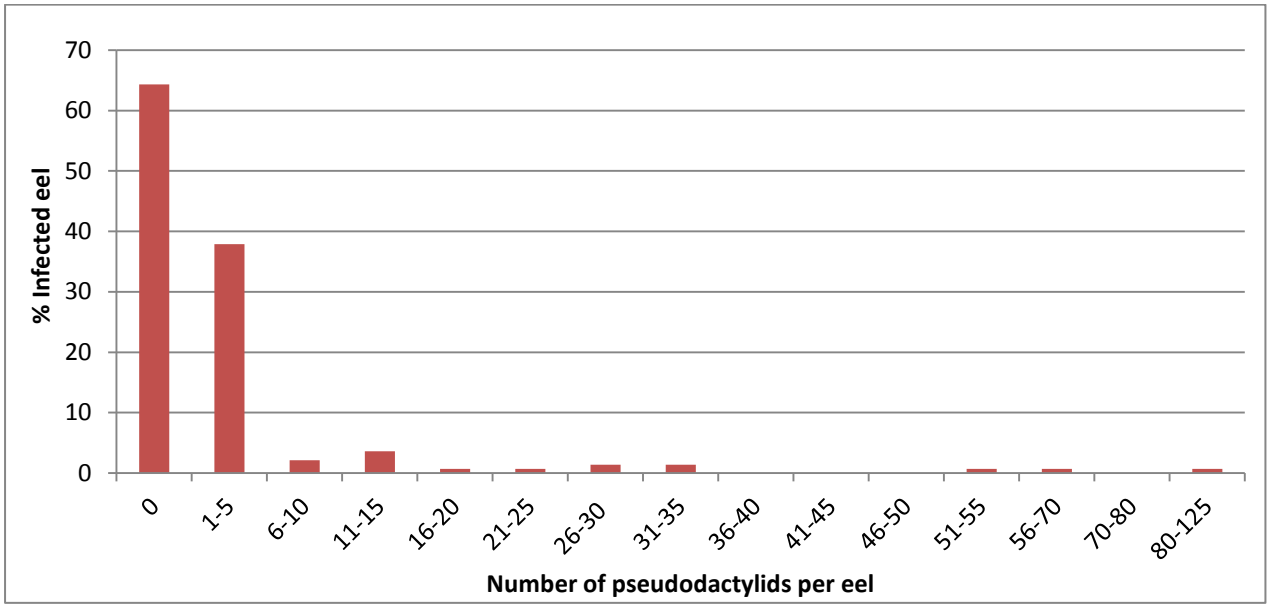

Figure 2.3 Summary of the dispersion of pseudodactylids within the sampled eel populations.

Upon analysis of the infection data at a regional level it was evident that all examined rivers in South East England and South Wales contained eel infected with pseudodactylids (Table 2.6). In North West England, only the River Gowy appeared to contain pseudodactylid-free eel. In contrast, the pseudodactylid-free condition was predominant in North Wales since only 1 catchment site (Clwyd-Elwy) out of the 4 rivers showed eel with a monogenean infection. Overall, eel sampled from South East England had the greatest prevalence, and also greatest mean abundance, of pseudodactylid infection. In contrast, the lowest prevalence and mean abundance was observed in eel sampled from North Wales.

Overall, the regional prevalence data was significantly different (p: 10^{-4}-0.0357) between the areas with the exception of the data observed for North West England and South Wales (p: 0.155). There were no significant differences observed in intensity of infection between the four geographic regions (p: 0.285-0.909). However, on analysis of pseudodactylid abundance, there were significant differences (p: 10^{-4}-0.0282) between the regions with the exception of the comparison between North West England and South Wales (p: 0.136).

Table 2.6 Regional infection data for pseudodactylids isolated from European eel sampled across England and Wales. ^No significant difference between these regions.

Regions	Number of river sites examined	Number of pseudodactylid-positive river sites	Number of examined eel	Number of infected eel	Pseudodactylid prevalence rate (%)	Number of pseudodactylids	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	4	50	15	30%^	273	5.5±19.56^	18.2±3.0^	1 to 125
South East England	2	2	20	17	85%	215	10.8±16.9	12.7±2.8^	1 to 56
North Wales	4	1	40	4	10%	17	0.4± 1.8	4.3± 4.7^	1 to 11
South Wales	3	3	30	14	46.7%^	114	3.8± 6.9^	8.1± 8.3^	1 to 29
Total	14	10	140	50	35.71%	619	4.4±13.9	12.4±21.3^	1 to125

2.4.1.2 Primary Infection data: the host

For the pseudodactylid infected eels, the body length ranged from 20 to 86 cm (mean = 36.8 ±17.5 cm) and body weight ranged from 7.7 to 1380 g (mean = 138±240.87 g). For uninfected eels, the body length ranged from 10 to 86 cm (mean = 28.97±13.44 cm) and body weight ranged from 1.2g to 1380g (mean = 156.57±282.4 g). The mean condition factor for infected eel is 0.15±0.05, whilst the mean average condition factor for the uninfected eel is 0.14±0.05 (Table 2.3 and 2.4). None of this data was statistically significant ($p < 0.03$).

Upon analysis of different categories of eel length it was apparent that mean pseudodactylid abundance and intensity increased as eel body length category increased (Table 2.7). Indeed, to corroborate this, only 1 pseudodactylid was recovered from the 26 specimens considered to form the smallest examined length category of eel. Differences in the intensity of infection were significant between the smallest length category and all the other length categories (p: 0.0001 – 0.013). In addition, the intensity of infection was also significantly different between the second smallest length category and the second largest length category (p = 0.025). There were no other significant differences in the intensity of infection between remaining length categories ($p > 0.05$). The prevalence of pseudodactylids also increased up to the eel category size 30.5 – 35cm and the prevalence of infection for the smallest length category of eel was significantly different to the pseudodactylid infection prevalence data observed for all other length categories (p: 0.0001 – 0.017). There was no significant difference in the prevalence of infection when comparisons were made between all the other length categories (p: 0.087 – 1.0).

Table 2.7 Pseudodactylid infection data with respect to category of eel body length.
*Significant difference ($p < 0.05$) when compared to the smallest length category. [#] Significant difference ($p < 0.05$) when compared to the smallest length category. [a] Significant difference ($p < 0.05$) when compared to the second smallest length category.

Body length range (cm)	Mean body length (±sd) (cm)	Number of examined eel	Number of infected eel	Pseudodactylid prevalence rate (%)	Number of pseudodactylids	Mean intensity (±sd)	Intensity range
10-20	16.9±3.0	26	1	3.80%	1	1	1
20.5-25	22.4±1.4	32	9	28.1%*	76	8.44±11.16[#]	1 - 29
25.5-30	27.9±1.4	32	15	46.9%*	177	11.8±15.71[#]	1 - 51
30.5-35	32.6±1.3	22	12	54.6%*	149	12.42±14.68[#,a]	1 - 56
35.5-86	53.9±17.4	28	13	46.4%*	216	16.62±35.34[#]	1 - 125

On analysis of the pseudodactylid infections with respect to eel weight categories (Table 2.8), a similar trend was noted as above for eel length. Prevalence of pseudodactylids increased up to the weight category 30.1- 60 g and the lowest intensity of infection was observed in the smallest eel. To corroborate this finding, the prevalence of infection for the smallest weight category of eel was significantly different to the pseudodactylid infection prevalence data observed for the largest two weight categories (p: 0.0008 – 0.005). In addition, the second smallest weight category had a significantly different prevalence of infection when compared to the second largest weight category ($p = 0.044$). There was no significant difference in the infection data when comparisons were made between all the other weight categories (p: 0.136 – 0.632). With respect to intensity of infection, there was a highly significant difference in parasite burden between the smallest weight category and the two largest weight categories (p: 0.003-0.0004). In addition there was a significant difference between the intensity data for the second smallest weight category and the second largest weight category (p=0.0435). There was no significant difference in the intensity data between all other classes of eel body weight (p>0.05).

Table 2.8 Pseudodactylid infection data with respect to category of eel body weight.
*Significant difference ($p < 0.05$) when compared to the smallest weight category. [b] Significant difference ($p < 0.05$) when compared to the second smallest weight category. [#] Significant difference ($p < 0.05$) when compared to the smallest weight category. [a] Significant difference ($p < 0.05$) when compared to the second smallest weight category.

Eel body weight range (g)	Mean body weight (±sd) (g)	Number of examined eel	Number of infected eel	Pseudodactylid prevalence (%)	Number of pseudodactylids	Intensity range	Mean intensity (±sd)
1.2 - 15	8.6±4.1	39	6	15.4%	43	1 - 29	7.2±10.8
15.1 - 30	22.1±4.4	32	9	28.1%	115	1 - 34	12.8±14.4
30.1 - 60	42.9±8.5	33	18	54.5%*[,b]	167	1 - 51	9.3±12.1[#,a]
60.1 - 1380	301.4±345.7	36	17	47.2%*	294	1-125	17.3±32.4[#]

With respect to eel condition factor, there was no significant difference in pseudodactylid prevalence of infection between the different categories of condition factor (Table 2.9) (p: 0.119 – 0.629). In addition, there was no significant difference when the intensity of infection was analysed between the different categories of condition factor (p: 0.182-0.798).

Table 2.9 Pseudodactylid infection data with respect to category of eel condition factor.

Condition factor range	Mean condition factor (±sd)	Number of eel examined	Number of infected eel	Pseudodactylid prevalence (%)	Number of pseudodactylids	Intensity range	Mean intensity (±sd)
0.04-0.12	0.09±0.03	34	11	32.4	84	1-29	7.6±10.1
0.13-0.14	0.13±0.00	38	10	26.3	234	1-125	23.4±37.9
0.15-0.16	0.15±0.00	28	13	46.4	75	1-22	5.8±6.0
0.17-0.29	0.19±0.03	40	16	40	226	1-56	14.1±19.8

2.4.2 *Pseudodactylogyrus* spp. morphological analysis

The two species of pseudodactylid documented in European eel are highly similar in appearance and can be morphologically very difficult to distinguish. As such, careful morphometric analysis of *Pseudodacylogyrus* spp. is crucial to species identity. An example of the difficulty of using a classical morphometric approach, particularly with samples subjected to repeat freeze–thaw cycles, is illustrated below (Figure 2.4). The body size of the pseudodactylid (extracted from eel sample CD9) was found to be 479µm in length which is within accepted limits for both *P. anguillae* and *P. bini* (section 2.2.3).

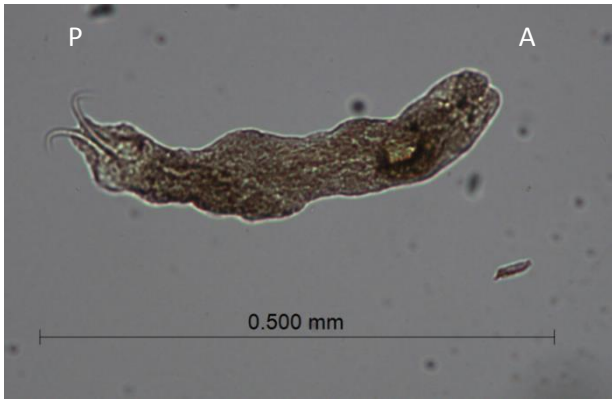

Figure: 2.4 A representative pseudodactylid (isolated from eel CD9). A: anterior end showing eye spots; P: posterior end showing the free hooks.

Moreover, measurements of the hamuli and connective bar were equally inconclusive. For example, the length of the hamuli was 62µm and the length and width measurements of the connecting bar were 46 µm and 10µm respectively (parasite isolated from eel sample CD9) (Figure 2.5, A, B & C). All these measurements are within acceptable ranges for *P. bini* and *P. anguillae* (see Table 2.1).

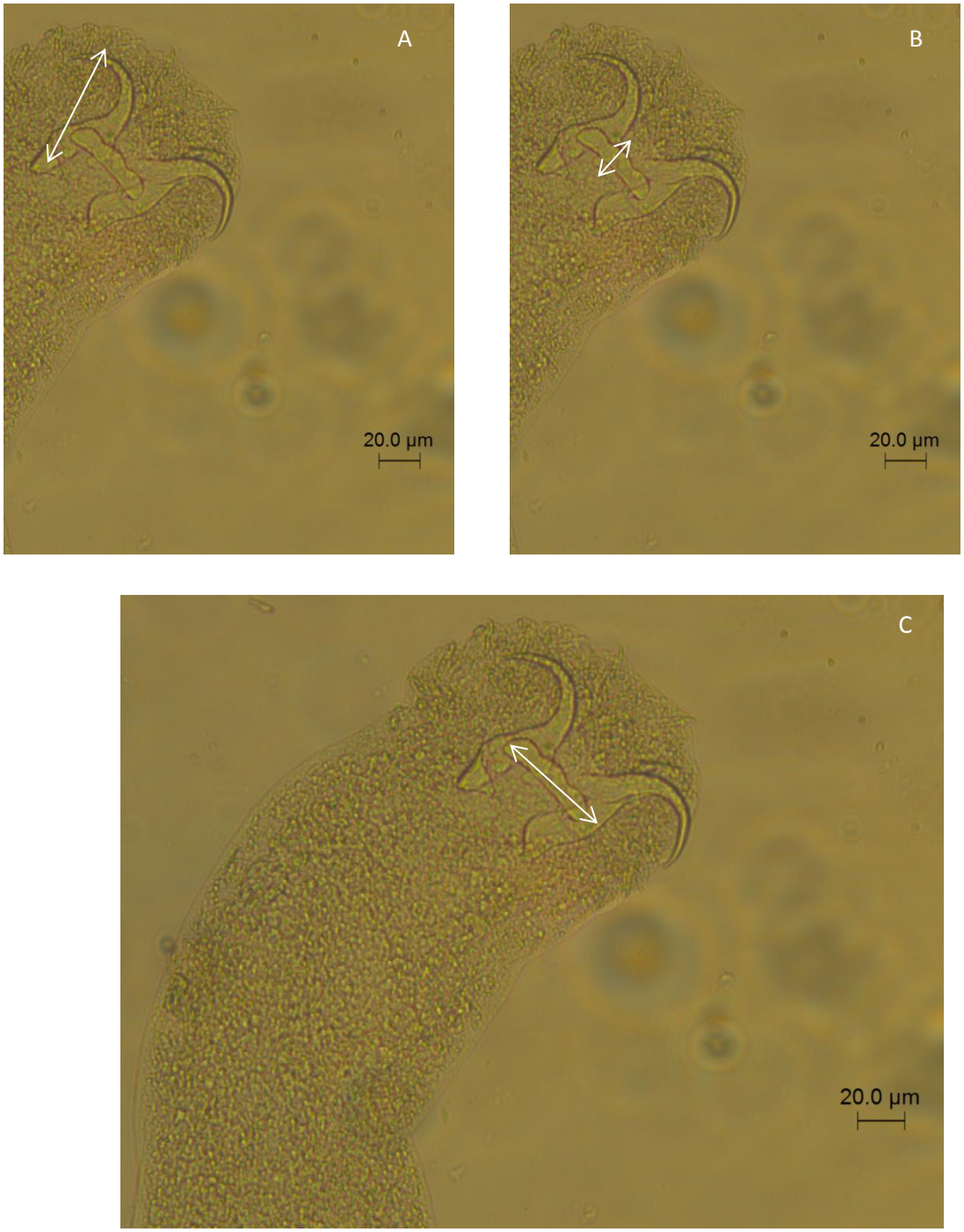

Figure 2.5 A representative pseudodactylid (isolated from eel CD9) showing the hamuli and measurements utilised for species identification attempts (x 400). (a) The arrow represents the length measurement (=62µm). (b) The arrow represents the width of the connective bar (11 µm). (c) The arrow represents the length of the connective bar (46 µm).

2.4.3 Molecular determination of pseudodactylid species

Given the difficulty in using morphological keys for pseudodactylid species confirmation a molecular diagnostic approach was employed. Examination of the NCBI GenBank database showed very limited entries for *P. anguillae* and *P. bini*. However, 18S rRNA gene sequences were present for both parasites and further analysis indicated that it would be possible to discriminate between the two pseudodactylids by PCR amplification of the respective 18S rRNA genes and subsequent restriction digestion of the products with EcoN1(Xag1) (Figure 2.6) showing alignment of the two 18S rRNA genes). If the source of DNA is *P. anguillae*, then the PCR product will be cut into two pieces by EcoNI, fragments sizes (358bp and 156bp). Whereas if *P. bini*, the PCR product will not be digested (514 bp). Total helminth genomic DNA was extracted from representative parasites and its 18S rRNA gene was PCR amplified. The resulting product was examined by agarose gel electrophoresis, (Figure 2.7) confirming the presence of a 514bp specific product. A PCR product of 514bp derived for *P. bini* well be expected to remain 514bp (i.e. uncut) following incubation with Xag1, in contrast, a *P. anguillae* derived PCR product well be cut by Xag1 into two fragments of 358bp and 156bp.

```
gi|13810563|dbj|AB060591.1|      AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTTAGCC 50
gi|14717824|dbj|AB065113.1|      AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTTAGCC 50
                                 **************************************************

gi|13810563|dbj|AB060591.1|      ATGCATGCCTCAGTACATAACCTTAACATGGTGAAACCGCGAATGGCTCA 100
gi|14717824|dbj|AB065113.1|      ATGCATGCCTCAGTACATAACCTTAACATGGTGAAACCGCGAATGGCTCA 100
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TTAAATCAGCTATGGTTCCTTGGATCATAATTCTACTACATGGATAACTG 150
gi|14717824|dbj|AB065113.1|      TTAAATCAGCTATGGTTCCTTGGATCATAATTCTACTACATGGATAACTG 150
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TAGTAATTCTAGAGCTAATACATGCCTCGATGCCCTGATCCGTAAGGTGA 200
gi|14717824|dbj|AB065113.1|      TAGTAATTCTAGAGCTAATACATGCCTCGATGCCCTGATCCGTAAGGTGA 200
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GGGGCGCAGTTATTAGATTACAAACCAAACGGGTTCGCCCGTGGTCTGTG 250
gi|14717824|dbj|AB065113.1|      GGGGCGCAGTTATTAGATTACAAACCAAACGGGTTCGCCCGTGGTCTGTG 250
                                 **************************************************

gi|13810563|dbj|AB060591.1|      ATGACTCTGGATAACTTTGTATGATCGCAGCTGGCCCTGTTGTCGGCGAT 300
gi|14717824|dbj|AB065113.1|      ATGACTCTGGATAACTTTGTATGATCGCAGCTGGCCCTGTTGTCGGCGAT 300
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GGATCCATCAAATATCTGCCCTATCAACTTTCGACGGTAGACGACATGCC 350
gi|14717824|dbj|AB065113.1|      GGATCCATCAAATATCTGCCCTATCAACTTTCGACGGTAGACGACATGCC 350
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TACCGTGGTTATGACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGG 400
gi|14717824|dbj|AB065113.1|      TACCGTGGTTATGACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGG 400
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCACGCAAAT 450
gi|14717824|dbj|AB065113.1|      GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCACGCAAAT 450
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TACCCACTCTCTGAACGAGGAGGTAGTGAAGATAAATATCGATACAGGAC 500
gi|14717824|dbj|AB065113.1|      TACCCACTCTCTGAACGAGGAGGTAGTGAAGATAAATATCGATACAGGAC 500
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TTATTATAGGCTCTGTAATCGAAATGAGTGCATTTTAAATCCTACAACGA 550
gi|14717824|dbj|AB065113.1|      TTATTATAGGCTCTGTAATCGAAATGAGTGCATTTTAAATCCTACAACGA 550
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT 600
gi|14717824|dbj|AB065113.1|      GGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAACTCCAGCT 600
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CCAATAGCATATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATC 650
gi|14717824|dbj|AB065113.1|      CCAATAGCATATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATC 650
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TGGGCCTGGCTCGATTGCTAGTCTGTGCGCGTGTGTGAAAGCATGAGTGT 700
gi|14717824|dbj|AB065113.1|      TGGGCCTGGCTCGATTGCTAGTCTGTGCGCGTGTGTGAAAGCATGAGTGT 700
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gi|13810563|dbj|AB060591.1|      GCGGGCTGGTATATTCGGGTCGGCTTCATAGCTGTGTCTGGCGGCCTTCA 750
gi|14717824|dbj|AB065113.1|      GCGGGCTGGTATATTCGGGTCGGCTTCATAGCTGTGTCTGGCGGCCTTCA 750
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CGGGCTGTCGGATGGTGTTAAGTGTCTGTGGCGTTCCCTCAGGGGGACGT 800
gi|14717824|dbj|AB065113.1|      CGGGCTGTCGGATGGTGTTAAGTGTCTGTGGCGTTCCCTCAGGGGGACGT 800
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TGCGGCTTAGCACTATGGTACGTTGATGCCCTTAACCGGGTGTCCGTAAC 850
gi|14717824|dbj|AB065113.1|      TGCGGCTTAGCACTATGGTACGTTGATGCCCTTAACCGGGTGTCCGTAAC 850
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TCGCAGCACGTTTACTTTGAACAAATTTGAGTGCTCAAAGCAGGCCGTTT 900
gi|14717824|dbj|AB065113.1|      TCGCAGCACGTTTACTTTGAACAAATTTGAGTGCTCAAAGCAGGCCGTTT 900
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gi|13810563|dbj|AB060591.1|      GTTGCCTGAAGATTCTTGCATGGAATAATGGAATAGGACTTTGGTCCTAT 950
gi|14717824|dbj|AB065113.1|      GTTGCCTGAAGATTCTTGCATGGAATAATGGAATAGGACTTTGGTCCTAT 950
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TTTGTTGGTTTGATAGAACCGAAGTAATGATAAAAAGAGACAGACGGGGG 1000
gi|14717824|dbj|AB065113.1|      TTTGTTGGTTTGATAGAACCGAAGTAATGATAAAAAGAGACAGACGGGGG 1000
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CATTTGTATGGCGGCGCTAGAGGTGAAATTCGTTGACCGTCGCCAGACAA 1050
gi|14717824|dbj|AB065113.1|      CATTTGTATGGCGGCGCTAGAGGTGAAATTCGTTGACCGTCGCCAGACAA 1050
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CCTAAAGCGAAAGCATCTGCCAAGTATGTTTTCATTTACCAGGAGCGAAA 1100
gi|14717824|dbj|AB065113.1|      CCTAAAGCGAAAGCATCTGCCAAGTATGTTTTCATTTACCAGGAGCGAAA 1100
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gi|13810563|dbj|AB060591.1|      GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACG 1150
gi|14717824|dbj|AB065113.1|      GTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACG 1150
                                 **************************************************

gi|13810563|dbj|AB060591.1|      ATGCCAACTGACGATCCGCGTAAGCTCTTCAATGAGCTCGCGGGCAGTCT 1200
gi|14717824|dbj|AB065113.1|      ATGCCAACTGACGATCCGCGTAAGCTCTTCAATGAGCTCGCGGGCAGTCT 1200
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CCGGGAAACCTGTAAGTCTATGGGTTCCGGGGAAAGTATGGTTGCAAAAC 1250
gi|14717824|dbj|AB065113.1|      CCGGGAAACCTGTAAGTCTATGGGTTCCGGGGAAAGTATGGTTGCAAAAC 1250
                                 **************************************************

gi|13810563|dbj|AB060591.1|      TGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCTTGCG 1300
gi|14717824|dbj|AB065113.1|      TGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCTTGCG 1300
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GCTTAATTTGACTCAACACGGGGAAACTCACCCGAACCGGACACTGGGAG 1350
gi|14717824|dbj|AB065113.1|      GCTTAATTTGACTCAACACGGGGAAACTCACCCGAACCGGACACTGGGAG 1350
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GATTGACAGAATGATTGCTCTTTCATGATTCAGTGGCTAGTGGTGCATGG 1400
gi|14717824|dbj|AB065113.1|      GATTGACAGAATGATTGCTCTTTCATGATTCAGTGGCTAGTGGTGCATGG 1400
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CCGTTCTTAGTTGGTGGTACGAACTGTCTGGTTAATTCCGATAACGAACG 1450
gi|14717824|dbj|AB065113.1|      CCGTTCTTAGTTGGTGGTACGAACTGTCTGGTTAATTCCGATAACGAACG 1450
                                 **************************************************

gi|13810563|dbj|AB060591.1|      AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 1500
gi|14717824|dbj|AB065113.1|      AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 1500
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GTGCTGGCGCCTTCCCTCGGGCAGGTGGTCGGGACAGGACCTCGTAAGCA 1550
gi|14717824|dbj|AB065113.1|      GTGCTGGCGCCTTCCCTCGGGCGGGTGGTCGGGACAGGACCTCGTAAGCA 1550
                                 ********************** ***************************

gi|13810563|dbj|AB060591.1|      GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 1600
gi|14717824|dbj|AB065113.1|      GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 1600
                                 **************************************************

gi|13810563|dbj|AB060591.1|      AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 1650
gi|14717824|dbj|AB065113.1|      AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 1650
                                 **************************************************

gi|13810563|dbj|AB060591.1|      AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 1700
gi|14717824|dbj|AB065113.1|      AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 1700
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 1750
gi|14717824|dbj|AB065113.1|      CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 1750
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 1800
gi|14717824|dbj|AB065113.1|      GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 1800
                                 **************************************************

gi|13810563|dbj|AB060591.1|      CCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTTTA 1850
gi|14717824|dbj|AB065113.1|      CCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTTTA 1850
                                 **************************************************

gi|13810563|dbj|AB060591.1|      GCAAGGCCATTGGATCGAGTCCATACGGTTCACGCTGTTTGGATTTGAGA 1900
```

```
gi|14717824|dbj|AB065113.1|     GCAAGGCCATTGGATCGAGTCCATACGGTTCACGCTGTTTGGATTTGAGA 1900
                                **************************************************

gi|13810563|dbj|AB060591.1|     AGATGGTCGAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTT 1950
gi|14717824|dbj|AB065113.1|     AGATGGTCGAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTT 1950
                                **************************************************

gi|13810563|dbj|AB060591.1|     CCGTAGGTGAACCTGCAGAAGGATC 1975
gi|14717824|dbj|AB065113.1|     CCGTAGGTGAACCTGCAGAAGGATC 1975
                                *************************
```

Figure 2.6 An alignment of the 18S rRNA sequences of *P. anguillae* (gi: 13810563) and *P. bini* (gi: 14717824) highlighting the one nucleotide difference at position 1523 bp that allows the *P. anguillae* sequence to be restricted with Xag I (5'-CCTNNNNNAGG-3'). The regions highlighted in yellow show the positions of the two PCR primers designed to allow the molecular diagnostic approach to pseudodactylid species identification.

2.4.4 Genomic DNA extraction from pseudodactylid samples

DNA extraction was performed on 35% (217/619) of the pseudodactylids isolated from 9 different catchment sites across England and Wales (Table 2.10). The only catchment site not examined was the River Bela.

Table 2.10 Summary of pseudodactylid genomic DNA extractions by catchment site.

River catchment Sites	Total number of pseudodactylids	number of DNA extractions	% of DNA extractions
Leven: England	232	48	20.7
Crouch: England	141	80	56.7
Crane: England	74	32	43.2
Hether Burn: England	33	14	42.4
Petteril: England	3	2	66.7
Cadoxton: Wales	60	20	33.3
Rhymney: Wales	53	10	18.9
Clwyd-Elwy: Wales	17	10	58.8
Taff: Wales	1	1	100
Total	619	217	35.3%

2.4.5 PCR-based diagnostics

In total, 172 pseudodactylid 18S rRNA PCR products of the expected size (514bp) were generated (Figure 2.7).

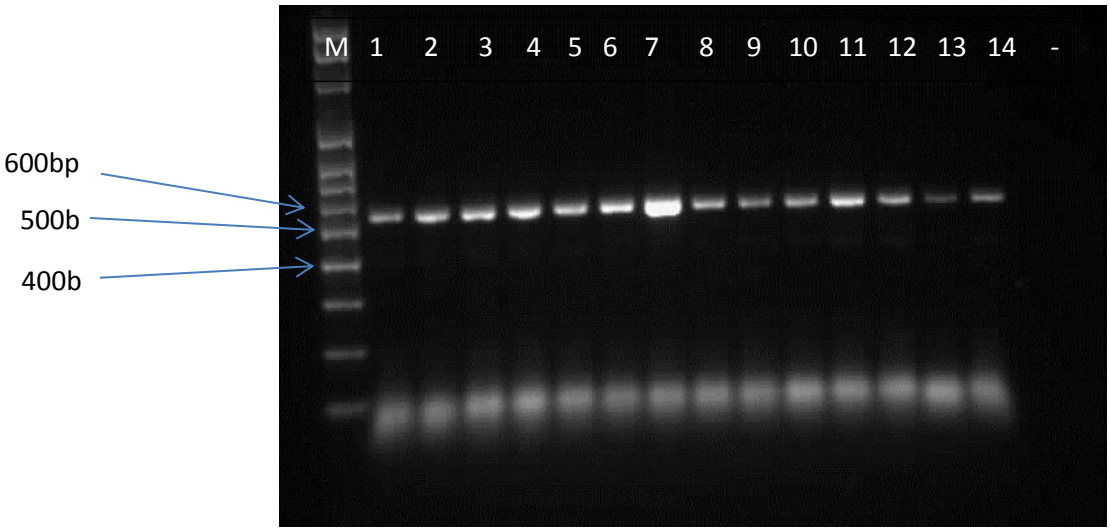

Figure 2.7 A representative agarose gel (1% w/v) showing PCR amplification of the 514bp fragment of the *Pseudodactylogyrus spp.* 18S rRNA gene. M: 100 bp Hyperladder (Bioline); lanes 1-14: PCR products derived from individual pseudodactylids isolated from eel sampled from the River Crane; –ve: negative control.

Seventy eight (45.3%) of the 172 PCR products subjected to Xag1 digestion showed clear restriction to produce the expected fragments of 350bp and 150bp. As such, these PCR products must have been derived from *P. anguillae* genomic DNA (Figure 2.8). The remaining 94 PCR products (54.7%) were not restricted with Xag1 and hence these must have been derived from *P. bini* genomic DNA. In all these cases, the control λ DNA was successfully restricted with Xag1. DNA sequence analysis of a small number of these PCR products confirmed interpretation of the restriction digests (Figure 2.9).

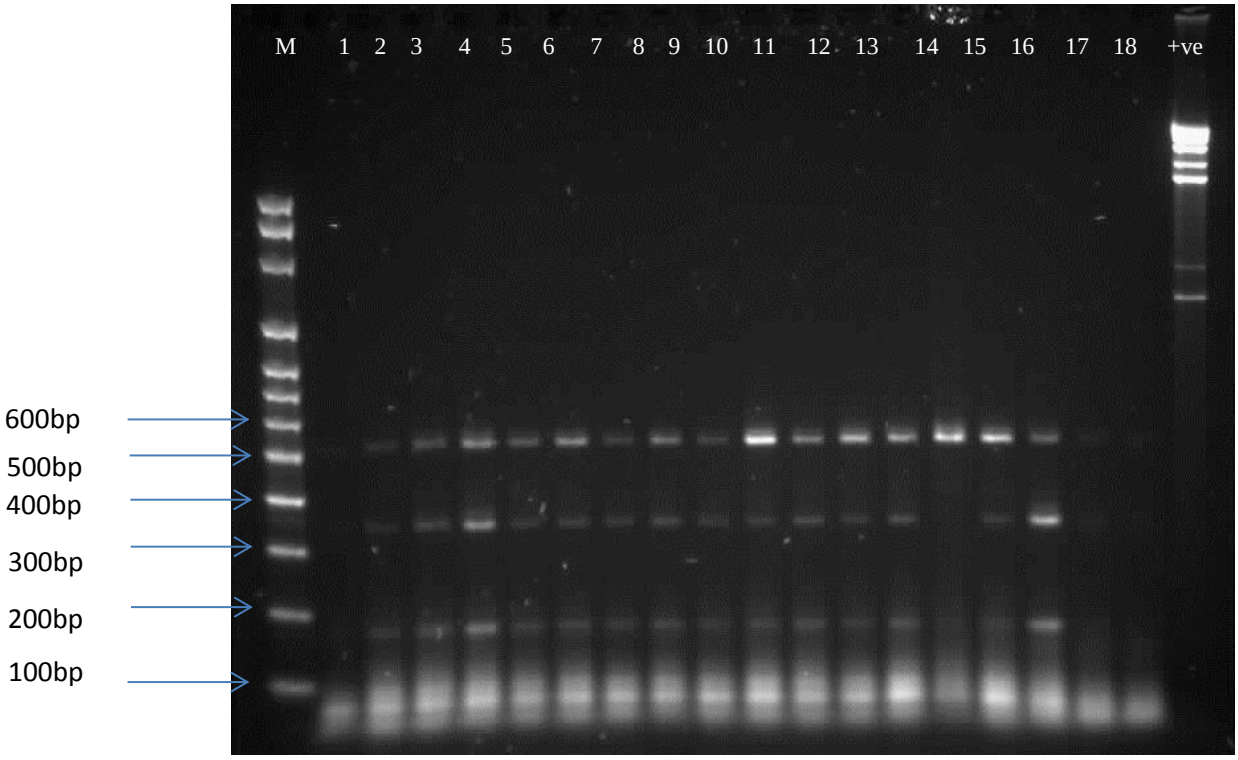

Figure 2.8 A representative agarose gel (1% w/v) showing Xag1 digestion of 18 S rRNA PCR products derived from pseudodactylids sampled from the River Leven. M: 100 by Hyperladder (Bioline); lanes 1-13 and 15-18 pseudodactylid 18S rRNA PCR products digested with Xag1 (= *P. anguillae);* lane 14: pseudodactylid 18S rRNA PCR products that is undigested with Xag1 (= *P. bini)*; +ve: λ DNA digested with Xag1.

Analysis of the Xag1 digestions by catchment site indicated that 3 rivers were infected with only *P. bini* and 3 rivers were infected with only *P. anguillae* (Table 2.11). These single infections were present in English and Welsh river systems. In the remaining three catchment sites, the data showed the presence of mixed pseudodactylid infections. Moreover, the river Leven had approximately equal numbers of *P. anguillae* and *P. bini*. In contrast, *P. bini* was the predominant species (92%) sampled from the River Crouch and *P. anguillae* was the predominant species (78%) from the River Cadoxton. In total, 55% of the pseudodactylids were confirmed *as P. bini* and 45% were *P. anguillae*. This is the first published report of *P. bini* existing in European eel in UK waters.

Table 2.11 Summary of the pseudodactylid molecular species confirmation approach.

Catchment Site	Number of PCR products analysed	Number of PCR products restricted by Xag1 (= *P. anguillae)*	Number of PCR products unrestricted by Xag1 (= *P. bini)*
Crouch; England	60	5	55
Leven; England	43	19	24
Crane; England	25	25	0
Hether Burn; England	14	14	0
Petteril; England	2	0	2
Clwyd-Elwy; Wales	10	0	10
Rhymney; Wales	8	8	0
Cadoxton; Wales	9	7	2
Taff; Wales	1	0	1
	172	78	94

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418204401_Pb1_F_A06             -------------------------GTCTGGTTAATTCCGATAACGAACG 25
418204401_Pb2_F_C06             -------------------------------------------------
gi|14717824|dbj|AB065113.1|     CCGTTCTTAGTTGGTGGTACGAACTGTCTGGTTAATTCCGATAACGAACG 1450
gi|13810563|dbj|AB060591.1|     CCGTTCTTAGTTGGTGGTACGAACTGTCTGGTTAATTCCGATAACGAACG 1450
417794001_pa3_F_E06             -----------------ACCAAACTGTCTGGTTAATTCCGATAACGAACG 33
417794001_pa4_F_G06             -------------------------TGTCTGGTTAATTCCGATAACGAACG 26

418204401_Pb1_F_A06             AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 75
418204401_Pb2_F_C06             --ACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 48
gi|14717824|dbj|AB065113.1|     AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 1500
gi|13810563|dbj|AB060591.1|     AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 1500
417794001_pa3_F_E06             AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 83
417794001_pa4_F_G06             AGACTCTAACCTGCTAAATAGTATGGTTGCAAAATATTACGTGGCCCCCT 76
                                  **********************************************

418204401_Pb1_F_A06             GTGCTGGCGCCTTCCCTCGGGCGGGTGGTCGGGACAGGACCTCGTAAGCA 125
418204401_Pb2_F_C06             GTGCTGGCGCCTTCCCTCGGGCGGGTGGTCGGGACAGGACCTCGTAAGCA 98
gi|14717824|dbj|AB065113.1|     GTGCTGGCGCCTTCCCTCGGGCGGGTGGTCGGGACAGGACCTCGTAAGCA 1550
gi|13810563|dbj|AB060591.1|     GTGCTGGCGCCTTCCCTCGGGCAGGTGGTCGGGACAGGACCTCGTAAGCA 1550
417794001_pa3_F_E06             GTGCTGGCGCCTTCCCTCGGGCAGGTGGTCGGGACAGGACCTCGTAAGCA 133
417794001_pa4_F_G06             GTGCTGGCGCCTTCCCTCGGGCAGGTGGTCGGGACAGGACCTCGTAAGCA 126
                                ********************* **************************

418204401_Pb1_F_A06             GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 175
418204401_Pb2_F_C06             GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 148
gi|14717824|dbj|AB065113.1|     GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 1600
gi|13810563|dbj|AB060591.1|     GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 1600
417794001_pa3_F_E06             GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 183
417794001_pa4_F_G06             GGTCAAACTTCTTAGAGGAACAGGCGCCAAAAAGGCGTACGAAAGAGAGC 176
                                **************************************************

418204401_Pb1_F_A06             AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 225
418204401_Pb2_F_C06             AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 198
gi|14717824|dbj|AB065113.1|     AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 1650
gi|13810563|dbj|AB060591.1|     AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 1650
417794001_pa3_F_E06             AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 233
417794001_pa4_F_G06             AATAACAGGTCTGTGATGCCCTAAGATGTCCGGGGCCGCACGCGTGCTAC 226
                                **************************************************

418204401_Pb1_F_A06             AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 275
418204401_Pb2_F_C06             AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 248
gi|14717824|dbj|AB065113.1|     AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 1700
gi|13810563|dbj|AB060591.1|     AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 1700
417794001_pa3_F_E06             AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 283
417794001_pa4_F_G06             AATGACGATGCTAGTGAGGATGATTCACTGGTCCGAAAGGATCGGTAAAA 276
                                **************************************************

418204401_Pb1_F_A06             CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 325
418204401_Pb2_F_C06             CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 298
gi|14717824|dbj|AB065113.1|     CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 1750
gi|13810563|dbj|AB060591.1|     CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 1750
417794001_pa3_F_E06             CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 333
417794001_pa4_F_G06             CTTTTCAATCATCGTCGTGCCTGGGATTGGGGTTTGCAATTGTCCCCCAT 326
                                **************************************************

418204401_Pb1_F_A06             GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 375
418204401_Pb2_F_C06             GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 348
gi|14717824|dbj|AB065113.1|     GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 1800
gi|13810563|dbj|AB060591.1|     GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 1800
417794001_pa3_F_E06             GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTGATTACGT 383
417794001_pa4_F_G06             GAACCAGGAATTCCTAGTAAGCACAAGTCATCACCTTGTGCTG------- 369
                                *****************************************
```

Figure 2.9 Sequence alignment of four 18S rRNA gene fragments derived from PCR amplification of individual pseudodactylids: Pb1 and Pb2 (from eel specimen C107), Pa3 (from eel specimen R8) and Pa4 (from eel specimen CD10). The data confirms the restriction analysis with Xag I: Pb1 and Pb2 = *P. bini* and Pa3 and Pa4 = *P. anguillae*. The 18S rRNA sequences for *P. bini* (gi: 14717824) and *P. anguillae* (gi: 13810563) are also shown.

2.5 Gill pathology

Prior to dissection, a number of the European eel specimens showed a clear mucosal secretion from the gill opening (Figure 2.10).

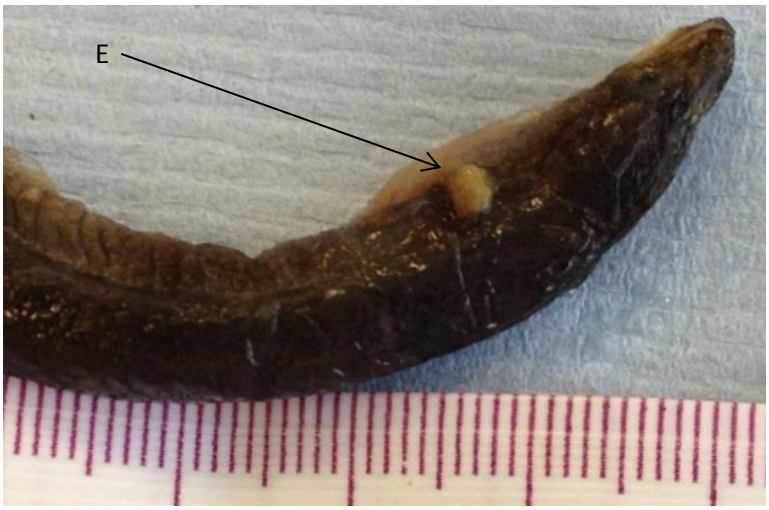

Figure 2.10 A representative image of the mucosal secretion observed from the gill opening (eel specimen R3 which was subsequently shown upon dissection to harbour 22 pseudodactylids). E: external gill opening with exudate.

Upon examination of the gill surface of each European eel it was difficult to ascertain the tissue damage and pathology caused directly by pseudodactylids since the hosts were subject to long-term storage and repeated freeze-thaw cycles which had contributed to some tissue deterioration. Consequently, the presence of exudate was utilised as the clear pathological indicator of parasite infection. Excessive exudate was clearly present on European eel examined from the Rivers Cadoxton, Rhymney and Clwyd-Elwy in Wales and the Rivers Leven and Petteril in England. However, in a small number of eel specimens from the Rivers Cadoxton and Rhymney exudate was present without subsequent detection of pseudodactylids. When present, the exudate covered the external surface of gills and also, it was present between the gill filaments (Figure 2.11). The exudate contained most of the pseudodactylids and hence it was not possible to associate any specific area of the gill with parasite preference.

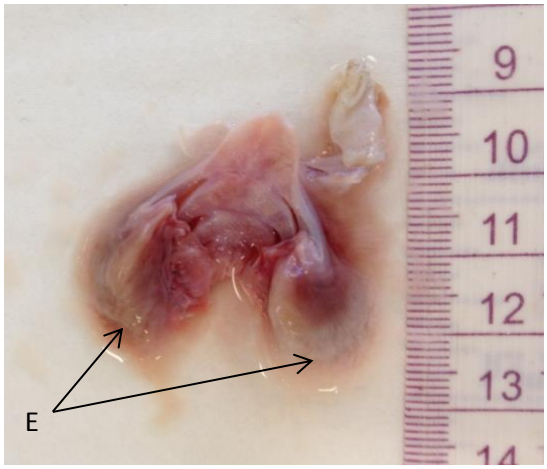

Figure 2.11 An image of the gross pathology of a pseudodactylid infected gill (eel RL2 subsequently shown to be infected with 125 pseudodactylids). E: excessive amounts of exudate covering the gill surface.

2.6 Discussion

Prior to initiation of this study, the only reports of pseudodactylid infection in UK eel showed that *P. anguillae* was present in specimens of *A. anguilla* sampled from three locations in Devon (Nie & Kennedy, 1991d; Kennedy, Nie, Kaspers, *et al.*, 1992). In the intervening years, there has been no further scientific study published that addresses the extent of pseudodactylid infection in UK eel. As such, the work presented in the chapter was aimed at establishing a more coherent and robust set of data that reports current levels of pseudodactylid infection in UK eel that were sampled from different regions of the United Kingdom. To this end, pseudodactylid infection was observed in eel specimens sampled from the majority of catchment sites in England and Wales.

The mode of acquisition of the eel specimens unfortunately did not permit a clear morphological characterisation of the two common pseudodactylid species reported in *A. anguilla*. Indeed, even when fresh parasite specimens are obtained, morphological distinction between *P. anguillae* and *P. bini* is often difficult; moreover, differences have even been reported for the same species at different geographical regions (Zolovs *et al.*, 2016). As such, a molecular-based diagnostic approach was developed that involved amplification of the 18S rRNA gene of pseudodactylids and then restriction of the resulting PCR product with the Xag I endonuclease. This novel approach allowed confirmation, as reported elsewhere (Copley & McCarthy, 2001; Kennedy, 2007b), that *P. bini* is indeed present in UK eel populations. Indeed, by screening 28% of the recovered pseudodactylids, it was apparent that *P. bini* was more prevalent that *P. anguillae*. There also appeared to be some catchment sites that contained eel harbouring single species infections whereas at other sites the hosts carried mixed infections. However, some of the single species infection data should be treated with caution; for example, only *P. bini* was characterised from eel present in the rivers Petteril and Taff but the analysis was based upon low numbers of parasites.

Overall, pseudodactylids were more prevalent in English rivers than Welsh rivers. However, given that the actual eel sampling dates are unknown, a degree of caution is necessary when interpreting the infection data. A similar pattern of the higher prevalence of the swim bladder nematode infection in the European eels in English rivers than Welsh rivers was reported (Ab-Aziz, 2012). Nonetheless, assuming that the sampling dates were within the same season, the data within this thesis shows that there were statistically significant differences in prevalence between most of the geographic regions. A similar profile of statistically significant differences was also observed for the regional parasite abundance data. As such, it is reasonable to conclude that pseudodactylids may have adapted to the different environmental conditions within these regions with varying levels of success. Interestingly, eel from the two most northern regions examined had the lowest prevalences of infection whereas eel from the most southern region had the greatest prevalence, and also abundance, of pseudodactylid infection. One possible explanation for this might be that the parasites are less well adapted to surviving in rivers that have a lower water temperature. Indeed, pseudodactylid eggs are reported as not able to hatch when the water temperature is below 10^{o}C (Buchmann, 1987). Interestingly, the most comprehensive data on water temperatures in UK rivers discusses the reasons for water temperature variation and notes that there are on average 153 days a year when water temperatures are $<10^{o}$C in the South of England whereas in the North West this increases to 181 days (Orr *et al.*, 2010). To this end, the parasite dynamics are likely to vary between the regions with the more southern rivers supporting enhanced parasite development and hence greater overall infection rates.

In terms of host factors, the data presented in this chapter highlights that when eel are less than 20cm in length the prevalence of infection with pseudodactylids is significantly less than occurs with larger eel. Moreover, the intensity of infection is also significantly less in the eel that are less than 20cm in length. Since body length is a reasonably good pseudomeasure of

age then this data most probably indicates that the smallest and hence youngest eel have incurred less exposure to the parasite (Buchmann, 1989b). A similar statistically significant infection profile is also observed when the data is analysed with respect to body weight. As the larger bodied fish will have an increased surface area of gill compared to the smaller eel then it is perhaps unsurprising that the bigger animals generally have a larger intensity of infection. Indeed, the intensity range increased as the overall size of the eel increased (Buchmann, 1988a; Mayo-Hernandez, Serrano, Penaver, *et al.*, 2015).

The condition factor is a useful measure of fish health and hence the infection data was also analysed with respect to the eel condition factor. No statistically significant data emerged from the analysis and hence it can be concluded that the overall health of the eel in this study was not directly impacted by pseudodactylid infection. In contrast to this data, a recent study documented that pseudodactylid infection decreased eel body condition in relation when the intensity of parasite infection increased (Gérard *et al.*, 2013). One reason for this anomaly might be that the Gerard *et al*., (2013) study was based upon analysis of migrating silver eel; none of the specimens in this thesis were migrating and only a few eel from the river Crane were likely to be at the silvering stage of development.

At the level of the individual host, it was evident that eel with a higher parasite burden often displayed a pathology that was characterised by the presence of exudate. This is indicative of the parasite causing damage to the gill as it penetrates the gill tissue with the hook in order to attach and feed. Indeed, as reported elsewhere, the parasite causes erosion and ulceration of the gill and the host defence is characterised by secretion of excessive amounts of exudate (Abdelmonem *et al.*, 2010). In this thesis, exudate was observed on the eel sampled from rivers shown to have hosts that were infected with only *P. bini* (rivers Clwyd-Elwy and Petteril), only *P. anguillae* (river Rhymney) and mixed species infections (rivers Cadoxton and Leven). As such, it is not possible to comment upon the relative pathological impact of

one species of pseudodactylid relative to the other. Moreover, this study was not focussed upon pathology and given the nature of specimen acquisition, it would be difficult to speculate further. However, other reports on pseudodactylid infections in *A. anguilla* conclude that *P. bini* is more pathogenic to eel than *P. anguillae* and this is a consequence of differences in the anatomy of their respective hooks (Arafa & Reda, 2012).

In summary, this chapter highlights that *P. anguillae* and also, *P. bini,* are present in European eel populations in the UK. Indeed, these parasites are present in the majority of rivers (10/14) from which eel were examined across England and Wales. Based upon analysis of the 140 eel in this thesis, the overall prevalence of pseudodactylids was 36% and the mean abundance and mean intensity data was 4.42 ±13.94 and 12.38 ±21.12 respectively. The data also show that infection differences occur between the geographic regions of the UK and that host factors may influence the infection.

CHAPTER THREE

Gastrointestinal Nematodes

3.1 Introduction

The presence of nematodes in fish hosts was recognized as early as the thirteenth century (Myers, 1976). In the last 50 years extensive evidence has accumulated recognizing the importance of fish nematodes; not least, that anisakid larvae on the viscera of many economically important fish species can impact the fish processing industry and cause public health concerns (Abollo *et al.*, 2001). In the past decade there has been a great increase in the number of non-native fish helminths introduced into western European waters as a result of uncontrolled movements of commercial fishes. A particularly pertinent example of a significant 'alien invader' nematode is *Anguillicoloides crassus* which was introduced into Western Europe as a result of uncontrolled intercontinental transfer of live eels for consumption and it is responsible for a significant pathology of the host swim bladder (Køie, 1991).

3.1.1 *Spinitectus inermis* (Zeder, 1800)

3.1.1.1 Introduction

The genus *Spinitectus* (Fourment, 1883) includes numerous species of medium sized nematodes parasitic within the digestive tract of fresh water and marine fishes, amphibians and mammal (Moravec, 2007). In Europe, two species of *Spinitectus* occur in fresh water fishes, *S. inermis* (Zeder, 1800) a parasite specific to the European eel and *S. gordoni*, a parasite of salmonids (Saraiva, Moravec, *et al.*, 2002).

Spinitectus inermis (Zeder, 1800) is a specific, widespread gastrointestinal nematode of *A. anguilla*. The parasite is widely distributed throughout the range of the European eel with widely varying prevalence data reported (eg. 1.8% to 43.3%) (Moravec, 1977; Kennedy, 1997a; Saraiva, Pereira, *et al.*, 2002; Norton, Rollinson, *et al.*, 2004; Kennedy, 2012; Moravec & Scholz, 2015). As such, it can be considered rare in some localities but in a few habitats the nematode may be common and occasionally it may be the dominant species in the host gut community (Kennedy, 2012).

3.1.1.2 Morphology

The body of the adult *S. inermis* is long (range, 6.7-7.9 mm) and slender (Rahman, 1964) and females are larger than males. The body of both sexes tapers slightly at the head. The female tail has a pointed spine that is present within a laterally serrated knob- like structure whereas male tail is spirally coiled. A characteristic feature of the male tail is the presence of eight pairs of papillae and one pair of spicules (Rahman, 1964). The cuticle is ringed transversely and bears 8-10 transverse rows of spines which are also characteristic of the genus (Rahman, 1964). The spines are very distinct anteriorly and become less prominent posteriorly; indeed, they are not visible posterior to the junction of the oesophagus and the intestine. The mouth

leads into a funnel vestibule and the oesophagus is long and is clearly marked into muscular and glandular regions (Sahay & Prasad, 1965).

3.1.1.3 Life Cycle

The definitive host *A. anguilla* acquires infection by feeding on the infected intermediate host; mayfly nymphs. The mayfly nymph ingests nematode eggs and the resulting first stage larvae penetrate the body cavity of the intermediate host. First stage larvae moult twice (on day 4 and 6 post infection at water temperatures of 20-25^{o}C (Saraiva, Moravec, *et al.*, 2002), to generate the infective third stage. In the infected eel, development to adulthood completes 28 post infection at 20^{o}C. The pre-patent period of *S. inermis* is estimated to be about two months (Saraiva, Moravec, *et al.*, 2002).

3.1.2 *Paraquimperia tenerrima* (Linstow, 1878)

3.1.2.1 Introduction

Paraquimperia tenerrima (Linstow) is a specific freshwater parasite of the European eel. *P. tenerrima* is a common specialist small nematode infecting the gut of eel from rivers of several European countries (Conneely & McCarthy, 1986; Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004; Shears & Kennedy, 2005; Moravec & Scholz, 2015). In some localities it is reported as common and occasionally as the dominant parasite of the gastrointestinal parasitic community of the European eel (Nie & Kennedy, 1991e).

3.1.2.2 Morphology

P. tenerrima is a small to medium size (range 3.8-7.7 mm) gastrointestinal nematode. The cuticle is thin and the lateral alae originate near the anterior extremity where they are broad and they become progressively narrow in the poster direction. The male and female tail is conical in shape and possesses a sharp cuticular tip (Moravec *et al.*, 2000).

3.1.2.3 Life Cycle

The life cycle of the gut nematode *P. tenerrima* is indirect and the intermediate host is the minnow *Phoxinus phoxinus* (Shears & Kennedy, 2005). Egg hatching is dependent upon water temperature; eggs fail to hatch below 10 ^{0}C and hatching occurs only at temperatures between 11 and 30 ^{0}C (Moravec, 1974). Survival of the free-living second stage larvae (L2) is also temperature dependent and maximal survival is reported between 10 and 20 ^{0}C (Shears & Kennedy, 2005). Survival of the eggs and the free-living L2 is unlikely to exceed a month at normal summer water temperatures. Free-living L2s are unable to infect eels directly.

P. tenerrima L2s infect minnows following ingestion and then exit the gut and migrate to the swimbladder. Following a moult, the L3 stage nematodes remain coiled within the swimbladder wall. Eel become infected by feeding on these minnows and completion of development to adulthood occurs in the eel gastrointestinal within about one month (Shears & Kennedy, 2005).

P. tenerrima prevalence and abundance within the European eel exhibit clear seasonality. Studies of the seasonal dynamics of *P. tenerrima* in European eel from three sites in Devon, southwest England, showed that infections occur in late winter to early spring, egg production proceeds throughout summer and then infection levels decline in autumn and early winter (Nie & Kennedy, 1991e).

3.1.3 *Raphidascaris acus* (Bloch, 1779)

3.1.3.1 Introduction

Raphidascaris acus (Bloch, 1779) is a common and widely distributed parasite of the digestive tract of various predatory fishes in Europe, Asia and North America (Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003; Moravec, 2004b; Moravec & Scholz, 2015). The most frequent definitive hosts of the small gut nematode are pike (*Esox lucius*) and brown trout (*Salmo troutta fario*), though the parasite is also commonly reported in salmonids and the European eel (Moravec, 1970). *R. acus* has recently been considered as a species complex since nematodes sampled from the Caspian Sea were morphologically identical to Czech and Canadian samples but genetically identical to Polish samples (Jahantab *et al.*, 2014).

3.1.3.2 Morphology

R. acus is a small to medium sized white coloured gastrointestinal nematode and the male is smaller 19.5-36 mm) than the female (20.2-44.6 mm). The cuticle has transverse striations and at the anterior end forms three lips with prominent lateral flanges. Lateral alae are distinct, starting from the lips and extending posteriorly to the mid part of tail. The tail is conical in both sexes and curves ventrally in the male (Jahantab *et al.*, 2014).

3.1.3.3 Life cycle

The life cycle of *R. acus* is indirect and involves obligate intermediate hosts. The L2 stage within the egg is ingested by a wide range of fish, or cyclostomes, that act as paratenic hosts (Smith, 1984; Moravec, 2004b). Development to the infective L3 stage occurs within the intermediate host and the European eel becomes infected via predation of the infected fish (Valtonen *et al.*, 1994). Completion of development occurs in the eel gut; however, some larvae become encapsulated within, or on, the liver, mesenteries, intestinal wall or other organs (Kennedy, 1974).

3.2 Objective

The main objective of this chapter is to perform a parasitological survey of the gastrointestinal nematode communities present in European eel sampled from rivers in England and Wales. To assist this analysis, molecular tools will be utilised to allow PCR amplification and DNA sequencing of the 18S rRNA gene of nematode samples.

3.3 Materials and Methods

3.3.1 Classical Morphological Examination

3.3.1.1 Sampling

Eel samples were acquired according to Chapter 1 (1.3).

3.3.1.2 Processing

Eel specimens were thawed and necropsies performed using a dissection kit, allowing the gastrointestinal tract to be removed and opened longitudinally. The gastrointestinal tract was dissected in a Petri dish containing distilled water under x 100 and x 160 magnification using a Wild Heerbrugg, M3B (Switzerland) dissecting microscope. A microscopic examination of the gut contents for the presence of nematodes was performed. In addition, the external surface of the gut was examined for pathological lesions or encapsulated larvae.

3.3.2. Prevalence and intensity

Any gastrointestinal nematodes were placed in a Petri dish containing distilled water. The total number of parasites was determined for each animal in order to allow a description of the primary infection data.

3.3.3 Imaging

Representative gastrointestinal nematodes were fixed in 10% (v/v) formalin and placed onto microscopic slides and covered with cover slide. A Leica Dulb M26 microscope was utilized in conjunction with the Leica Application suite, version 3.5 and digital camera DFC31FX to capture images of the parasites to assist in identification.

3.3.4 Molecular parasitological analysis

Given the difficulty in morphological identification of some of the small size gastrointestinal nematodes a molecular identification was carried out based on the DNA sequence of the 18S rRNA gene. To accomplish this, a selection of gastrointestinal nematodes were stored in

tubes containing 70% (v/v) ethanol and maintained at -20^{o}C until required. In addition, there were cysts present in the gastrointestinal tract of some large eels (river Crane). Some cysts were removed from the gut wall of these eels and they were cut using sterile scissors into small pieces for storage in 70% (v/v) ethanol at -20^{o}C until required for DNA extraction.

3.3.4.1 DNA Extraction from gastrointestinal nematodes

DNA was extracted from an isolated gastrointestinal nematode, and also from cyst fragments, using the PureLink Genomic DNA Kit (Invitrogen by Life Technologies) as described by the manufacturer. Briefly, a gut nematode was placed in a 1.5 ml eppendorf tube and 180 µl PureLink Genomic Digestion Buffer and 20 µl Proteinase K (20 mg / ml) was added for each sample. The sample was then processed according to section 2.3.4.1.

3.3.4.2 DNA quality control

For quantification of DNA recovery and purity assessment aliquots were analysed using the NanoDrop Lite Instrument (ThermoFisher Scientific) (2.3.4.2).

3.3.5 Polymerase chain reaction

3.3.5.1 *Spinitectus inermis*

Inspection of the NCBI GenBank database revealed limited molecular information for *Spinitectus* species as 18S rRNA sequences were only available for *S. tabascoensis* (JF803922.1), *S. petterae* (DQ813447.1) and *S. carolini* (DQ503464.1). PCR primers were designed using Primer 3 software (Untergrasser et al., 2012) to amplify a predicted 435 bp fragment of the 18S rRNA gene of *S. inermis*. The primer sequences were as follows: SpiniF, 5'- GAAAGTCAGAGGTTCGAAGGC -3' and SpiniR: 5'- ACAACATGCGGCTTAACACC -3'. Oligonucleotides were synthesized by Eurofins MWG

Operon and re-suspended in PCR-grade H_2O to a stock concentration of 10 pM and stored at -20^{o}C until required.

3.3.5.2 *Paraquimperia tenerrima*

Inspection of the NCBI GenBank database revealed a single 18S rRNA gene sequence available for the eel parasite *Paraquimperia africana* (JF803925.1). PCR primers were designed using Primer 3 to amplify a predicted 662 bp fragment of the 18S rRNA gene of *Paraquimperia tenerrima*. The primer sequences were as follows: ParaqF, 5'- CGCCCTAGTTCTGACCGTAA -3' and ParaqR, 5'- GGACTGAGCCGTTTCGAGAA -3'. Oligonucleotides were synthesized by Eurofins MWG Operon and resuspended in PCR-grade H_2O to a stock concentration of 10 pM and stored at -20^{o}C until required.

3.3.5.3 Cyst nematodes

It was not possible to morphologically determine the likely identification of the encysted larvae nematode species within the large eels. Consequently, PCR was attempted using the ParaqF and ParaqR primers (see above) in an attempt to amplify a nematode 18S rRNA PCR product that could be subjected to DNA sequencing.

3.3.6 PCR Profile

The PCR was carried out according to section 2.3.5.1; however, 2 µl of gastrointestinal nematode genomic DNA and nematode primers were utilised in place of the pseudodactylid equivalents. The PCR cycling profile for isolated nematodes consisted of an initial denaturation step at 94^{o}C for 5 minutes, followed by denaturation of 94^{o}C for 30 seconds, annealing at 57^{o}C for 30s and extension at 72^{o}C for 30s. This cycle profile was repeated a total of 36 times, followed by a final extension of 10 minutes at 72^{o}C. The PCR profile for the encysted larvae was the same, except the annealing temperature was reduced to 55^{o}C. All

PCR reactions were performed on a MultiGene machine (Labnet International. Inc.)

3.3.7 Agarose gel electrophoresis

PCR products were analysed by agarose gel electrophoresis (2.3.6).

3.3.8 PCR product purification

PCR products were purified from the agarose gel using the Isolate II PCR and Gel Purification Kit (BioLine) as described by the manufacturer (2.3.7).

3.3.9 DNA sequencing

Recovery of purified PCR products was assessed using the NanoDrop Lite Instrument (ThermoFisher Scientific) (2.3.4.2). PCR products were then prepared for sequencing as stated in section 2.3.7. All data was analysed according to section 2.3.7. In addition, a phylogenetic analysis was conducted for novel sequences using ClustalW2 (Larkin *et al.*, 2007) and MEGA 6.0 (Tamura *et al.*, 2013).

3.3.10 Statistical analyses

The Fisher's exact test was used to compare differences in prevalence between geographic regions and also between host factors (length, weight and condition factor). The Mann-Whitney test was used to assess the significance of differences in intensity of infection between the different geographic regions and also between host factors (length, weight and condition factor). All tests were conducted using Minitab 16 (licensed to the University of Salford).

3.4 Results

3.4.1 Primary infection data: the environment

In total, 47 of the 140 (33.57%) European eel examined contained gastrointestinal nematodes. The infected eel were sampled from 12 out of the 14 (85.7%) river systems surveyed across England and Wales (Table 3.1). No gastrointestinal nematodes were recovered from eel sampled from the River Crouch in England and the River Rhymney from Wales. At sites positive for gastrointestinal nematodes, the prevalence ranged from 10 to 80%. The River Leven showed the highest prevalence of infection (80%), while the lowest prevalence (10%) was observed in the Rivers Gowy, Taff and Cadoxton.

Table 3.1 Summary of the primary infection data for gastrointestinal nematodes isolated from European eel sampled across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence rate (%)	Total number of parasites	Mean parasitic abundance (± sd)	Mean infection intensity (± sd)
Leven RL: England	80	35	3.5±4.97	4.37±5.23
Petteril RP: England	70	75	7.5±7.94	10.71±7.38
Bela B: England	70	40	4.0±5.73	5.73±6.15
Crane CN: England	40	26	2.6±6.53	6.5±9.71
Hether Burn HB: England	20	16	1.6±3.40	8.0±1.43
Gowy RG: England	10	5	0.5±1.58	5.0±0
Crouch C: England	0	0	0	0
Clwyd-Meirchion MC: Wales	60	14	1.4±1.7	2.3±1.6
Clwyd-Elwy CE: Wales	40	7	0.7±1.05	1.75±0.95
Dee-Eitha D: Wales	40	29	2.9±7.17	7.25±10.59
Mawddach-eden M; Wales	20	10	0.7±1.88	4.7±3.53
Taff TB: Wales	10	1	0.1±0.31	1.0±0
Cadoxton CD: Wales	10	5	0.5±1.58	5.0±0
Rhymney R: Wales	0	0	0	0
	33.57±27.63	263	1.88±4.46	5.60±6.23

Overall, 263 gastrointestinal nematodes were collected from the eel (mean abundance = 1.9 ±4.5; mean intensity = 5.6 ±6.2). The number of gastrointestinal nematodes extracted from individual eel ranged between 1 and 23; the latter being isolated from a fish sampled from the Dee-Eitha (specimen D5). A single gastrointestinal nematode was extracted from 34.0% of infected animals (16/47). Moreover, the majority 83.0% (39/47) of the infected hosts carried less than 10 gastrointestinal nematodes and hence only 17.0% (8/47) of the infected eels harboured 11+ gastrointestinal nematodes (Tables 3.2 & 3.3). With respect to the sampling sites, the River Petteril yielded most gastrointestinal nematodes (n = 75) and the lowest number was recovered from the River Taff (n = 1).

Table 3.2 Eel morphometric data for animals with gastrointestinal nematode infections sampled from sites across England. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Number of gastrointestinal Nematodes
Leven: RL	RL1	21.0	13.9	0.16	8
	RL2	52.0	187.0	0.13	1
	RL3	27.0	35.8	0.18	16
	RL4	22.5	21.3	0.19	2
	RL6	35.0	71.3	0.17	3
	RL8	29.5	29.5	0.11	3
	RL9	30.0	38.1	0.14	1
	RL10	26.0	22.3	0.13	1
Petteril: RP	RP1	36.0	110.7	0.24	22
	RL2	31.5	82.8	0.28	17
	RL4	28.0	35.3	0.16	4
	RL6	27.5	38.2	0.18	4
	RL8	43.0	101.1	0.07	11
	RL9	33.0	98.3	0.27	14
	RL10	26.0	29.7	0.17	3
Bela: B	B1	30.0	42.0	0.16	5
	B2	32.0	49.1	0.15	4
	B3	27.0	33.0	0.17	1
	B4	19.5	11.9	0.16	1
	B7	24.0	22.2	0.16	6
	B8	28.0	24.5	0.18	19
	B10	49.0	209.0	0.18	4
Crane: CN	CN5	70.0	668.6	0.19	21
	CN6	74.0	839.7	0.21	1
	CN8	77.0	661.9	0.14	1
	CN10	74.0	743.4	0.18	3
Hether Burn: HB	HB5	25.0	18.6	0.12	9
	HB10	16.5	11.1	0.25	7
Gowy: RG	RG8	33.0	62.2	0.17	5

Table 3.3 Eel morphometric data for animals with gastrointestinal nematode infections sampled from sites across Wales. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Number of gastrointestinal nematodes
Clwyd-Meirchion: MC	MC2	30.0	38.8	0.14	5
	MC3	35.0	54.6	0.13	1
	MC5	19.5	21.8	0.29	3
	MC6	18.5	9.0	0.14	1
	MC7	22.0	12.0	0.11	3
	MC8	15.5	5.3	0.14	1
Clwyd-Elwy: CE	CE1	39.0	72.9	0.12	1
	CE3	31.0	46.0	0.15	1
	CE5	29.0	39.3	0.16	2
	CE9	20.0	10.9	0.14	3
Dee-Eitha: D	D1	28.5	27.6	0.13	1
	D5	14.0	2.4	0.09	23
	D9	14.5	1.2	0.04	4
	D10	25.5	21.6	0.13	1
Mawddach-eden: M	M6	28.0	36.9	0.17	1
	M9	23.0	15.5	0.13	9
Taff: TB	TB4	22.0	15.6	0.15	1
Cadoxton: CD	CD3	28.5	21.4	0.06	5

The mean intensity of gastrointestinal nematode infection in eels examined from different catchment sites ranged from 1 to 10.7±7.4 parasites per eel; the former from the river Taff in Wales and the latter from the river Petteril in England. The mean parasitic abundance in infected eels from different river sites surveyed ranged from 0.1±0.3 to 7.5±7.9 nematode per eel; the former from the river Taff and the latter from the river Petteril (Table.3.1). Overall, the parasites were over-dispersed (dispersion index = 11.1) (Figure 3.1)

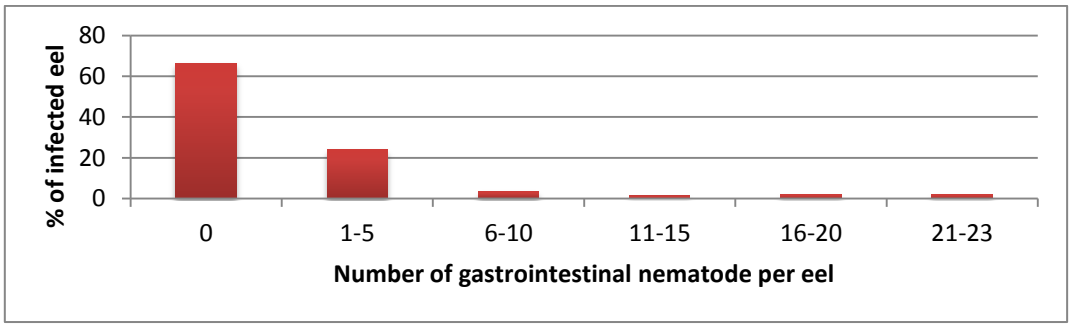

Figure 3.1 Summary of the dispersion of gastrointestinal nematodes within the sampled eel populations.

Upon analysis of the infection data at a regional level it was evident that all examined rivers in North West England and North Wales contained eel infected with gastrointestinal nematodes (Table 3.4). The rivers Crouch (South East England) and Rhymney (South Wales) appeared to contain eel completely lacking gastrointestinal nematode infections. Overall, eel sampled from North West England had the greatest prevalence, and also greatest mean abundance and mean intensity of gastrointestinal nematode infection. In contrast, the lowest prevalence and mean abundance was observed in eel sampled from South Wales.

Table 3.4 Regional infection data for gastrointestinal nematodes isolated from European eel sampled across England and Wales.

Regions	Number of examined river sites	Number of gut nematode-positive river sites	Number of examined eel	Number of infected eel	Nematode Prevalence rate (%)	Number of gut nematode	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	5	50	25	50%	171	3.42±5.54	6.84±6.3	1 - 22
South East England	2	1	20	4	20%	26	1.3±4.69	6.5±9.71	1 - 21
North Wales	4	4	40	16	40%	60	1.5±3.92	3.75±5.57	1 - 23
South Wales	3	2	30	2	6.7%	6	0.28±0.92	3±2.8	1 - 5
Total	14	12	140	47	33.57%	263	1.88±4.46	5.6±6.23	1 - 23

Overall, the regional prevalence data was significantly different between North West England and South East England ($p = 0.031$) and North Wales and South Wales ($p = 0.001$). Also, the prevalence data was significant differently between North West England and South Wales ($p < 10^{-4}$). There were no significant differences observed in the gastrointestinal nematode prevalence data between eels from South East England and North ($p = 0.153$) and South Wales ($p = 0.201$), and also, between North West England and North Wales ($p = 0.397$).

On analysis of gastrointestinal nematode intensity of infection data there was a significant difference between North West England and North Wales (p = 0.0396). There were no significant differences observed in intensity of infection between any of the other geographic regions (p: 0.4474 – 1.0). With respect to the abundance data, there was a significant difference between North West England and South East England (p = 0.036), North West England and South Wales (p = 0.0003), and also between North and South Wales (p = 0.002). There were no significant differences in abundance between North West England and North Wales (p = 0.204), South East England and North Wales (p = 0.1435), and South East England and South Wales (p = 0.166).

3.4.1.1 Primary Infection data: the host

For the gastrointestinal nematode infected eels, body length ranged from 14 to 77 cm (mean = 32.2 ±15.3 cm) and body weight ranged from 1.2 to 839.7 g (mean = 105.0±199.2 g). For uninfected eels, body length ranged from 10 to 86 cm (mean = 29.9±14.7 cm) and body weight ranged from 1.2g to 1380g (mean = 90.0±219.6 g). For the gastrointestinal nematode infected eels the condition factor ranged from 0.04 to 0.29 (mean = 0.16±0.05). For uninfected eels, the condition factor ranged from 0.05 to 0.23 (mean = 0.14±0.03) (Table 3.2 and 3.3).

Upon analysis of the different categories of eel body length it was apparent that the lowest prevalence was found in the smallest length category and the highest prevalence in the 25.5-30 cm category (Table 3.5). However, there were no statistically significant differences between the respective length categories (p: 0.2724 – 1). In addition, there were no significant differences in mean intensity of infection between the different eel body length categories (p: 0.0953 – 1.000) (Table 3.5).

Table 3.5 Gastrointestinal nematode infection data with respect to eel body length category.

Body length range (cm)	Mean body length (±sd) (cm)	Number of examined eel	Number of infected eel	Gastrointestinal nematode prevalence rate (%)	Number of gastrointestinal nematode	Mean intensity (±sd)	Intensity range
10-20	16.9±3.0	26	7	26.9%	40	5.7±7.9	1 - 23
20.5-25	22.4±1.4	32	9	28.1%	46	5.1±3.1	1 - 9
25.5-30	27.9±1.4	32	14	43.8%	48	3.4±3.9	1 - 16
30.5-35	32.6±1.3	22	7	31.7%	45	6.4±6.4	1 - 17
35.5-86	53.9±17.4	28	10	35.7%	84	8.4±9	1 - 21

Upon analysis of the different categories of eel condition factor it was apparent that the highest prevalence of gastrointestinal nematode infection was in the highest category whilst the lowest prevalence was found in the smallest condition factor category (Table 3.6). However, no significant differences in prevalence were found between the different condition factor categories (p: 0.0518 – 1). The lowest mean intensity of infection was found in the second smallest category whilst the highest mean intensity occurred in the largest condition factor category and this difference was significant (p = 0.002). In addition, there was a significant difference in mean intensity of infection between the two smallest condition factor categories (p = 0.007). There were no significant differences in mean intensity of infection between all other combinations of condition factor category (p: 0.0681 – 0.8886).

Table 3.6 Gastrointestinal nematode infection data with respect to eel condition factor category. [#] Statistically significant difference ($p<0.05$) in mean intensity with respect to the data observed for the condition factor category 0.13-0.14.

Condition factor range	Length range (cm)	Mean length (±sd) (cm)	Eel examined	Infected eel	Prevalence rate (%)	Number of nematodes	Intensity range	Mean intensity(±sd)
0.04-0.12	10-43	23.59±8.24	34	8	23.5%	59	1 - 23	7.38±7.13[#]
0.13-0.14	11 - 86	29.89±14.67	38	12	31.6%	26	1 - 9	2.17±2.48
0.15-0.16	19 - 35	27.18±4.38	28	9	32.1%	32	1 - 8	3.56±2.50
0.17-0.29	14.5-86	40.03±19.33	40	19	47.5%	146	1 - 22	8.11±7.64[#]

3.4.2 *Spinitectus inermis* (Zeder, 1800)

3.4.2.1 Morphological examination

The *S. inermis* parasites were identified based upon characteristic morphological features, as highlighted in the images below (Figures 3.2 – 3.4).

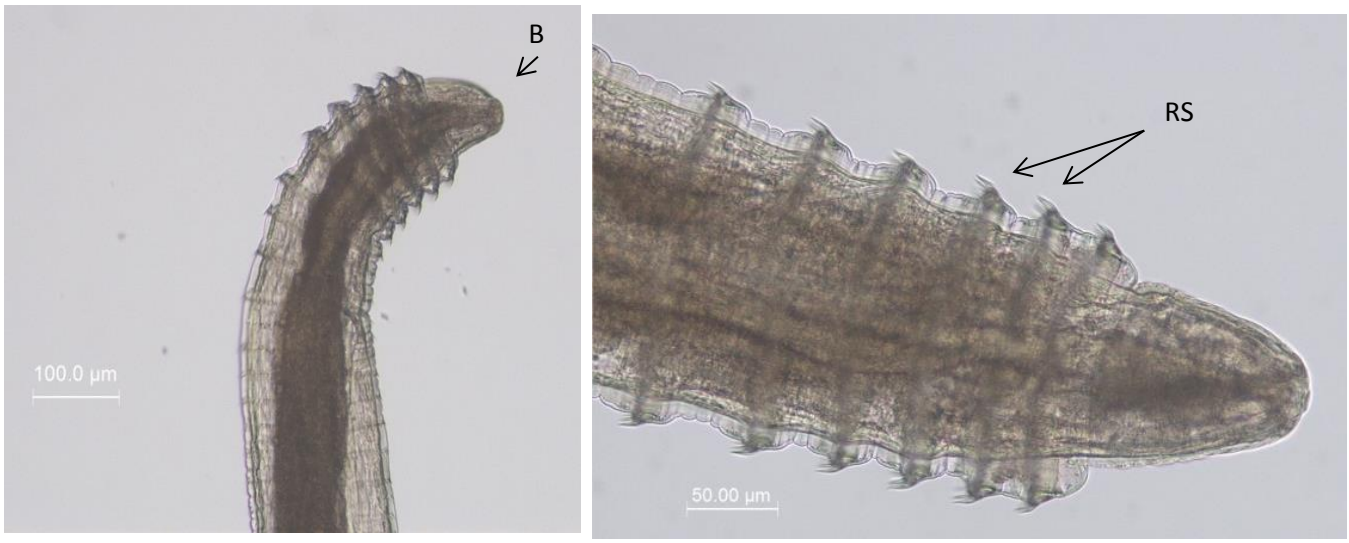

Figure 3.2 Representative images of the anterior end of *S. inermis*, showing the characteristic funnel shaped buccal capsule (BC) and the ringed transverse rows of spines (RS) on the lateral margins of the body (parasite extracted from eel D5 sampled from the River Dee-Eitha).

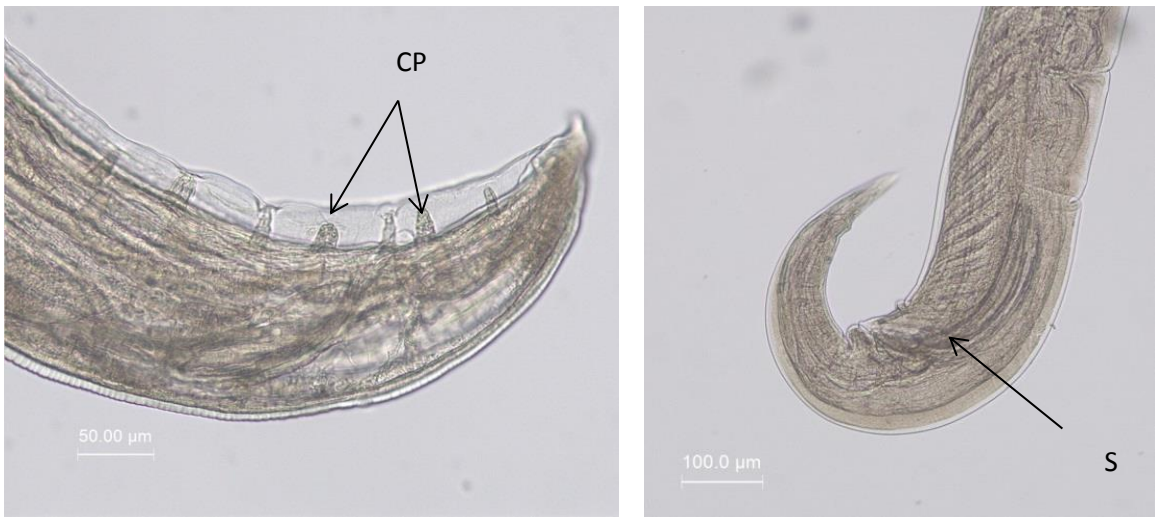

Figure 3.3 Representative images of the posterior end of a male *S. inermis* showing the characteristic caudal papillae (CP) and the spicules (S) (parasite extracted from eel D5 sampled from the River Dee-Eitha).

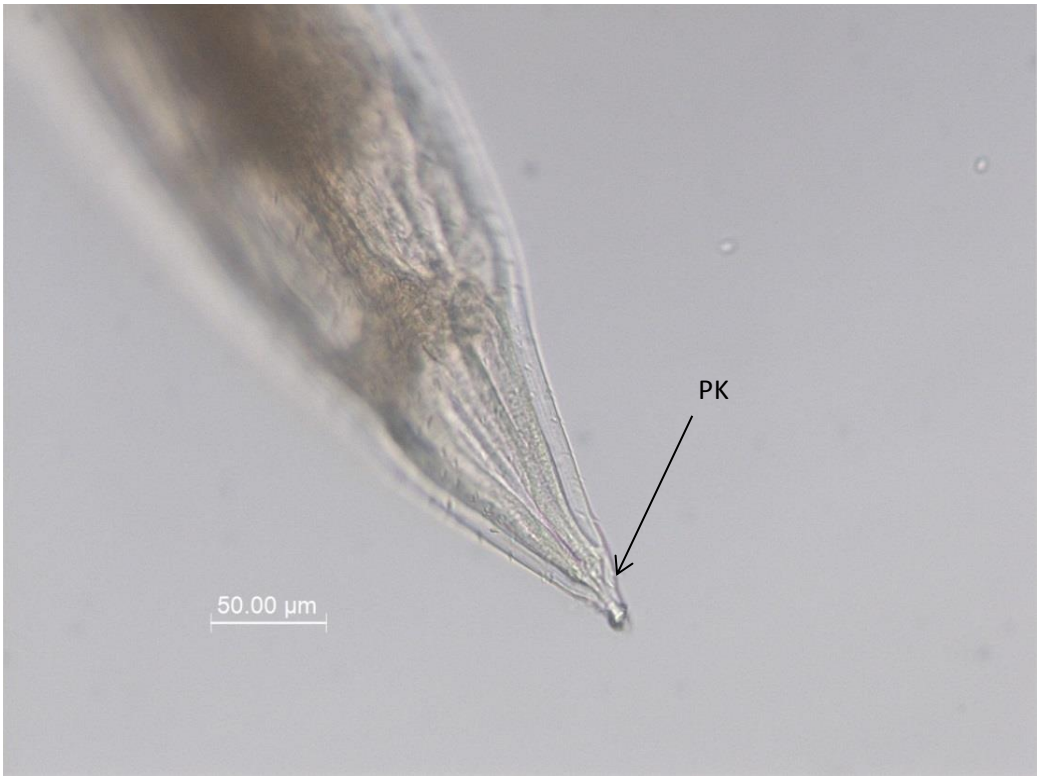

Figure 3.4 Representative image of the posterior end of a female *S. inermis* showing the characteristic pointed knob (PK) (parasite extracted from eel D5 sampled from the River Dee-Eitha).

3.4.2 Primary infection data: the environment

Examination of eel gastrointestinal tracts confirmed the presence of *S. inermis* nematodes in 9 of the 140 (6.4%) European eel specimens. The positive infections were observed in eel sampled from only two English rivers and two Welsh rivers (Table 3.7). There was no statistical significance between the prevalence of *S. inermis* at the four positive catchment sites ($p > 0.30$).

The total number of *S. inermis* collected was 61 parasites (mean intensity = 6.8 ±7.6 [61/9]; mean abundance = 0.44 ± 2.47 [61/140]). Overall, the parasites were over-dispersed (dispersion index =13.8); the number of nematodes per eel ranged from 1 (specimen RL2) to 23 (specimen D5) and the majority (77.8%) of the infected eels harboured less than 8 nematodes (Table 3.7). There was no significant difference in the mean intensity of *S. inermis*

between eel from the four catchment sites (*p*: 0.31 – 0.91). *S. inermis* was always isolated from European eel as a mixed infection with other small gastrointestinal nematodes (Table 3.7).

Table 3.7 *S. inermis* abundance and intensity data at the infected catchment sites. Table also includes data for total gastrointestinal nematode infections. Ten eels were examined from each site.

Location	Prevalence of gastrointestinal nematodes (%)	Total number of gastrointestinal nematodes	*Spinitectus* prevalence (%)	Total number of *Spinitectus*	*Spinitectus* mean abundance ±(sd)	*Spinitectus* mean intensity ±(sd)
Dee-Eitha: Wales	40	29	20	27	2.7 ± 7.24	13.5 ± 13.43
Clwyd-Elwy: Wales	40	7	20	5	0.5 ± 1.08	2.5 ± 0.70
Hether Burn: England	20	16	10	7	0.7 ± 2.21	7
Leven: England	80	35	40	22	2.2 ± 4.96	5.5 ± 7.04
	45%	87	22.5%	61	1.53 ±4.48	6.8 ±7.61

3.4.2.1 Primary infection data: the host

For *S. inermis* infected eels, the body length ranged from 14.0 to 52.0 cm (mean = 25±11.74 cm) and body weight ranged from 1.2 to 187.0g (mean = 37.73±57.68 g). For uninfected eels, the body length ranged from 10 to 86 cm (mean = 31.10±14.10 cm) and body weight ranged from 1.2g to 1380g (mean = 98.55±217.90 g). The mean condition factor for infected eel was 0.14±0.06, whilst the mean condition factor for the uninfected eel was 0.15±0.04 (Table 3.8). Upon statistical analysis there was a marginal significant difference ($p = 0.0497$) between the body length of infected and non-infected eels. No significant differences in body weight ($p = 0.173$) and condition factor ($p = 0.085$) were observed between the *S. inermis* infected and uninfected eels.

Table 3.8 Eel morphometric data for animals with *Spinitectus inermis* infections sampled from sites across England and Wales. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Number of *Spinitectus* /eel
Dee-Eitha: Wales	D5 D9	14.0 14.5	2.4 1.4	0.09 0.04	23 4
Clwyd-Elwy: England	CE5 CE9	29.0 20.0	39.3 10.9	0.16 0.14	2 3
Hether Burn: England	HB10	16.5	11.1	0.25	7
Leven: England	RL2 RL3 RL4 RL8	52.0 27.0 22.5 29.5	187.0 35.8 21.3 29.5	0.13 0.18 0.19 0.11	1 16 2 3

3.4.3 Molecular confirmation of *Spinitectus inermis* infection

Genomic DNA was extracted and purified from three parasites (isolated from eel specimens D5, CE5 and RL2) suspected to be *S. inermis.* The 18S rRNA was PCR amplified and the resulting 435 bp products subjected to DNA sequencing. The BlastN analysis showed that the three sequences were identical and novel and that the most similar sequence was the 18S rRNA gene of *S. carolini* (Table 3.9). The novelty of the sequence is a consequence of there being a 'C' at position 286 bp (Figure 3.5) that distinguishes it from the published 18S rRNA sequences of other *Spinitectus* spp.; there is an absence of published data for the 18S rRNA gene data of *S. inermis*. In addition, there is a 'G' at position 309 bp of the sequenced sample which contrasts with the 'A' at this position in the 18S rRNA sequences deposited for *S. carolini* and *S. tabascoensis*. As such, the molecular analysis complements the morphological interpretation that the eel infection was indeed due to *S. inermis*.

Table 3.9 BlastN anaylsis of the 18S RNA gene fragment derived from a nematode isolated from eel specimen D5 (equivalent data was generated for PCR products derived from nematodes sampled from eels CE5 and RL2).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Spinitectus carolini 18S ribosomal RNA gene, partial sequence	686	686	99%	0.0	99%	DQ503464.1
*Spinitectus tabascoe*nsis voucher IPCAS N-928 small subunit ribosomal RNA gene, partial sequence	677	677	99%	0.0	98%	JF803922.1
Spinitectus petterae isolate LV29 18S ribosomal RNA gene, partial sequence	652	652	99%	0.0	97%	DQ813447.1

```
420125701_SP1_F_A06             -------------------TACGCCTTGACGGGCAGCTTCCCGGAAACGA 31
gi|339787469|gb|JF803922.1|     GCGTTCCGTCGGTGGTAAATACGCCTTGACGGGCAGCTTCCCGGAAACGA 1012
gi|95116612|gb|DQ503464.1|      GCGTTCCGTCGGTGGTAAATACGCCTTGACGGGCAGCTTCCCGGAAACGA 1047
gi|459650384|gb|KC291616.1|     GCGTTCCGT-GGTGGTAAATACGCCTTGACGGGCAGCTTCCCGGAAACGA 168
                                          ********************************

420125701_SP1_F_A06             AAGTCTTTCGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGA 81
gi|339787469|gb|JF803922.1|     AAGTCTTTCGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGA 1062
gi|95116612|gb|DQ503464.1|      AAGTCTTTCGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGA 1097
gi|459650384|gb|KC291616.1|     AAGTCTTTCGGTTCCGGGGGAAGTATGGAAGCAAAGCTGAAACTTAAAGA 218
                                ***************************  ******************

420125701_SP1_F_A06             AATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACT 131
gi|339787469|gb|JF803922.1|     AATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACT 1112
gi|95116612|gb|DQ503464.1|      AATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACT 1147
gi|459650384|gb|KC291616.1|     AATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACT 268
                                **************************************************

420125701_SP1_F_A06             CAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTG 181
gi|339787469|gb|JF803922.1|     CAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTG 1162
gi|95116612|gb|DQ503464.1|      CAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTG 1197
gi|459650384|gb|KC291616.1|     CAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTG 318
                                **************************************************

420125701_SP1_F_A06             AGAGCTCTTTCTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTG 231
gi|339787469|gb|JF803922.1|     AGAGCTCTTTCTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTG 1212
gi|95116612|gb|DQ503464.1|      AGAGCTCTTTCTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTG 1247
gi|459650384|gb|KC291616.1|     AGAGCTCAAACTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTG 368
                                *******   ************************************

420125701_SP1_F_A06             GTGGAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTA 281
gi|339787469|gb|JF803922.1|     GTGGAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTA 1262
gi|95116612|gb|DQ503464.1|      GTGGAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTA 1297
gi|459650384|gb|KC291616.1|     GTGGAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTA 418
                                **************************************************

420125701_SP1_F_A06             CTAACTAGTCACCGGATGATTGCGTCCGGTGAGACTTCTTAGAGGGACAA 331
gi|339787469|gb|JF803922.1|     CTAAATAGACACTGGATGATTGCGTCCAGTGAGACTTCTTAAAGGGACAA 1312
gi|95116612|gb|DQ503464.1|      CTAAATAGTCACTGGATGATTGGGTCCAGTGAGACTTCTTAGAGGGACAA 1347
gi|459650384|gb|KC291616.1|     CTAAATAGTCATCGGATGAAGAAGGTCGTGGAGACTTCTTAGAGGGACAA 468
                                **** *** **  ******    *  *   *********** ********

420125701_SP1_F_A06             GCGGTGCTTAGCCGCATGAAGTTGAGCAATAACAGGTCTGTGATGCCCTT 381
gi|339787469|gb|JF803922.1|     GCGGTGCTTAGCCGCATGAAGTTGAGCAATAACAGGTCTGTGATGCCCTC 1362
gi|95116612|gb|DQ503464.1|      GCGGTGCTTAGCCGCATGAAGTTGAGCAATAACAGGTCTGTGATGCCCTT 1397
gi|459650384|gb|KC291616.1|     GCGGTGTTAAGCCGCATGTTGTTGAGCAATAACAGGTCTGTGATGCCCTT 518
                                ****** * *********  ****************************

420125701_SP1_F_A06             AGATGTCCAA---------------------------------------- 391
gi|339787469|gb|JF803922.1|     AGATGTCCAGGGCTGCACGCGCGCTACACTGGAGGAATCAGCGTGCATTA 1412
gi|95116612|gb|DQ503464.1|      AGATGTCCAGGGCTGCACGCGCGCTACACTGGAGGAATCAGCGTGCATTA 1447
gi|459650384|gb|KC291616.1|     AGATGTCCAGG---------------------------------------- 529
                                *********
```

Figure 3.5 The 391 bp 18S rRNA DNA sequence (420125701_SP1_F_A06) from the nematode isolated from eel sample D5 aligned with *Spinitectus* spp. 18S rRNA sequences deposited in GenBank: *S. carolini* (gi: 95116612), *S. tabascoensis* (gi: 339787469) and *S. petterae* (gi: 459650384).

A phylogenetic analysis of the 18S rRNA gene fragments for *Spinitectus* spp. and the novel sequence characterised from nematode D5/2010/03 shows that the latter is indeed most related to *S. tabascoensis* and *S. carolini* (Figure 3.6).

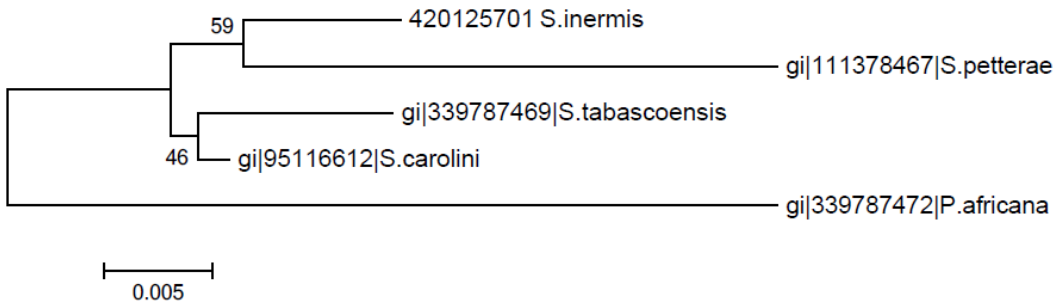

Figure 3.6 Phylogram constructed using MEGA 6.0 of the genus *Spinitectus* derived using the *S. inermis* 18S rRNA gene fragment (420125701_SP1_F_A06) and the published *Spinitectus* spp. 18S rRNA gene fragments: *S. carolini* (gi: 95116612), *S. tabascoensis* (gi: 339787469) and *S. petterae* (gi: 459650384). The 18S rRNA gene fragment from *Paraquimperia africana* (gi: 339787472) was utilized as the outgroup.

3.4.4 *Paraquimperia tenerrima* and *Raphidascaris acus*

3.4.4.1 Morphological examinations

The small gastrointestinal nematode *Paraquimperia tenerrima* was identified based upon characteristic morphological features, as highlighted in the images below (Figure 3.7).

Figure 3.7 Representative images of *P. tenerrima* showing the characteristic cuticular alae (CA) at the anterior end and the sharp tail tip (ST) at the posterior (parasite extracted from eel RP1 sampled from the River Petteril).

The other small gastrointestinal nematode frequently observed was *Raphidascaris acus* and this was identified based upon the characteristic morphological features highlighted in the images below (Figure 3.8).

Figure 3.8 Representative images of the anterior and posterior ends of *Raphidascaris acus* showing the characteristic features of the head end (HE) and the tail ending (TE) (parasite extracted from eel B8 sampled from the River Bela).

3.4.4.2 Primary infection data: the environment

Examination of the eel gastrointestinal tracts confirmed the presence of small size nematodes in 38 out of 140 European eel specimens (27.1%) (Tables 3.10 and 3.11). Moreover, these parasites were present in eel sampled from 12 of the 14 sites (Table 3.12). The greatest prevalence of small gastrointestinal nematodes was observed in eel sampled from the Rivers Bela and Petteril. In contrast, no small gastrointestinal nematodes were observed in eel sampled from the rivers Rhymney and Crouch.

Eel sampled from the majority (8/12) of the catchment sites positive for gastrointestinal nematodes were not co-infected with *S. inermis*. In the remaining sites harbouring eel positive for gastrointestinal nematode infection (4/12), the small gastrointestinal nematodes were present as a co-infection with *S. inermis*.

Table 3.10 Host and small gastrointestinal nematode data for the 6 positive catchment sites in England. Ten eels were examined from each site.

Catchment Sites Location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of small nematode
Leven: RL	RL1	21.0	13.9	0.16	8
	RL6	35.0	71.3	0.17	3
	RL9	30.0	38.1	0.14	1
	RL10	26.0	22.3	0.13	1
Petteril: RP	RP1	36.0	110.7	0.24	22
	RP2	31.5	82.8	0.28	17
	RP4	28.0	35.3	0.16	4
	RP6	27.5	38.2	0.18	4
	RP8	43.0	101.1	0.07	11
	RP9	33.0	98.3	0.27	14
	RP10	26.0	29.7	0.17	3
Bela: B	B1	30.0	42.0	0.16	5
	B2	32.0	49.1	0.15	4
	B3	27.0	33.0	0.17	1
	B4	19.5	11.9	0.16	1
	B7	24.0	22.2	0.16	6
	B8	28.0	24.5	0.18	19
	B10	49.0	209.0	0.18	4
Crane: CN	CN5	70.0	668.6	0.19	21
	CN6	74.0	839.7	0.21	1
	CN8	77.0	661.9	0.14	1
	CN10	74.0	743.4	0.18	3
Hether Burn: HB	HB5	25.0	18.6	0.12	9
Gowy: RG	RG8	33.0	62.2	0.17	5

Table 3.11 Host and small gastrointestinal nematode data for the 6 positive catchment sites from Wales. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of small nematode
Clwyd-Meirchion: MC	MC2	30.0	38.8	0.14	5
	MC3	35.0	54.6	0.13	1
	MC5	19.5	21.8	0.29	3
	MC6	18.5	9.0	0.14	1
	MC7	22.0	12.0	0.11	3
	MC8	15.5	5.3	0.14	1
Clwyd-Elwy: CE	CE1	39.0	72.9	0.12	1
	CE3	31.0	46.0	0.15	1
Dee-Eitha: D	D1	28.5	27.6	0.13	1
	D10	25.5	21.6	0.13	1
Mawddach-eden: M	M6	28.0	36.9	0.17	1
	M9	23.0	15.5	0.13	9
Taff: TB	TB4	22.0	15.6	0.18	1
Cadoxton: CD	CD3	28.5	21.4	0.06	5

Table 3.12 Primary infection data for small gastrointestinal nematode infection in European eel samples from English and Welsh rivers. Ten eels were examined at each catchment site.

Eel Catchment Sites	Prevalence Rate (%)	Total number of parasite	Mean Parasitic Abundance ±(sd)	Mean Infection Intensity ±(sd)
Leven RL: England	40	13	1.3 ± 2.54	3.25 ± 3.30
Petteril RP: England	70	75	7.5±7.94	10.71±7.38
Bela B: England	70	40	4.0±5.73	5.73±6.15
Crane CN: England	40	26	2.6±6.53	6.5±9.71
Hether Burn HB: England	10	9	0.9 ± 2.84	9.0
Gowy RG: England	10	5	0.5±1.58	5.0
Crouch C: England	0	0	0	0
Clwyd-Meirchion MC: Wales	60	14	1.4±1.7	2.3±1.6
Clwyd-Elwy CE: Wales	20	2	0.2 ± o.42	1 ± 0.0
Dee-Eitha D: Wales	20	2	0.2 ± 0.42	1.0
Mawddach-eden M; Wales	20	10	0.7±1.88	4.7±3.53
Taff TB: Wales	10	1	0.1±0.31	1.0
Cadoxton CD: Wales	10	5	0.5±1.58	5.0
Rhymney R: Wales	0	0	0	0
	27.14%	202	1.44± 3.87	5.31±5.93

The total, 202 small gastrointestinal nematodes were collected from the eels surveyed in this study (mean intensity of infection = 5.31 ±5.93 [202/38]; mean abundance = 1.44 ± 3.87 [202/140]). Eel sampled from the River Petteril had the greatest mean intensity of infection (10.71±7.38). The number of nematodes isolated from individual eel ranged from 1 (specimen TB4) to 22 (specimen RP1) per eel. The majority (32/38, 84.21%) of the small gastrointestinal nematode infected eels harboured less than 10 parasites. Moreover, almost 40% (15/38) of the infected eels harboured a single small gastrointestinal nematode. In contrast, 15.79% (6/38) of the infected hosts harboured greater than 10 small gastrointestinal

nematodes. The small gastrointestinal nematodes were therefore over-dispersed in the eel population (dispersion index = 39.5).

At the regional level, all the geographic areas contained eel with small gastrointestinal nematode infections (Table 3.13). Interestingly, comparing the South of England with the North West of England, and also, South Wales with North Wales, the more northerly of these sites showed a higher prevalence of small gastrointestinal infections in eel. Indeed, this difference was statistically significant for the Welsh comparison ($p = 0.018$), though not for the English comparison ($p = 0.16$). There was also a statistically significant difference in prevalence of small gastrointestinal nematodes between eels sampled from North West England and South Wales ($p = 0.0015$).

With respect to small gastrointestinal abundance, there was a statistically significant difference between North West England and South Wales ($p = 0.0013$) and also, between North West England and South East England ($p = 0.0417$). All other comparisons of small gastrointestinal nematode abundance were not statistically significant ($p > 0.05$).

Table 3.13 Regional infection data for small gastrointestinal nematodes isolated from European eel sampled across England and Wales. [#] Statistically significant difference in prevalence (p<0.05) with respect to South Wales. * Statistically significant difference in mean abundance (p<0.05) with respect to South Wales. [a] Statistically significant difference in mean abundance (p<0.05) with respect to North West England.

Regions	Number of examined river sites	Number of examined eel	Number of infected eel	Small nematodes prevalence (%)	Number of small nematodes	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	50	20	40%[#]	142	2.84±5.28[*]	7.1±6.32	1 - 22
South East England	2	20	4	20%	26	1.3±4.69[a]	6.5±9.71	1 - 21
North Wales	4	40	12	30%[#]	28	0.7±1.69	2.33±1.46	1 – 9
South Wales	3	30	2	6.7%	6	0.2±0.92	3±2.83	1 - 5
Total	14	140	38	27.1%	202	1.44 ± 3.87	5.31 ±5.93	1 - 22

3.4.4.3 Primary infection data: the host

For the small gastrointestinal nematode infected eels, the body length ranged from 15.5 to 86.0 cm (mean = 35.21±17.53 cm) and body weight ranged from 5.3 to 839.7g (mean = 138±240.87 g). For uninfected eels, the body length ranged from 10 to 86 cm (mean = 28.97±13.44 cm) and body weight ranged from 1.2g to 1380g (mean = 78.28±199.07 g). The mean condition factor for infected eel was 0.16±0.05, whilst the mean condition factor for the uninfected eel was 0.14±0.04 (Tables 3.11 and 3.12).

Upon analysing the prevalence data with respect to body length category (Table 3.14) there were no statistically significant differences observed (p>0.05). With respect to mean intensity of infection, the only significant difference was between the two categories of eel body length between 20 and 30 cm (p = 0.0301).

Table 3.14 Small gastrointestinal nematode infection data with respect to eel body length category.

Body length range (cm)	Number of examined eel	Number of infected eel	Small nematodes prevalence rate (%)	Number of small nematodes	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	3	11.5	3	0.12±0.33	1	1
20.5-25	32	8	25	44	1.38±2.81	5.5±3.02	1-9
25.5-30	32	11	34.4	27	0.84±1.55	2.75	1-5
30.5-35	22	7	31.8	45	2.05±4.6	6.43±6.43	1-17
35.5-86	28	9	31.1	83	2.96±6.64	9.22±9.15	1-21

With respect to condition factor, there was a significant difference in prevalence data between the smallest and largest categories of condition factor (p = 0.036) (Table 3.15). Comparisons of all other categories of condition factor showed no statistical significance in prevalence data (p: 0.22 – 1.0). The mean intensity of infection for the smallest category of condition factor was significantly different to the data for the two categories of eel with eel condition factors between 0.13 and 0.16. In addition, the eel within the greatest category of condition factor had a significantly greater mean intensity of small gastrointestinal nematodes compared to the eel within the condition factor category 0.13-0.14.

Table 3.15 Small gastrointestinal nematode infection with respect to eel condition factor category. # Statistically significant difference in prevalence (p<0.05) relative to the lowest condition factor. * Statistically significant difference in mean intensity (p<0.05) relative to the lowest condition factor. [a] Statistically significant difference in mean intensity (p<0.05) relative to the condition factor category 0.13-0.14.

Condition factor range	Length range (cm)	Mean length (±sd) (cm)	Eel examined	Infected eel	Prevalence rate (%)	Number of small nematodes	Intensity range	Mean intensity(±sd)
0.04-0.12	10-43	23.6±8.2	34	5	17.7	29	1-11	5.8±4.1
0.13-0.14	11-86	29.9±14.7	38	10	36.3	22	1-9	2.2±2.7*
0.15-0.16	19-35	27.2±4.4	28	8	28.6	30	1-8	3.8±2.6*
0.17-0.29	14.5-86	40.0±19.3	40	15	37.5[#]	121	1-22	15.1±7.9[a]

3.4.5 Molecular identification of the small nematodes

Genomic DNA was extracted and purified from 12 randomly selected small gastrointestinal nematodes (isolated from eel specimens across four sites: CD3, B2, B3**,** CN5, RP2 and RP9). The 18S rRNA was PCR amplified and the resulting 662 bp products subjected to DNA sequencing. The BlastN analysis showed that 50% (6/12) of the sequences were identical to the 18S rRNA gene of *Raphidascaris acus* (Figure 3.9). The other 6 sequences generated were identical and novel and most similar to the 18S rRNA DNA sequence for *Paraquimperia africana* (Table 3.16 and Figure 3.10). The novelty of these sequences is a consequence of there being a 'A' at position 240 bp (Figure 3.10) that distinguishes it from the 18S rRNA sequence of *P. africana.* As such, the molecular analysis complements the morphological approach and the *R. ascus* and *P. tennerima* nematodes were likely to be present in approximately equal proportions in the sampled eel. Not surprisingly, examples of mixed *R. ascus* and *P. tennerima* were evident in individual eel (Table 3.17).

```
421377801_9_PaF_A06           ----------------------------------------GTATGGTTGCA 11
gi|95116608|gb|DQ503460.1|    CAGCTTCCCGGAAACGAAAGTCTTTCGGTTCCGGGGGAAGTATGGTTGCA 1100
                                                                     ***********

421377801_9_PaF_A06           AAGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCC 61
gi|95116608|gb|DQ503460.1|    AAGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCC 1150
                              **************************************************

421377801_9_PaF_A06           TGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCG 111
gi|95116608|gb|DQ503460.1|    TGCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCG 1200
                              **************************************************

421377801_9_PaF_A06           TGAGGATTGACAGATTGATAGCTCTTTCTTGATTCGGTGGTTGGTGGTGC 161
gi|95116608|gb|DQ503460.1|    TGAGGATTGACAGATTGATAGCTCTTTCTTGATTCGGTGGTTGGTGGTGC 1250
                              **************************************************

421377801_9_PaF_A06           ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTTATTCCGATAACG 211
gi|95116608|gb|DQ503460.1|    ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTTATTCCGATAACG 1300
                              **************************************************

421377801_9_PaF_A06           AGCGAGACTCTAGCCTACTAAATAGTCATCGGATAAATACGTCTGGAAGA 261
gi|95116608|gb|DQ503460.1|    AGCGAGACTCTAGCCTACTAAATAGTCATCGGATAAATACGTCTGGAAGA 1350
                              **************************************************

421377801_9_PaF_A06           CTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTGAGCAATAACA 311
gi|95116608|gb|DQ503460.1|    CTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTGAGCAATAACA 1400
                              **************************************************

421377801_9_PaF_A06           GGTCTGTGATGCCCTTAGATGTCCAGGGCTGCACGCGCGCTACACTGGAG 361
gi|95116608|gb|DQ503460.1|    GGTCTGTGATGCCCTTAGATGTCCAGGGCTGCACGCGCGCTACACTGGAG 1450
                              **************************************************

421377801_9_PaF_A06           GAATCAGCGTGCTGTAACCATTGCCGAAAGGTATTGGTAACCCCTTGAAA 411
gi|95116608|gb|DQ503460.1|    GAATCAGCGTGCTGTAACCATTGCCGAAAGGTATTGGTAACCCCTTGAAA 1500
                              **************************************************

421377801_9_PaF_A06           ATCCTCCGTGATCGGGATCGGGAATTGCAATTATTTCCCTTGAACGAGGA 461
gi|95116608|gb|DQ503460.1|    ATCCTCCGTGATCGGGATCGGGAATTGCAATTATTTCCCTTGAACGAGGA 1550
                              **************************************************

421377801_9_PaF_A06           ATTCCTAGTAAGTGTGAGTCATCAGCTCACGTTGATTACGTCCCTGCCCT 511
gi|95116608|gb|DQ503460.1|    ATTCCTAGTAAGTGTGAGTCATCAGCTCACGTTGATTACGTCCCTGCCCT 1600
                              **************************************************

421377801_9_PaF_A06           TTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCGTTTT--------- 552
gi|95116608|gb|DQ503460.1|    TTGTACACACCGCCCGTCGCTGCCCGGGACTGAGCCGTTTCGAGAAAAGC 1650
                              ****************************************
```

Figure 3.9 The 18S rRNA DNA sequence (421377801_9_PaF_A06) derived from a small gastrointestinal nematode (CN5 (2)) isolated from eel sample CN5 aligned with the *Raphidascaris acus* 18S rRNA sequence deposited in GenBank (gi: 95116608).

Table 3.16 BlastN analysis of the 18S RNA gene fragment derived from a nematode isolated from eel specimen CD3 (equivalent data was generated for 5 other PCR products derived from nematodes sampled from eels B2, B3 and CN5).

worm I.D	Sequence I.D	Description	Max score	Total score	Query cover	E value	Ident	Accession
CD3.3	421377801_1_PaF_A05	*Paraquimperia africana* small subunit ribosomal RNA gene, partial sequence	1009	1009	100%	0.0	99%	JF803925

```
421377801_1_PaF_A05             ----------------------------CAAAGCTGAAACTTAAAGGAAT 22
gi|339787472|gb|JF803925.1|     TCTTCCGGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAAT 1050
                                                           **********************

421377801_1_PaF_A05             TGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAA 72
gi|339787472|gb|JF803925.1|     TGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAA 1100
                                **************************************************

421377801_1_PaF_A05             CACGGGAAAACTCACCCGGCCCGGACACCGTGAGGATTGACAGATTGAGA 122
gi|339787472|gb|JF803925.1|     CACGGGAAAACTCACCCGGCCCGGACACCGTGAGGATTGACAGATTGAGA 1150
                                **************************************************

421377801_1_PaF_A05             GCTCTTTCTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTG 172
gi|339787472|gb|JF803925.1|     GCTCTTTCTTGATTCGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTG 1200
                                **************************************************

421377801_1_PaF_A05             GAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTACTA 222
gi|339787472|gb|JF803925.1|     GAGTGATTTGTCTGGTTTATTCCGATAACGAGCGAGACTCTAGCCTACTA 1250
                                **************************************************

421377801_1_PaF_A05             AATAGTGACTGGATACTTAAGTCCAGAATACTTCTTAGAGGGACAAGCGG 272
gi|339787472|gb|JF803925.1|     AATAGTGACTGGATACTTGAGTCCAGAATACTTCTTAGAGGGACAAGCGG 1300
                                ****************** ******************************

421377801_1_PaF_A05             TGTTCAGCCGCACGAAATTGAGCAATAACAGGTCTGTGATGCCCTTAGAT 322
gi|339787472|gb|JF803925.1|     TGTTCAGCCGCACGAAATTGAGCAATAACAGGTCTGTGATGCCCTTAGAT 1350
                                **************************************************

421377801_1_PaF_A05             GTCCGGGGCTGCACGCGCGCTACACTGGAGGAATCAGCGTGCTGTAACCA 372
gi|339787472|gb|JF803925.1|     GTCCGGGGCTGCACGCGCGCTACACTGGAGGAATCAGCGTGCTGTAACCA 1400
                                **************************************************

421377801_1_PaF_A05             TTGCCGAAAGGCATTGGTAACCCCTTGAAAATCCTCCGTGATCGGGATCG 422
gi|339787472|gb|JF803925.1|     TTGCCGAAAGGCATTGGTAACCCCTTGAAAATCCTCCGTGATCGGGATCG 1450
                                **************************************************

421377801_1_PaF_A05             GGAATTGCAATTATTTCCCTTGAACGAGGAATTCCCAGTAAGTGTGAGTC 472
gi|339787472|gb|JF803925.1|     GGAATTGCAATTATTTCCCTTGAACGAGGAATTCCCAGTAAGTGTGAGTC 1500
                                **************************************************

421377801_1_PaF_A05             ATCAGCTCACGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGC 522
gi|339787472|gb|JF803925.1|     ATCAGCTCACGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGC 1550
                                **************************************************

421377801_1_PaF_A05             TGCCCGGGACTGAGCCGTTTCGAGAAA---------------------- 549
gi|339787472|gb|JF803925.1|     TGCCCGGGACTGAGCCGTTTCGAGAAAAGCGGGGACTGCTGATTTGAGGC 1600
                                ***************************
```

Figure 3.10 The 549 bp 18S rRNA DNA sequence (421377801_1_PaF_A05) from a small gastrointestinal nematode (CD3 (1)) isolated from eel sample CD3 aligned with the *Paraquimperia africana* 18S rRNA sequence deposited in GenBank (gi: 339787472).

Table 3.17 Molecular identification of 12 individual small gastrointestinal nematodes based upon BlastN analysis of the 18S RNA gene fragment.

Eel catchment sites	Individual eel code and total number of small gastrointestinal nematodes in parenthesis	Small gastrointestinal nematode code	Most similar, or identical, BlastN output
Petteril RP: England	RP2 (17)	RP2 (1)	*R. acus*
		RP2 (2)	*R. acus*
	RP9 (14)	RP9 (2)	*P. africana*
		RP9 (3)	*P. africana*
		RP9 (5)	*R. acus*
		RP9 (8)	*R. acus*
Bela B: England	B2 (4)	B2 (2)	*P. africana*
	B3 (1)	B3 (1)	*P. africana*
Crane CN: England	CN5(21)	CN5 (1)	*P. africana*
		CN5(2)	*R. acus*
Cadoxton CD: Wales	CD3(5)	CD3 (1)	*P. africana*
		CD3 (3)	*P. africana*

3.4.6 Gut pathological response to nematode infection

Upon dissection and analysis of the gut of eels sampled from the River Crane it was apparent that the majority (70%) exhibited an encystment response to nematode infection (Figure 3.11). The number of encysted nematodes ranged from 1 to 8 per eel. No gut encystment response was observed in any of the eel specimens from the remaining sampling sites.

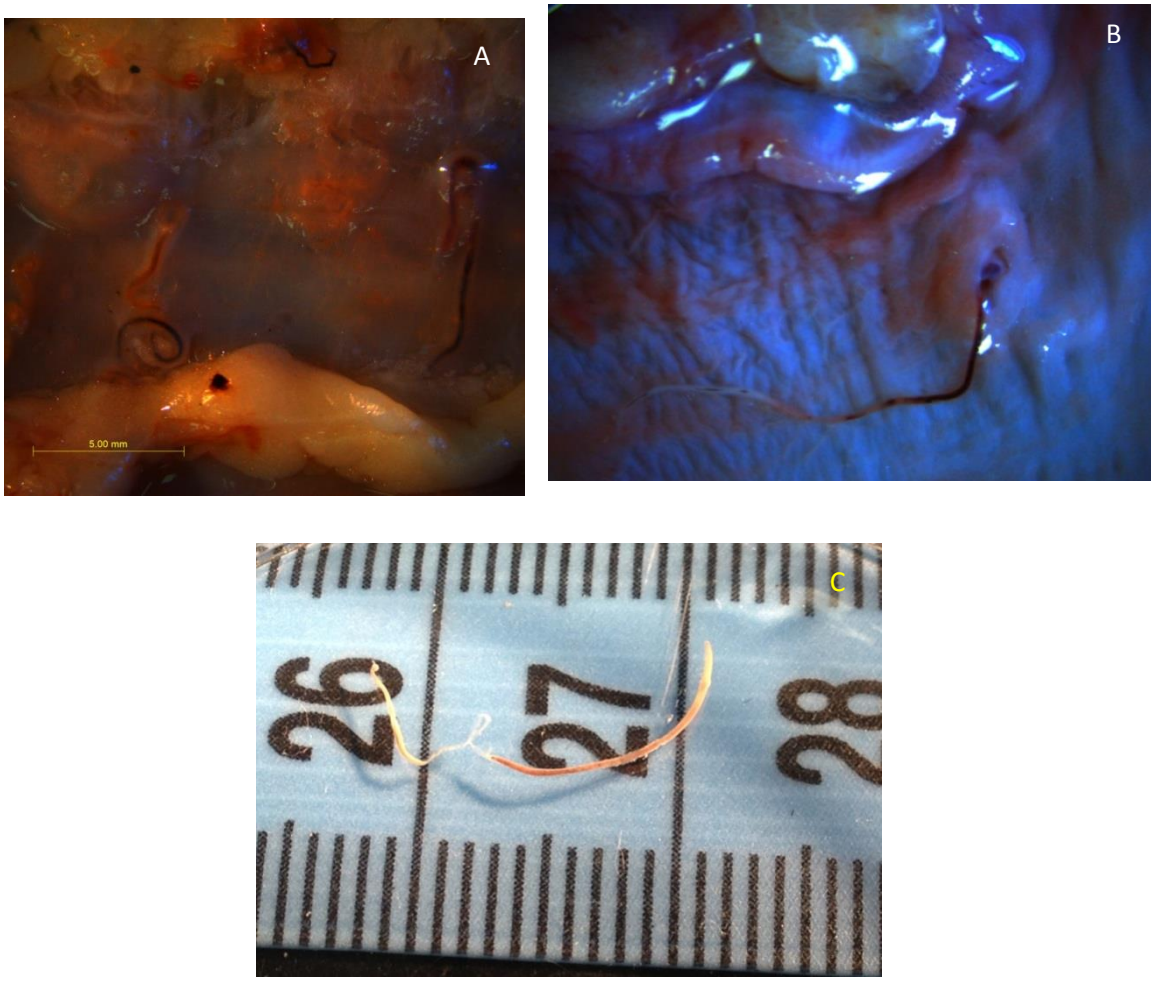

Figure 3.11 Representative images of (A) the encystment of nematodes with the eel gut wall (CN4), (B) a partially encysted nematode and (C) a nematode upon extraction from the gut wall that morphologically resembles an L3 stage *A. crassus*.

Morphological analysis of the extracted nematodes indicated that they were of two distinct species and these were suspected to be *R. acus* and the swim bladder nematode *A. crassus*.

3.4.6.1 Molecular identification of the gut encysted nematodes

Genomic DNA was extracted from 6 encapsulated worms randomly removed from the gut wall of eel specimens CN4 (n=2), CN5 (n=2) and CN10 (n=2). A fragment of the 18S rRNA gene was PCR amplified from the genomic DNA and the resulting 662 bp products were subjected to DNA sequence analysis. The BlastN analysis showed that the sequence was 99.7% identical to the *A. crassus* 18S rRNA gene (Table 3.18) and hence one of the encysted nematodes was, as suspected from the earlier morphological analysis, most likely to be *A. crassus*.

Table 3.18 BlastN analysis of the 18S RNA gene fragment derived from encapsulated larvae isolated from eel specimen CN10.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anguillicola crassus from Czech Republic small subunit ribosomal RNA gene, partial sequence	551	551	99%	0.0	99%	DQ490223.1
Anguillicola crassus small subunit ribosomal RNA gene, partial sequence	551	551	99%	0.0	99%	DQ118535.1

The remaining 5 sequences were all identical; BlastN analysis and sequence alignment (Table 3.19, Figure 3.12) confirmed that these nematodes were either *R. acus,* or *Hysterothylacium deardorffoverstreetorum*. The latter is a nematode currently only reported from cutlass fish (*Trichiurus lepturus*) (Knoff *et al.,* 2012), stripped weakfish (*Cynoscion guatucupa*) (Fontenelle *et al.*, 2013) and *Paralichthys isosceles* (Knoff *et al.*, 2012); all captured off the coast of Brazil. Moreover, the species of *Hysterothylacium* known to infect eel is *H. aduncum* (Køie, 1993; Alves *et al.*, 2002) and the 18S rRNA sequence of this nematode is dissimilar to that of *H. deardorffoverstreetorum* and hence the samples derived from this study. Consequently, the 5 encysted nematodes examined by PCR and sequencing can be confidently predicted to be *R. acus*.

Table 3.19 BlastN analysis of the 18S RNA gene fragment derived from encapsulated larvae isolated from eel specimen CN10 (equivalent data was generated for 4 other PCR products derived from encysted nematodes removed from the gut wall of eel specimens CN4 (n=2) and CN5 (n=2).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Hysterothylacium deardorffoverstreetorum 18S ribosomal RNA gene, partial sequence	1035	1035	100%	0.0	100%	JF718550.1
Raphidascaris acus 18S ribosomal RNA gene, partial sequence	1035	1035	100%	0.0	100%	DQ503460.1

```
gi|380084914|gb|JF718550.1|      ACCAAAGCTCCGAATTTTTGACGAGCGCATCTATTAGATTAAAACCAATC 170
gi|95116608|gb|DQ503460.1|       ACCAAAGCTCCGAATTTTTGACGAGCGCATCTATTAGATTAAAACCAATC 200
420573301_An4_F_C01              ---------------------------------TTAGATTAAAACCAATC 17
                                                                  *****************

gi|380084914|gb|JF718550.1|      GGGTTTCGGCCCGTTTGTTGGTGACTCTGAATAACTATAGCTGATCGCAT 220
gi|95116608|gb|DQ503460.1|       GGGTTTCGGCCCGTTTGTTGGTGACTCTGAATAACTATAGCTGATCGCAT 250
420573301_An4_F_C01              GGGTTTCGGCCCGTTTGTTGGTGACTCTGAATAACTATAGCTGATCGCAT 67
                                 **************************************************

gi|380084914|gb|JF718550.1|      GGTCTAGAACCGGCGACGTGTCTATCAAGTGTCTGCCTTATCAACTGTCG 270
gi|95116608|gb|DQ503460.1|       GGTCTAGAACCGGCGACGTGTCTATCAAGTGTCTGCCTTATCAACTGTCG 300
420573301_An4_F_C01              GGTCTAGAACCGGCGACGTGTCTATCAAGTGTCTGCCTTATCAACTGTCG 117
                                 **************************************************

gi|380084914|gb|JF718550.1|      ATGGTAGTTTATGTGCCTACCATGGTTGTAACGGGTAACGGAGAATAAGG 320
gi|95116608|gb|DQ503460.1|       ATGGTAGTTTATGTGCCTACCATGGTTGTAACGGGTAACGGAGAATAAGG 350
420573301_An4_F_C01              ATGGTAGTTTATGTGCCTACCATGGTTGTAACGGGTAACGGAGAATAAGG 167
                                 **************************************************

gi|380084914|gb|JF718550.1|      GTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG 370
gi|95116608|gb|DQ503460.1|       GTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG 400
420573301_An4_F_C01              GTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG 217
                                 **************************************************

gi|380084914|gb|JF718550.1|      GCAGCAGGCGCGCAAATTACCCACTCTCGGCATGAGGAGGTAGTGACGAA 420
gi|95116608|gb|DQ503460.1|       GCAGCAGGCGCGCAAATTACCCACTCTCGGCATGAGGAGGTAGTGACGAA 450
420573301_An4_F_C01              GCAGCAGGCGCGCAAATTACCCACTCTCGGCATGAGGAGGTAGTGACGAA 267
                                 **************************************************

gi|380084914|gb|JF718550.1|      AAATAACGAGACCGTTCTCTATGAGGCCGGTTATCGGAATGGGTACAATT 470
gi|95116608|gb|DQ503460.1|       AAATAACGAGACCGTTCTCTATGAGGCCGGTTATCGGAATGGGTACAATT 500
420573301_An4_F_C01              AAATAACGAGACCGTTCTCTATGAGGCCGGTTATCGGAATGGGTACAATT 317
                                 **************************************************

gi|380084914|gb|JF718550.1|      TAAACCCGTTAACGAGGATCTATGAGAGGGCAAGTCTGGTGCCAGCAGCC 520
gi|95116608|gb|DQ503460.1|       TAAACCCGTTAACGAGGATCTATGAGAGGGCAAGTCTGGTGCCAGCAGCC 550
420573301_An4_F_C01              TAAACCCGTTAACGAGGATCTATGAGAGGGCAAGTCTGGTGCCAGCAGCC 367
                                 **************************************************

gi|380084914|gb|JF718550.1|      GCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCTGCGGTTAAAAA 570
gi|95116608|gb|DQ503460.1|       GCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCTGCGGTTAAAAA 600
420573301_An4_F_C01              GCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCTGCGGTTAAAAA 417
                                 **************************************************

gi|380084914|gb|JF718550.1|      GCTCGTAGTTGGATCTGCGCCTCAGGACTTGGTCCGCCCACTGGGTGAGA 620
gi|95116608|gb|DQ503460.1|       GCTCGTAGTTGGATCTGCGCCTCAGGACTTGGTCCGCCCACTGGGTGAGA 650
420573301_An4_F_C01              GCTCGTAGTTGGATCTGCGCCTCAGGACTTGGTCCGCCCACTGGGTGAGA 467
                                 **************************************************

gi|380084914|gb|JF718550.1|      ACTGGGCTCCTGGGCTAGTTCTGCTGGTTTTCCCTACGTTGCCTTCATCG 670
gi|95116608|gb|DQ503460.1|       ACTGGGCTCCTGGGCTAGTTCTGCTGGTTTTCCCTACGTTGCCTTCATCG 700
420573301_An4_F_C01              ACTGGGCTCCTGGGCTAGTTCTGCTGGTTTTCCCTACGTTGC-------- 509
                                 ****************************************
```

Figure 3.12 The 509 bp 18S rRNA DNA sequence (420573301_An4_F_C01) derived from an encapsulated nematode removed from the gut wall from eel sample CN10 aligned with the *Hysterothylacium deardorffoverstreetorum* (gi: 380084914) and *Raphidascaris acus* (gi: 95116608) 18S rRNA gene sequences deposited in GenBank.

3.5 Discussion

Gastrointestinal nematodes are common infections in most animals and in mammals, there is increasing evidence to suggest that there is a health benefit to harbouring such parasites (Wills-Karp *et al.*, 2001; Okada *et al.*, 2010). However, in fish, there is much less known about the interactions that occur between different species of gastrointestinal nematodes and also, between these parasites and the host immune system. As such, the work carried out in this chapter attempted to describe some baseline data on gastrointestinal nematode infections in European eel sampled from river systems in the United Kingdom. As highlighted in previous chapters, the mode of acquisition of the eel was not ideal and hence caution is necessary when interpreting the significance of some of the primary infection data.

Perhaps not surprisingly, the data showed that gastrointestinal infections in the European eel are common and widespread in UK rivers and as observed in other studies, the parasites are over-dispersed amongst the population (Nie & Kennedy, 1991c). Interestingly, there appeared to be a significant difference in the prevalence of gastrointestinal nematode infections between the regions; the more northern sampling areas of England and Wales had the greater percentage of infection relative to the respective southern regions. This is in contrast to the trend observed with the gill monogenean parasite (Chapter 2) and is most likely explained by life-cycle differences since the pseudodactylid has a direct life-cycle whereas the gastrointestinal nematodes have obligatory intermediate host stages. Moreover, the gastrointestinal nematode abundance data was significantly greater for eel sampled from rivers in North West England compared to two of the three other regions; the exception being North Wales. The reason for this is probably due to the relatively high prevalence of gastrointestinal nematode infection observed in eel from North Wales which indicates an environment conducive to transmission of the parasites to the definitive host and hence enhanced burden. Upon examination of the parasite infection data with respect to host

factors, there was no significant impact upon prevalence, or intensity, with respect to eel body length. However, significance was established when the data was examined with respect to host condition factor. Indeed, perhaps most convincingly, the greatest intensity of infection was observed in eel with the greatest category of condition factor (0.17-0.29) and this contrasted with a significantly lower infection intensity observed in eel that comprised a relatively low condition factor category (0.13-0.14). As such, one conclusion from this would be that gastrointestinal nematodes appear not to affect the overall body condition of eel (Kennedy, 2007a; Marcogliese & Pietrock, 2011; Mayo-Hernaez *et al.*, 2015).

An examination of the gastrointestinal nematode community that infected the eels showed that it comprised of three species; *S. inermis* and the relatively small nematodes *P. tenerrima* and *R. acus*. These species have been reported to infect the European eel elsewhere (Kennedy, 1974; Esch *et al.*, 1988; Kennedy, 1993b; Kennedy, 2012) and hence their identification in UK eel specimens is not surprising. *S. inermis* was the least common gastrointestinal nematode in the sampled population since it was only recovered from two catchment sites in England and two in Wales. Moreover, this parasite was never recovered exclusively from the eel gastrointestinal tracts since the smaller nematodes were also always present. Perhaps worthy of further investigation was the finding that there appeared to be a significant difference in body length between *S. inermis* infected and uninfected eels; albeit, this was only marginally significant. The 18S rRNA sequence derived from samples of *S. inermis* was novel and hence provides useful molecular assistance to identifying this parasite. Moreover, the sequence data has assisted a phylogenetic analysis of the genus since *S. inermis* separates the clade formed by *S. carolini* and *S. tabascoensis* from *S. petterae*.

A regional analysis of the infection data for the small gastrointestinal nematodes *P. tenerrima* and *R. acus* reinforced the earlier more general finding that the more northerly sites have a higher prevalence of infection. Indeed, the eel sampled from North Wales and North West

England had a significantly greater prevalence of these small gastrointestinal nematodes compared to South Wales. Further corroboration for a regional difference was provided by significant differences in the abundance data between North West England and South Wales and also, North West England and South East England. As commented above, these differences are most likely reflecting regional variations in the physical and biological properties of the rivers and how these factors interact to support the intermediate and paratenic hosts and the transmission of the parasite to the eel.

Interestingly, there appeared to be a significant difference in intensity of infection with *P. tennerima* and *R. acus* between two relatively similar categories of eel body size; 20.5-25cm and 25.5-30cm. This result was somewhat surprising and most probably reflects for some unknown reason that the 25.5-30 cm body length category of eel harboured fewer parasites than expected since the overall trend was for the larger fish to contain more gastrointestinal nematodes.

Interestingly, the eel with the lowest condition factor category had the second greatest intensity of infection with small gastrointestinal nematodes and as a result, the data was significantly different to categories of eel with greater condition factors. As such, this lends itself to the intriguing proposal that the greater numbers of *P. tennerima* and *R. acus* may have contributed to the lowered condition factor in these hosts. The alternative and perhaps stronger argument given that the greatest intensity data is observed in the highest category of condition factor, is that the eel with the lowest condition factor are simply more susceptible to increased numbers of these parasites in their gastrointestinal tracts.

In characterising the *P. tennerima* infections novel 18S rRNA sequence data was generated. Firstly, this facilitated an overall comparison of the relative contributions of *P. tenerrima* and *R. acus* to the overall gastrointestinal nematode community; based on a random sample of 12 nematodes extracted from six hosts across 4 catchment sites, these species were present in

equal numbers. Secondly, the DNA sequence data should assist a phylogenetic analysis of the genus *Paraquimperia*.

In body length and body weight, eel from the River Crane were the largest (Chapter 1). Interestingly, the majority of eel from this catchment site contained encysted nematodes within their gastrointestinal tracts whereas this pathological feature was absent in all the other eel specimens. The identity of the encysted nematodes was confirmed following PCR amplification and DNA sequencing of the 18S rRNA gene of a random sample of parasites extracted from the cysts. The greatest proportion of encysted nematodes were identified as *R. acus* and the other parasite identified was *A. crassus*. Interpretation of this observation is that the largest and hence oldest eel have developed a degree of immunity to infection with both of these parasites. Indeed, this type of immunity to *A. crassus* has previously been reported (Knopf *et al.*, 2000).

In summary, data in this Chapter has shown that three species of gastrointestinal nematode; *S. inermis, P. tennerima* and *R. acus,* are commonly found in *A. anguilla* present in rivers across England and Wales. Some interesting regional data was observed that would be worthy of further exploration given that the precise sampling dates are unknown. Moreover, certain host factors are also worthy of further investigation given the observed infection profiles. The encystment observed in the largest eel certainly corroborates other reports that these hosts are able to mount an immune response to certain gastrointestinal nematode infections and in particular, a limited response to *A. crassus*. The precise details of this immune response and indeed, how gastrointestinal nematodes may influence the immune status of the European eel more generally, remain topics worthy of considerable more study.

CHAPTER FOUR

Other Gastrointestinal Helminths

4.1 INTRODUCTION

4.1.1 Acanthocephalan parasites

Acanthocephala, the thorny-headed worms, is a phylum of gastrointestinal parasites of vertebrates that utilise arthropods as intermediate hosts (Crompton & Nickol, 1985). The phylum includes approximately 1298 described species, and it is divided into four classes: Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala and Polyaacanthocephala (Amin, 2013). In addition to the species of intermediate and final host, these classes are distinguished by morphological characteristics that include the location of lacunar canals, the persistence of ligament sacs in females, the number and type of cement glands in males and the number and size of proboscis hooks (Bullock & Schmidt, 1969; Amin, 1987; García-Varela *et al.*, 2000; Wayland, 2010). The name of the phylum refers to the thorny retractable proboscis that anchors the adult worm to the intestine of the final host. Superficially, the acanthocephalan body consists of the anterior proboscis, a neck and a trunk (Figure 4.1). The proboscis size, shape and number of hooks are important taxonomic characters(Wayland, 2010). The trunk, or metasoma, contains the reproductive system and also functions in absorbing and distributing nutrients from the host's intestinal content. The proboscis and trunk are separated by the neck (Roberts & Janovy, 2005).

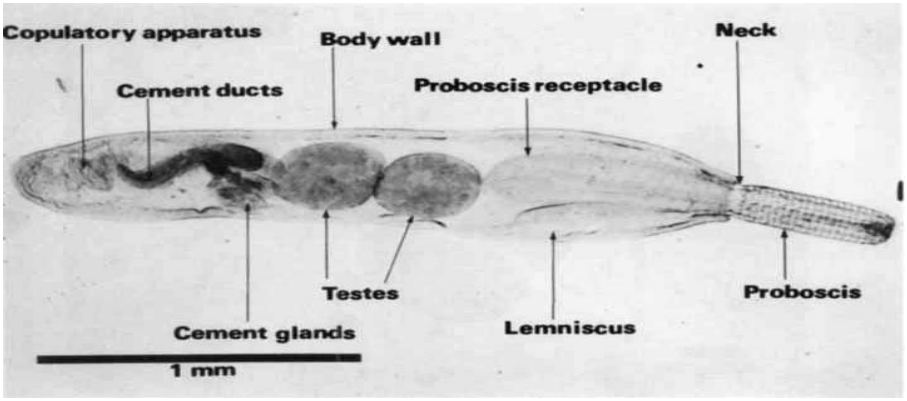

Figure 4.1 An annotated image of a male *A. clavula* (Brown *et al.*, 1986).

4.1.1.1. Fish acanthocephalans

Six species of acanthocephalan, *Pomphorhvnchus laevis* (Miller, 1776), *Neoechinorhynchus rutili* (Miller, 1780), *Acanthocephulus anguillae* (Muller, 1780), *A. lucii* (Muller, 1780), *A. clavula* (Dujardin, 1845), and *Echinorhynchus truttae* (Schrank, 1788), representing four genera, are recorded as parasitic of British freshwater fishes, including the European eel (Kennedy, 1974; Brown *et al.*, 1986; Lyndon & Kennedy, 2001). One of these species, *P. laevis*, is noted as a category 2 parasite and movement of fish infected with this parasite is stringently regulated by the Salmon and Freshwater Fisheries Act 1975 and the Diseases of Fish Act 1983 (Environment Agency UK, 2014). Another species, *E. salmonis*, is believed to have been incorrectly identified in the past, as its intermediate host, *Pontoporeia afinis*, does not occur in the British Isles (Brown *et al.*, 1986).

None of these acanthocephalan species is rare in the UK; however, any may be rare locally. Due primarily to the utilization of migratory fish hosts and also, common and widespread crustaceans as intermediate hosts, these parasites are considered successful colonizers. As the six species appear to exhibit partial resource partitioning within the hosts, at either, or both, the larval and adult stages, the potential for competition is reduced and hence colonization and parasite survival is facilitated. (Kennedy, 1985; Lyndon & Kennedy, 2001).

Mixed species infections are very rarely encountered in the UK since most freshwater localities contain, or are almost completely dominated by, only a single species of acanthocephalan (Kennedy, 1985). In addition, studies carried out on eels in Ireland have shown that although *A. lucii* was the dominant acanthocephalan and most commonly found as a single-species infection, mixed infections were observed with *A. anguillae*. Within the eel intestine, the distribution of these two species overlapped considerably, though there was no evidence to indicate competitive displacement of one species by the other (Kennedy & Moriarty, 1987; Bates & Kennedy, 1991).

4.1.1.2 General life cycle of acanthocephalan parasites

Acanthocephalans have indirect life cycles since the parasite must develop via intermediate arthropod hosts, including amphipods, isopods, ostracods, copepods, insects, and myriapods, and then mature within the vertebrate definitive host (Figure 4.2). Gravid females release eggs that exit the fish host via the faecal route and these are ingested by an arthropod intermediate host. The eggs hatch and the larvae enter the haemocoel and develop into the infective cystacanth. There is also field and lab-based evidence to suggest that some gravid female *A. dirus* may exit the fish host with the faeces and that their eggs may hatch within a sediment-dwelling intermediate host following ingestion of the worm; this alternative may occur with other acanthcephalan species (Kopp *et al.*, 2011).

Completion of the life cycle occurs when the vertebrate definitive host ingests the infected arthropod intermediate host, allowing the cystacanth to complete maturation to the adult within the fish intestine. Larval acanthocephalans that share the same intermediate host may, or may not, also share the same definitive hosts. Moreover, associations among acanthocephalan species in the intermediate hosts are not random, and are likely to have occurred due to selection favouring certain pathways of transmission (Dezfuli *et al.*, 2000).

The life cycle of some acanthocephalans may involve paratenic hosts since some vertebrate fish are prey for larger fish species that may be definitive hosts (Médoc *et al.*, 2011). In such paratenic hosts, the larvae of some acanthocephalans migrate from the gut lumen to encyst in the peritoneal cavity and viscera, where they remain as immature worms until ingested by the final host (Crompton & Nickol, 1985). Post-cyclic transmission has also been described for some species of acanthocephalans since an infected definitive host may be ingested by a predator and the worm may survive and parasitize the larger fish (Nickol & Crompton, 1985). Indeed, Lassiere and Crompton (1988) transmitted *N. rutili* from *Gasterosteus aculeatus* to rainbow trout (Lassiere & Crompton, 1988) and Kennedy (1999) demonstrated that post-

cyclic transmission of *P. laevis* occurred when rainbow trout predated infected *Cottus gobio*, *Noemacheilus barbatulus*, *Phoxinus phoxinus* and *Leuciscus cephalus* (Kennedy, 1999).

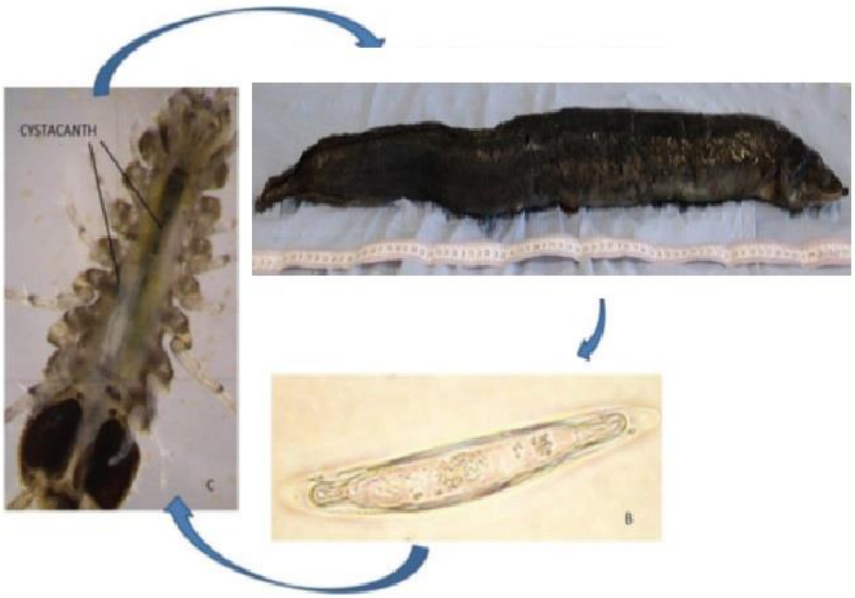

Figure 4.2 The life cycle of *Acanthocephalus lucii*. (A) The European eel is often a definitive fish host. The adult worms reproduce in the intestine of the eel and (B) shelled acanthor larvae called "eggs" are shed into the water with the fish faeces. Isopods (*Asellus aquaticus*) serve as intermediate hosts and while foraging they ingest the eggs. In the intestine of the isopod the acanthor larva hatches and penetrates into the haemocoel, whereupon it starts to grow and (C) develop to the cystacanth stage, which is infective to the fish host. When a cystacanth-infected isopod is predated by a fish, the cycle is completed. In the picture above (C) two larvae can be seen in the haemocoel from the abdominal side of the isopod host. Image modified from ((Hasu, 2013).

Many parasites with complex life cycles increase the chances of reaching a final host by adapting strategies to manipulate their intermediate host's appearance, condition or behaviour (Bakker *et al.*, 1997; Bollache *et al.*, 2002). Indeed, acanthocephalans, including *P. laevis*, increase their chance of transmission to a final host using olfactory-triggered manipulation of the anti-predator behaviour of the arthropod (Baldauf *et al.*, 2007). In addition, acanthocephalan parasites may also induce physiological changes in the intermediate host that may be favourable to the parasite; for example, influencing the re-allocation of resources such as lipids and glycogen (Bauer *et al.*, 2000; Plaistow *et al.*, 2001; Tain *et al.*, 2007).

4.1.1.3 Morphology of recognised acanthocephalans of *A. anguilla*

Anatomical variation occurs and certain characters show considerable variation between individuals. For example, acanthocephalans of the same species may vary greatly in size even within an individual fish (Brown *et al.*, 1986; Brown, 1987). One of the most useful characters in species identification is the arrangement of the proboscis armature, in combination with the size, shape and arrangement of other anatomical parts including the proboscis, neck and testes (Brown *et al.*, 1986; O'Mahony, Kennedy, *et al.*, 2004).

The identification of the intermediate host species is often a useful characteristic to aid parasite identification; however, it is possible for two, or more species of acanthocephalan to use the same arthropod host (Dezfuli *et al.*, 2000). The species of fish host is often of little value for identification of the parasites since acanthocephalans infect a variety of fish species (Médoc *et al.*, 2011); albeit they grow to maturity and reproduce in a narrower range of host species (Hine & Kennedy, 1974b; Brown *et al.*, 1986). Historically, mixed species infections are very rarely encountered in the UK since most freshwater localities are dominated by only a single species of acanthocephalan (Kennedy, 1985). Site specificity and distribution of acanthocephalans within the intestines of *A. anguilla* is also not informative with respect to identification of the worm since any preference tends to be masked by a general distribution (Kennedy, 1985).

4.1.1.3.1 *Pomphorhvnchus laevis* (Muller, 1776)

Species of the genus *Pomphorhynchus* (Monticelli, 1905) are largely parasites of freshwater fishes; 23 species are recognized of which one species, *P. dubious* (Kaw 1941), occurs in amphibians and four species occur in marine or coastal waters (Amin *et al.*, 2003).

Of concern, given that *P. laevis* is a category 2 parasite, is that this worm species is one of the most abundant and widely distributed acanthocephalan parasites of freshwater fishes in Europe (Kennedy *et al.*, 1989; Moravec & Scholz, 1991; Perrot-Minnot, 2004). *P. laevis* is

able to develop in a variety of amphipod crustacean species (eg. *Gammarus pulex* and *G. roeseli*), which act as intermediate hosts (Kennedy *et al.*, 1989). A recent study of P. laevis intermediate hosts in France has shown that *G. fossarum* is likely to be the preferred intermediate host (Bauer & Rigaud, 2015).

The worms are usually orange in colour and body size may vary between 4 and 25 mm in length which is an indicator of age (Brown *et al.*, 1986). The neck is long and the proboscis bulb is localized to the base of the proboscis (Figure 4.3). The elaborate proboscis consists of 13 to 20 longitudinal rows of hooks and within each row there can be between 11 and 13 hooks (Brown, 1987; O'Mahony, Kennedy, *et al.*, 2004). There are 7 anterior hooks that are large, with well-developed posteriorly split roots, whereas the posterior hooks are smaller, with poorly-developed roots (Amin *et al.*, 2003).

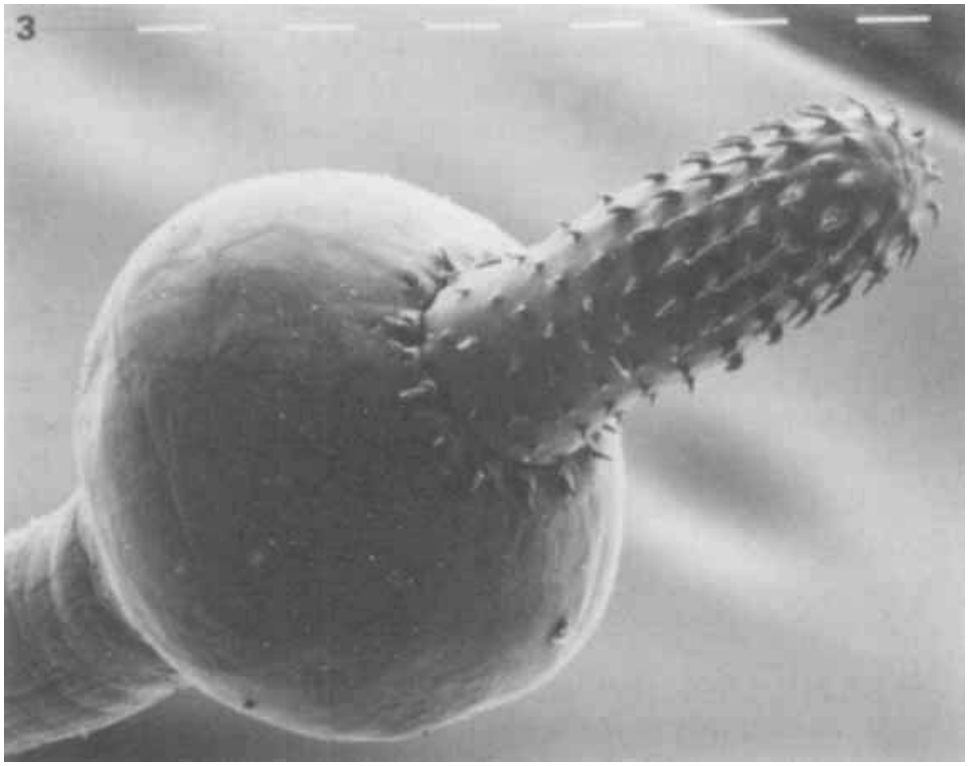

Figure 4.3 Scanning electron micrograph of the proboscis and the proboscis bulb of *P. laevis* (- - - Markers = 100 µm) (modified from Brown et al., 1986).

P. laevis is a common endoparasite of freshwater fish and it has the ability to survive, though not necessarily mature, in virtually every species of freshwater fish (Hine & Kennedy, 1974a; Kennedy *et al.*, 1978). As such, post-cyclic transmission of the parasite is most likely important (Kennedy, 1999).

Three biologically distinct strains of *P. laevis* have been recognized within the UK (Kennedy *et al.*, 1978; Kennedy, 1984b; Brown, 1987). The so-called British strain was shown to dominate three river systems: the Thames, the Severn and the Hampshire Avon and Stour. Within these rivers, the preferred definitive hosts were shown to be chub (*Leuciscus cephalus*) and barbel (*Barbus barbus*) and the preferred intermediate host was identified as *G. pulex.* In addition, *P. laevis* was also confirmed to infect chub, bullhead (*Cottus gobio*) and minnow (*Phoxinus phoxinus*) sampled from the river Lugg (Kennedy *et al.*, 1989). The marine strain of *P. laevis* is known to occur in the Baltic and North Seas and in the lower reaches of estuaries opening into them (Kennedy, 1984b). The intermediate hosts utilized are *G. locusta* and *G. zaddachi*, and the preferred definitive host is flounder, (*Platichthys flesus*), although it can also mature in plaice (*Pleuronectes platessa*) (Kennedy *et al.*, 1989). The marine strain of *P. laevis* has been recorded in the UK and the parasite has been sampled from migratory fish, or fish able to survive periods in estuarine and marine waters; these include *A. anguilla*, salmon and flounder (Kennedy *et al.*, 1989). The Irish strain of *P. laevis* is thought to have originated from the introduction of infected cyprinids in the 17th century and it is now widely distributed throughout the south of the country; prevalences of 22.6% and 100% have been recorded in *A. anguilla* and brown trout (*S. trutta*) respectively (Kennedy *et al.*, 1989; Kennedy, 1996). The definitive host of the Irish strain of *P. laevis* is recognized as *S. trutta* and the preferred intermediate host as *G. duebeni*. (Brown *et al.*, 1986; Kennedy *et al.*, 1989; Evans *et al.*, 2001). A more recent analyses, based upon morphological and molecular data, confirmed the strain variation and suggested that the

differences were based upon host species preferences as opposed to geographic distribution (O'mahony, Bradley, *et al.*, 2004; O'Mahony, Kennedy, *et al.*, 2004).

P. laevis causes local damage to the intestinal wall of fish, the extent of which can vary between different host species (Hine & Kennedy, 1974a). Cystacanths and adults of *P. laevis* release proteolytic enzymes and trypsin-like proteases and these have been shown to be necessary for the complete and rapid perforation of the fish intestinal wall (Polzer & Taraschewski, 1994). Studies have shown that *P. laevis* infection stimulates a localized cellular response involving mucosal cells and mast cells, which form a barrier to protect the intestinal mucosa (Dezfuli *et al.*, 2002; Dezfuli *et al.*, 2011; Bosi & Dezfuli, 2015). The mast cells are the dominant immune cells that respond to *P. laevis* infection and recent analysis in the three-spined stickleback (*Gasterosteus aculeatus*) has shown the worm becomes encapsulated by fibres containing epitheloid cells and mast cells and that some of the latter are able to penetrate the parasite tegument (Dezfuli *et al.*, 2015).

4.1.1.3.2 *Neoechinorhynchus rutili* (Muller, 1780)

Members of the genus *Neoechinorhynchus* (Stiles and Hassail, 1905), are primarily parasites of freshwater fishes but some also infect reptiles and amphibians (Amin & Heckmann, 1992). *N. rutili* is a widely distributed acanthocephalan worm that parasitizes the small intestine of many freshwater fish species of the Northern hemisphere; it has been recorded from a wide range of definitive hosts throughout Europe, Asia and North America (Walkey, 1967; Moravec & Scholz, 1994; Lyndon & Kennedy, 2001).

In the UK, *N. rutili* is common and widespread, and it utilizes the ostracods *Cypria ophthalmica* and *Candona candida* as intermediate hosts (Walkey, 1967; Bibby, 1972; Brown *et al.*, 1986). The parasite is usually white in colour and the body ranges from 2 to 20 mm in length (Figure 4.4). The neck is short and the proboscis is small with six longitudinal rows of hooks, each of which contain two large and one small hook (Brown *et al.*, 1986).

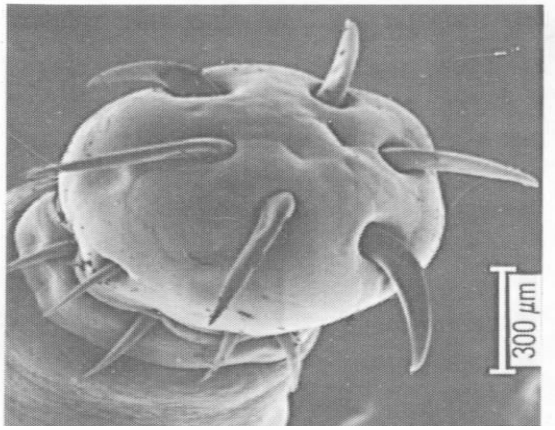

Figure 4.4 Scanning electron micrograph of the proboscides of *N. rutili* collected from an alder fly larva *(Sialis lularia)* (modified from (Lassiere, 1988).

N. rutili not only has the capacity to transmit via the ostracod route (Walkey, 1967; Valtonen, 1979), since as observed for other acanthocephalans, a post transmission life cycle from prey to predator fish is also possible (Lassiere & Crompton, 1988). Moreover, a further life-cycle variation is reported to occur with *N. rutili* since field and laboratory investigations have confirmed that the parasite may infect the alder fly, *Sialis lutaria*, and then subsequently complete its life-cycle in a final host (Lassiere, 1988).

4.1.1.3.3 *Acanthocephalus anguillae* (Muller, 1780)

A. anguillae occurs in the intestine of a variety of European fish including members of the families Anguillidae, Salmonidae and Cyprinidae (Kennedy, 1985; Tarachewski, 1988; Taraschewski, 1989). In the UK, the definitive hosts are known to include chub and eels, although mature specimens have also been recorded from roach (*Rutilus rutilus*) and stone loach (*Barbatula barbatula*) whilst in Ireland, where chub are absent, mature specimens have been reported solely from eels (Kennedy *et al.*, 1989). The intermediate hosts are species of freshwater crustacean such as *Asellus aquaticus* (Kennedy *et al.*, 1989; Dezfuli *et al.*, 1994).

A. anguillae is usually white in colour, the body size may be up to 30 mm in length and the hooks on the proboscis are long and prominent (Brown *et al.*, 1986). In total, there are 10 longitudinal rows of proboscis hooks with between five and seven hooks in each row (Figure

4.5). The anterior and posterior hooks are reported to be 120 µm and 64 µm long respectively and the roots of the hooks possess well developed lateral processes (Brown *et al.*, 1986; Amin *et al.*, 2008).

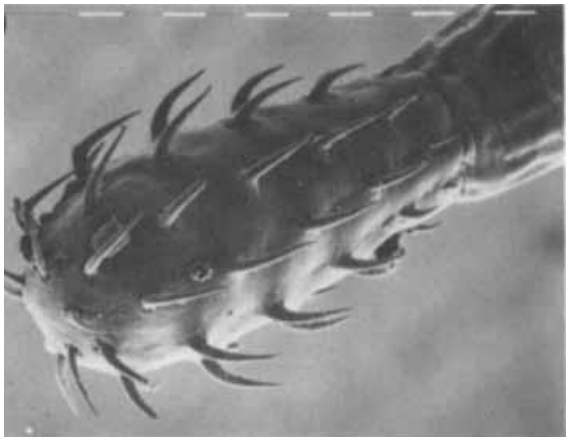

Figure 4.5 Scanning electron micrograph of the lateral view of the proboscis of *A. anguilla* showing the longitudinal rows of proboscis hooks (- - - Markers = 63 µm). Modified from (Brown *et al.*, 1986).

Previous studies suggest that the distribution of *A. anguillae* is localized and restricted in both the UK and Ireland (Kennedy & Moriarty, 1987; Kennedy *et al.*, 1989). Indeed, the parasite was only reported in fish sampled from rivers flowing eastwards into the North Sea, including the rivers Trent and Thames, whereas in Ireland, records indicate that it is mostly confined to the river Shannon system (Kennedy & Moriarty, 1987; Kennedy *et al.*, 1989).

4.1.1.3.4 *Acanthocephalus lucii* (Muller, 1780)

A. lucii is one of the most widely distributed species of acanthocephalans infecting freshwater fishes throughout Europe (Amin *et al.*, 2011). In the UK, *A. lucii* is commonly found in the gastrointestinal tract of many freshwater fish species and it is widely distributed (Kennedy, 1985; Brown *et al.*, 1986). The intermediate host is an isopod crustacean, most commonly *A. aquaticus* (Lee, 1981; Brattey, 1986) and the definitive host becomes infected via the ingestion route (Lee, 1981; Brattey, 1988).

A. lucii is white in colour and up to 15 mm in length. The hooks on the proboscis are slightly less prominent than other species and the number range from 12 to 16 longitudinal rows; there are between seven and nine hooks within each row and they lack prominent lateral processes (Figure 4.6) (Brown *et al.*, 1986). Hooks are uniformly slender in shape throughout the length of the proboscis (Amin *et al.*, 2011).

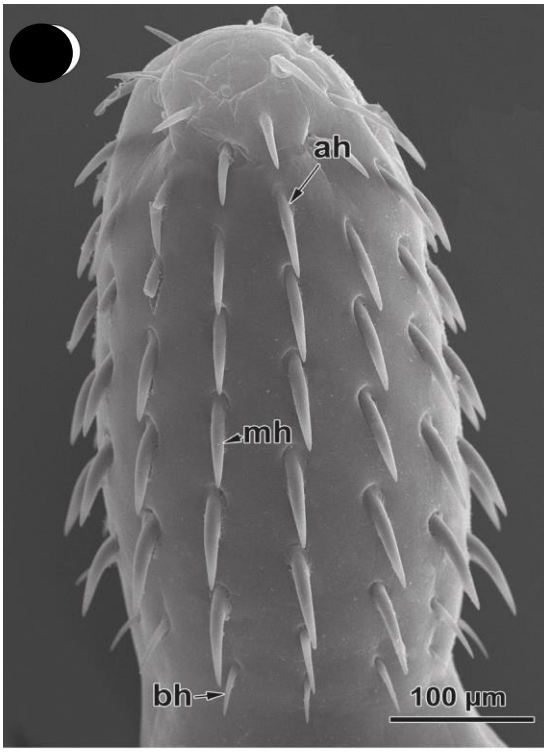

Figure 4.6 Scanning electron micrograph view of the proboscis and proboscis hooks of *A. lucii* from *Perca fluviatilis* (ah = apical hooks; bh = basal hooks; mh – middle hook). Modified from (Brázová *et al.*, 2014).

4.1.1.3.5 *Acanthocephalus clavula* (Dujardin, 1845)

In contrast to the above acanthocephalans, the preferred definitive host of *A. clavula* is the European eel; however, the parasite is also able to grow and reproduce in other species of fish (Kennedy & Lord, 1982; Kennedy, 1984a). Nonetheless, in some species (eg. brown trout) the female *A. clavula* often fail to reach sexual maturity which is indicative of the fish being an accidental host (Byrne *et al.*, 2004). In the UK, *A. clavula* is a common eel parasite that is reported to utilize *A. meridianus* as the intermediate host (Kennedy, 1984a; Brown *et al.*, 1986).

A. clavula is white in colour and relatively small in size since adults grow up to a maximum of 5 mm in length. There are 16 to 18 rows of longitudinal hooks on the proboscis and each of these has between 12 and 14 hooks (Brown *et al.*, 1986).

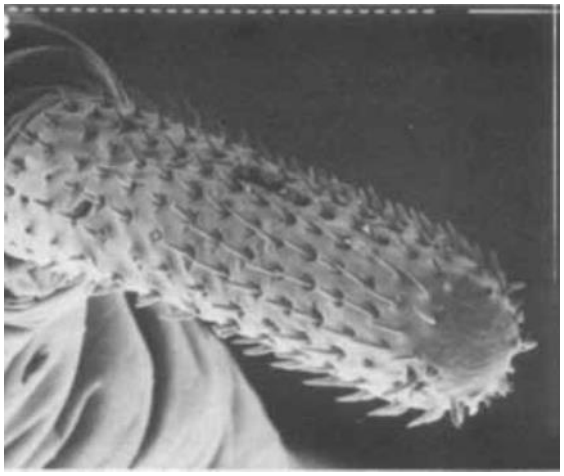

Figure 4.7 Scanning electron micrograph of the proboscis of *A. clavula* showing the longitudinal rows of proboscis hooks (- - - Markers 8 µm). Modified from (Brown *et al.*, 1986).

4.1.1.3.6 *Echinorhynchus truttae* (Schrank, 1788)

E. truttae is a acanthocephalan of fish throughout Europe and UK focused studies have highlighted that it is commonly associated with salmonids such as brown trout, *S. trutta*, and rainbow trout, *Oncorhychus mykiss* (Awachie, 1965; Dorucu & Huntingfordl, 1995) , in addition to infecting eels (Norton *et al.*, 2003). Several reports however state that the European eel is most likely to be an accidental host of *E. truttae* since it is not commonly reported in *A. anguilla* (Moravec, 2004a; Thielen, Muenderle, *et al.*, 2007).

The most common intermediate host is reported to be the freshwater shrimp *G. pulex* (Awachie, 1965, 1966; Kennedy, 1985). *E. truttae* often has a pale orange/pink colouration and it has 20 to 22 longitudinal rows of proboscis hooks; each row has between 13 and 16 hooks (Brown *et al.*, 1986) (Figure 4.8).

Figures 4.8 Scanning electron micrograph of an adult male specimen of *E. truttae* from Kessler's sculpin (*Leocottus kesslerii*), showing the anterodorsal hump. Modified from (Amin *et al.*, 2015).

4.1.2 Tapeworms

Tapeworm (Platyhelminthes, Cestoda) infections are highly prevalent worldwide (Budke *et al.*, 2009). These parasites are passively transmitted between hosts and infect virtually every vertebrate species (Bogitsh *et al.*, 2013). The zoonotic helminth infections, echinococcosis and cysticercosis are caused by the larval stages of the cestodes, *Echinococcus spp*. and *Taenia solium*; they are among the most neglected severe parasitic diseases in humans (Garcia *et al.*, 2007). Despite causing a considerable global burden of ill health in humans and a substantial financial burden on the livestock industry (Torgerson & Macpherson, 2011), tapeworm infections are often difficult to treat (Tsai *et al.*, 2013).

4.1.2.1 Fish tapeworms

Diphyllobothriosis, a human disease caused by tapeworms of the genus *Diphyllobothrium*, is the most important fish-borne zoonosis caused by a cestode parasite and it has been estimated that 20 million people are affected globally (Scholz *et al.*, 2009). Infection occurs through eating raw or undercooked fish harbouring plerocercoid larvae, which subsequently develop into adult tapeworms in the small intestine after ingestion.

About 18 species of *Diphyllobothrium* have been reported to infect humans and *D. latum* and *D. nihonkaiense* are the most common; the definitive host is usually a pike, perch or burbot (Zhang *et al.*, 2015). Diphyllobothriosis is known to occur widely across the world (Yera *et al.*, 2006; Jackson *et al.*, 2007; Lee *et al.*, 2007; Wicht *et al.*, 2008; Mercado *et al.*, 2010; Chen *et al.*, 2014; Zhang *et al.*, 2015) and the disease is generally considered to be a mild illness, though occasionally infection can cause malignant anaemia due to the consumption of vitamin B12 by the tapeworm (Zhang *et al.*, 2015). Several outbreaks caused by *Diphyllobothrium* infection have had significant impact on public health and local economies (Mello Sampaio *et al.*, 2005; Zhang *et al.*, 2015).

4.1.2.2 Life Cycle

The life cycle of *D. latum* starts when immature eggs are passed in the faeces of an infected human. The eggs mature in water within three weeks and form oncospheres which develop into larvae, called coracidia. After ingestion by a suitable freshwater crustacean such as a copepod, the coracidia develop into procercoid larvae. Following ingestion of the copepod by a suitable second intermediate host, typically minnows and other small freshwater fish, the procercoid larvae are released from the crustacean and penetrate the gut (von Bonsdorff & Bylund, 1981). These larvae migrate to muscle tissue whereupon they develop into plerocercoid larvae, known as sparganum, that are infective to humans (Kuchta *et al.*, 2013). Usually, a third intermediate host is needed because humans tend not to consume raw species of small fish like the minnow. Consequently, human infection is most often via consumption of infected raw predator fish such as trout, walleyed pike or perch (Unsworth, 1944).

The adult tapeworm attaches to the intestinal mucosa by means of the two bothria. Adults live for up to 20 years and can reach more than 10 m in length and contain more than 3,000 proglottids. Up to 1 million eggs per day can be discharged from the proglottids and are passed in the feces (Bylund, 2003; CDC, 2012).

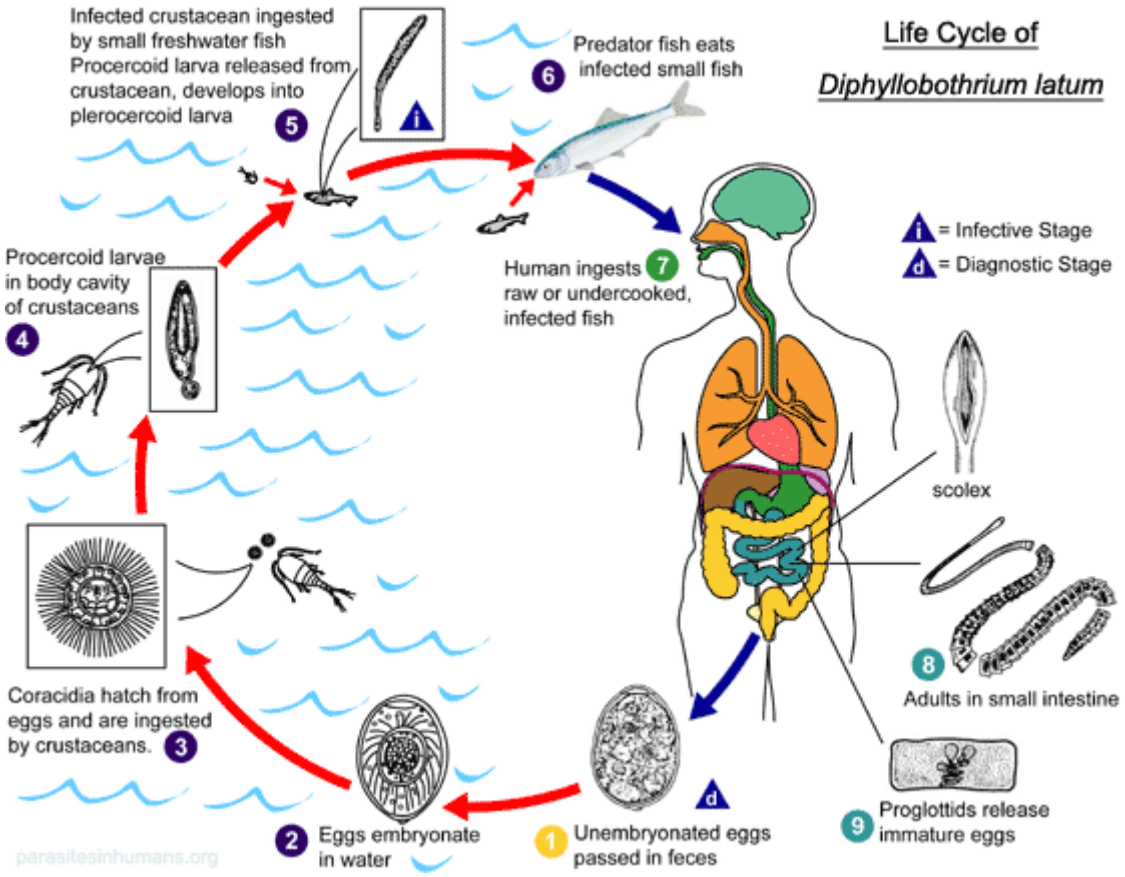

Figure 4.9 Life cycle of the tapeworm *D. latum* (CDC, 2012).

4.1.2.3 *Bothriocephalus claviceps* (Goeze, 1782)

The order Bothriocephalidea is one of the major cestode groups and it consists mostly of parasites of marine and freshwater fish, though some genera are reported as specific to mammals and less frequently to birds, reptiles and amphibians (Kuchta *et al.*, 2008; Kuchta *et al.*, 2012). Bothriocephalidea are characterised mainly by the possession of two bothria on the scolex, which is an attachment organ formed by a longitudinal groove or depression of different shape and depth, on the ventral and dorsal surfaces of the scolex (Kuchta *et al.*, 2008). The genus *Bothriocephalus* consists of about 70 species most of which are intestinal parasites of marine fish throughout the world, though some species occur in freshwater fish and also, a few amphibians (Kuchta *et al.*, 2008).

B. claviceps is a specific eel tapeworm (Nie & Kennedy, 1992b) and infection is reported across Europe, including the UK, and also across North America, Africa and Japan (Chubb *et al.*, 1987; Nie & Kennedy, 1992b; Scholz, 1997b; Borgsteede, Haenen, De Bree, *et al.*, 1999; Norton *et al.*, 2003; Scholz *et al.*, 2004; Kuchta *et al.*, 2012). *B. claviceps* requires the copepod, *Cyclops vicinus*, as the obligatory intermediate host in order to complete its life cycle (Nie & Kennedy, 1993). In the UK eel, *B. claviceps* reaches a maximum size and becomes gravid mainly during the summer months (Nie & Kennedy, 1992) Some fish, such as perch (*Perca fluviatilis)* and guppies (*Poecilia reticulate)* have been described as acting as paratenic hosts of this tapeworm (Nie & Kennedy, 1993; Scholz, 1997a).

4.1.2.3.1 Morphology

B. claviceps is a large tapeworm that may measure up to 54 cm long, and the proglottides are wider than they are long. The scolex is elongated and is longer than it is wide and it tapers posteriorly (Chubb *et al.*, 1987). Moreover, the scolex consists of a prominent terminal disc that is wider than the bothrial region and also, two long, wide and shallow dorsoventral bothria (Figure 4.10). A neck is absent and the testes are spherical to oval in shape and

medullary positioned, whereas the vitelline follicles are numerous and oval or spherical, smaller and arranged in two lateral bands (Scholz, 1997b).

Figure 4.10 Scanning electron micrograph of *B. claviceps* showing the long scolex and the bi-lobed apical disc with an indentation above each bothrium (arrowed) (Chubb *et al.*, 1987).

4.1.2.4 *Proteocephalus macrocephalus* (Creplin, 1825)

Tapeworms of the order Proteocephalidea are cosmopolitan parasites of freshwater fish, amphibians and reptiles (Rego *et al.*, 1998) and the highest number of species belongs to the genus *Proteocephalus* (Kodedová *et al.*, 2000). The morphology of the scolex is one of the most important characteristics used for the classification of Proteocephalidean cestodes at generic and family levels since some features such as the shape and size of the scolex, together with shape and size of the apical sucker, are stable and species specific (Scholz *et al.*, 1998).

4.1.2.4.1 Morphology

P. macrocephalus is a specific tapeworm of the European eel in the UK and many other European countries (Nie & Kennedy, 1991b; Scholz *et al.*, 1997; Borgsteede, Haenen, Bree, *et al.*, 1999). *P. macrocephalus* is a large tapeworm with a spherical to globular short scolex that lacks a central glandular area. The scolex is separated from the strobila by a distinct neck and the suckers are relatively large (Figure 4.11). The shape, size and tegument ultrastructure

of adult *P. macrocephalus* is variable in length to width ratio but overall, the parasite is almost a regular oblong shape (Scholz *et al.*, 1997; Žďárská & Nebesářová, 1999).

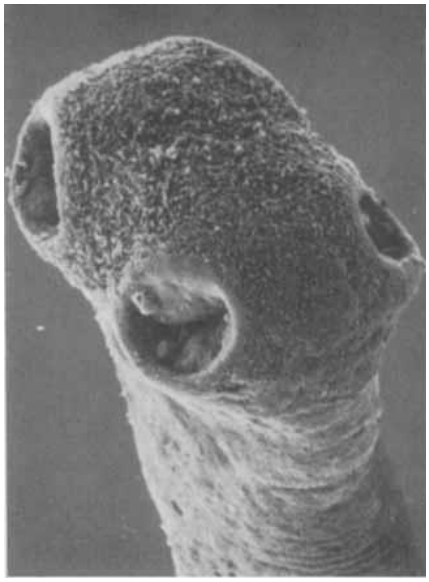

Figure 4.11 Scanning electron micrograph of *P. macrocephalus* scolex (Chubb *et al.*, 1987).

4.2 Objectives

The main objective of the work presented in this chapter was to screen the gastrointestinal tracts of the eel specimens sampled from rivers across England and Wales in order to provide some baseline infection data on acanthocephalans and cestodes. In doing so, species characterization was attempted based upon morphological descriptors of these parasites. To assist this species identification, a molecular-based approach was attempted based upon PCR amplification of a fragment of the 18S rRNA gene of some of the parasites.

4.3 Materials and Methods

4.3.1 Morphological Examination

4.3.1.1. Sampling

Eel samples were acquired according to Chapter 1 (1.3).

4.3.1.2 Processing

Eel specimens were thawed and necropsies performed using a dissection kit, allowing the gastrointestinal tract to be removed and opened longitudinally. The gastrointestinal tract was dissected in a Petri dish containing distilled water under x 100 and x 160 magnification using a Wild Heerbrugg, M3B (Switzerland) dissecting microscope (section 3.3.1.2). A microscopic examination of the gut contents for the presence of acanthocephalan and tapeworm parasites was performed. In addition, the external surface of the gut was examined for pathological lesions, or protruded and attached parasites.

4.3.2 Prevalence and intensity

Acanthocephalan and tapeworm parasite prevalence and intensity data were determined according to Chapter 2 (2.3.2).

4.3.3 Imaging

Images of the characteristic organs of representative acanthocephalan and tapeworm parasites were acquired according to Chapter 2 (2.3.3).

4.3.4 Molecular analysis of acanthocephalans

4.3.4.1 DNA extraction from acanthocephalan and cestode parasites

DNA was extracted from individual acanthocephalan, or cestode parasites, using the PureLink Genomic DNA Kit (Invitrogen by Life Technologies). Briefly, an individual acanthocephalan was placed in a petri dish and cut into small pieces using a sterile scalpel. The cut specimen was then placed in a 1.5 ml eppendorf tube and 180 µl PureLink Genomic

Digestion Buffer and 20 µl Proteinase K (20 mg / ml) was added and the DNA extracted according to Chapter 2 (2.3.4.1).

4.3.4.2 DNA Quality control

Recovery of acanthocephalan and cestode DNA was assessed using the NanoDrop Lite Instrument (ThermoFisher Scientific) as described earlier (2.3.3.2).

4.3.5 Polymerase chain reaction

4.3.5.1 PCR primer design for acanthocephalan analysis

The GenBank database was inspected for acanthocephalan DNA sequence deposits and the 18S rRNA genes from a variety of species were extracted and aligned using ClustalW (Larkin *et al.*, 2007). PCR primers were designed to highly conserved regions of the acanthocephalan 18S rRNA gene with the assistance of Primer 3 (Untergasser *et al.*, 2012). The resulting oligonucleotide sequences were as follows: AcanthoF, 5'-ACTGCGAAAGCATTTGCCAA-3' and AcanthoR, 5'-CGCGYTTACTAGGAATTCCT-3'. Oligonucleotides were synthesized by Eurofins MWG Operon and re-suspended in PCR-grade H_2O to a stock concentration of 10 pM and stored at -20^{o}C until required.

4.3.5.2 PCR primer design for cestode analysis

The 18S rRNA gene sequences of *B. claviceps and P. macrocephalus* were extracted from GenBank and aligned using ClustalW (Larkin *et al.*, 2007). PCR primers were designed to amplify a 741 bp, and 786 bp, fragment respectively of the *B. claviceps* and *P. macrocephalus* 18S rRNA genes using Primer 3 (Untergasser *et al.*, 2012). The oligonucleotide sequences were as follows (5'-3'): BothrioF, CATGAGCGAAAGTCAGAGGC and BothrioR, AAAGGGCAGGGACGTAATCA; ProteoF,CATGAGCGAAAGTCAGAGGC and ProteoR, AAAGGGCAGGGACGTAATCA.

The oligonucleotides were synthesized by Eurofins MWG Operon and re-suspended in PCR-grade H_2O to a stock concentration of 10 pM and stored at -20^{o}C until required.

4.3.6 PCR profiles for acanthocephalan and cestode analyses

PCR was carried out using either purified acanthocephalan, or cestode genomic DNA, with the appropriate primers; reagents were added according to Chapter 2 (2.3.5.1). For acanthocephalan analysis, the PCR cycling profile consisted of an initial denaturation step at 94^{o}C for 5 minutes, followed by denaturation of 94^{o}C for 30 seconds, annealing at 65^{o}C for 30s and extension at 72^{o}C for 30s. This cycle profile was repeated a total of 36 times, followed by a final extension of 10 minutes at 72^{o}C. For cestode analysis, the PCR was as above except that the primer primer annealing temperature was increased to 68^{o}C. All PCRs were performed on a MultiGene thermocycler (Labnet International. Inc).

4.3.7 Agarose gel electrophoresis

PCR products were analysed by agarose gel electrophoresis as described in Chapter 2 (2.3.6).

4.3.8 PCR product purification

PCR products were purified from the agarose gel using the Isolate II PCR and Gel Purification Kit (BioLine) as described in Chapter 2 (2.3.7).

4.3.9 DNA sequencing

Recovery of purified PCR products was assessed using the NanoDrop Lite Instrument (ThermoFisher Scientific) (2.3.4.2). PCR products were then prepared for sequencing as described earlier (2.3.7). All data was analysed according to section 2.3.7.

4.3.10 Statistical analyses

The Fisher's exact test was used to compare differences in prevalence between geographic regions and also between host factors (length, weight and condition factor). The Mann-Whitney test was used to assess the significance of differences in intensity of infection

between the different geographic regions and also between host factors (length, weight and condition factor). All tests were conducted using Minitab 16 (licensed to the University of Salford).

4.4 Results

4.4.1 Acanthocephalans: primary infection data

In total, 42 of the 140 (30%) European eel examined contained acanthocephalan parasites within their gastrointestinal tracts. These acanthocephalan infected eel were sampled from 8 (57.1%) of the examined river systems surveyed across England and Wales (Table 4.1). Acanthocephalans were not recovered from eels sampled from the River Hether Burn in England and the Rivers Mawddach-eden, Clwyd-Meirchion, Dee-Eitha, Clwyd-Elwy and Cadoxton from Wales. At sites positive for acanthocephalans, the prevalence ranged from 10 to 100%.

Table 4.1 Primary infection data for acanthocephalan parasites in European eel sampled from 14 catchment sites across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence (%)	Total number of parasites	Mean parasitic abundance (± sd)	Mean infection intensity (± sd)
Crane: CN England	100	208	20.8±17.5	20.8±17.5
Crouch: C England	100	177	17.7±17.2	17.7±17.2
Petteril: RP England	30	4	0.4±0.7	1.3±0.6
Leven: RL England	20	4	0.4±0.9	2.0±1.4
Gowy: RG England	20	4	0.4±0.9	3.0±2.8
Bela: B England	10	4	0.4±1.3	4.0±0.0
Hether Burn: HB England	0	0	0	0
Mawddach-eden: M Wales	0	0	0	0
Clwyd-Meirchion: MC Wales	0	0	0	0
Dee-Eitha: D Wales	0	0	0	0
Clwyd-Elwy: CE Wales	0	0	0	0
Rhymney: R Wales	90	50	5.0±4.3	5.5±4.1
Taff: TB Wales	50	18	1.8±2.9	3.6±3.4
Cadoxton: CD Wales	0	0	0	0
	30±39.0	469	3.4±10.7	10.9±17.1

4.4.1.2 Primary infection data: the environment

Overall, 469 acanthocephalans were collected from the eel specimens (mean abundance = 3.4±10.7; mean intensity = 11.2±17.2) and the parasites were over-dispersed (dispersion index = 34) (Figure 4.12). The numbers of acanthocephalans extracted from individual eel ranged between 1 and 81; the latter being isolated from a fish sampled from the river Crouch (specimen C131). A single acanthocephalan was extracted from 26.2% (11/42) of the infected animals. Moreover, the majority 69% (29/42) of the infected hosts carried less than 10 acanthocephalans and hence only 21% (13/42) of the infected eels harboured 10 or greater of these parasites (Tables 4.2 and 4.3). With respect to the sampling sites, the River Crane yielded most acanthocephalans (n = 208; mean abundance and intensity = 20.8±17.5) and the lowest number of parasites was recovered from the Rivers Petteril, Leven, Gowy and Bela (n = 4; mean abundance, 0.4±0.9 and intensity = 3.0±2.8).

Table 4.2 Eel morphometric and associated acanthocephalan infection data for positive sampling sites in Wales. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of acanthocephalan
Rhymney: R Wales	R1	32	48.9	0.15	1
	R2	31	48.6	0.16	10
	R3	30	42.3	0.16	3
	R4	31.5	67.2	0.22	9
	R5	27	29.3	0.15	2
	R6	30	39.7	0.15	0
	R7	29	53.5	0.22	1
	R8	30	43.9	0.16	7
	R9	23.5	12.7	0.10	12
	R10	23.5	18.5	0.14	5
Taff: TB Wales	TB1	20	10.2	0.13	9
	TB2	25	33.5	0.21	1
	TB3	28	33	0.15	0
	TB4	22	15.6	0.15	0
	TB5	21.5	15.5	0.16	0
	TB6	24	24.4	o.18	1
	TB7	25	21.6	0.14	5
	TB8	28	35.2	0.16	0
	TB9	19	10.6	0.15	2
	TB10	25	24.7	0.16	0

Table 4.3 Eel morphometric and associated acanthocephalan infection data for positive sampling sites in England. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of acanthocephalan
Crane: CN England	CN1	70	682.3	0.21	56
	CN2	74	702.5	0.17	24
	CN3	86	1380.3	0.22	19
	CN4	77	1058.8	0.23	39
	CN5	70	668.6	0.19	6
	CN6	74	839.7	0.21	20
	CN7	86	805.7	0.13	30
	CN8	77	661.9	0.14	1
	CN9	69	563.5	0.17	1
	CN10	74	743.4	0.18	12
Crouch: C England	C157	33	65.8	0.18	15
	C107	32	47	0.14	2
	C117	28	27.5	0.13	2
	C59	32	50.1	0.15	5
	C74	50	209.8	0.17	53
	C116	29	36.4	0.15	1
	C71	46	198.2	0.19	12
	C131	39	112	0.19	81
	C40	32	43.9	0.13	2
	C48	31	44.7	0.15	4
Petteril: RP England	RP1	36	110.7	0.24	0
	RP2	31.5	82.8	0.28	0
	RP3	18.5	8.2	0.13	0
	RP4	28	35.3	0.16	0
	RP5	35.5	74.9	0.17	0
	RP6	27.5	38.2	0.18	2
	RP7	14.5	5.2	0.17	0
	RP8	43	101.1	0.07	1
	RP9	33	98.3	0.27	0
	RP10	26	29.7	0.17	1
Leven: RL England	RL1	21	13.9	0.16	0
	RL2	52	18.7	0.13	0
	RL3	27	35.8	0.18	1
	RL4	22.5	21.3	0.19	0
	RL5	34	55.5	0.14	3
	RL6	35	71.3	0.17	0
	RL7	27	26.3	0.13	0
	RL8	29.5	29.5	0.11	0
	RL9	30	38.1	0.14	0
	RL10	26	22.3	0.13	0
Gowy: RG England	RG1	39	73.6	0.12	0
	RG2	37	61.7	0.12	0
	RG3	36	63.2	0.14	0
	RG4	36	58.9	0.13	0
	RG5	40	65.7	0.10	3
	RG6	32	41.8	0.13	0
	RG7	35	70.5	0.16	0
	RG8	33	62.2	0.17	0
	RG9	26	27.1	0.15	0
	RG10	27	24.4	0.12	1
Bela: B England	B1	30	42	0.16	0
	B2	32	49.1	0.15	0
	B3	27	33	0.17	0
	B4	19.5	11.9	0.16	0
	B5	21	13.2	0.14	0
	B6	23	13.3	0.11	0
	B7	24	22.2	0.16	0
	B8	48	24.5	0.18	4
	B9	24	18.3	0.13	0
	B10	49	209.0	0.18	0

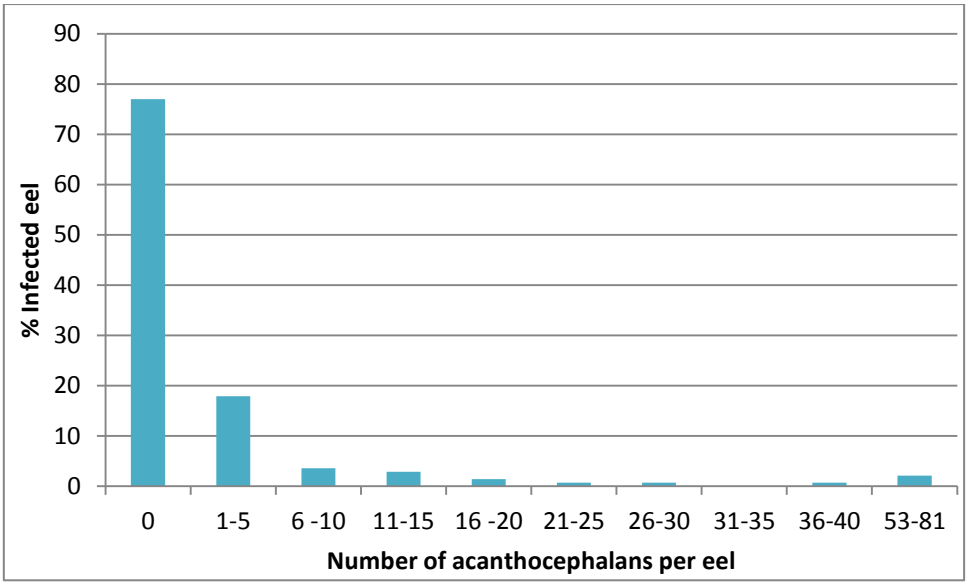

Figure 4.12 Summary of the dispersion of acanthocephalans within the eel study population.

Upon analysis of the infection data at a regional level it was evident that all examined rivers in South East England contained eel infected with acanthocephalan parasites (Table 4.4). Indeed, eels sampled from South East England had the greatest prevalence, and also the greatest mean abundance, and intensity of acanthocephalan infection. In contrast, all sites examined in North Wales showed absence of acanthocephalan infection. The majority of rivers in North West England and South Wales contained eel with acanthocephalan infections; only specimens from the Hether Burn and Cadoxton were lacking acanthocephalans in these respective regions. Overall, the regional acanthocephalan prevalence data was significantly different (p: 10^{-4}-0.0043) between the four geographic regions.

With respect to the abundance data, there were highly significant differences between South East England and the other examined regions (p: 0.001 – 10^{-4}). There was also a highly significant difference between abundance of acanthocephalans in rivers from North West England and South Wales ($p = 10^{-4}$). The intensity of acanthocephalan infection was also

significantly different between eel specimens examined from rivers in South East England compared to those from North West England (p = 0.0095) and South Wales (p = 0.0428).

Table 4.4 Regional infection data for acanthocephalans isolated from European eel sampled across England and Wales.

Regions	Number of river sites examined	Number of acanthocephalan positive sites	Number of examined eels	Number of infected eels	Prevalence (%)	Number of acanthocephalans	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	4	50	8	16	16	0.3±0.9	2±1.2	1 – 4
South East England	2	2	20	20	100	384	19.2±22.4	19.2±22.4	1 – 81
North Wales	4	0	40	0	0	0	0	0	0
South Wales	3	2	30	14	46.7	68	2.3±3.6	4.9±3.9	1- 12
Total	14	8	140	42	30.7	470	3.4±10.7	10.9±17.1	1-81

4.4.1.3 Primary infection data: the host

For the acanthocephalan infected eels, the body length ranged from 19 to 86 cm (mean = 41.7 ±20.5 cm) and body weight ranged from 10.2 to 1380 g (mean = 236.9±346.7 g). For uninfected eels, the body length ranged from 10 to 52 cm (mean = 25.9±8.0 cm) and body weight ranged from 1.2g to 209g (mean = 34.3±36.9 g). These differences in body length and weight between the infected and uninfected eels were highly significant ($p = 10^{-4}$). The mean condition factor for acanthocephalan infected eels was 0.16±0.04, whilst the mean condition factor for the uninfected eels was 0.14±0.04 (Tables 4.2 and 4.3); this was also highly significant (p = 0.0002).

Upon analysis of different categories of eel length it was apparent that prevalence, as well as mean acanthocephalan abundance and intensity, generally increased as the eel body length category increased (Table 4.5). Indeed, the prevalence of acanthocephalan infection was significantly different (p: 0.0420 – 0.0005) between the largest length category of eel and the two smallest length categories. There were no other significant differences in prevalence of infection between remaining length categories (p: 0.05778 - 0.7195). With respect to intensity

of acanthocephalan infection, there was a significant difference (p=0.0031), between the largest length category of eel and the category that included the eel of median size (25.5-30 cm). There were no other significant differences (p: 0.08 – 1.0) in the intensity of infection between the remaining length categories.

Table 4.5 Acanthocephalan infection data with respect to eel body length category.

Body length range (cm)	Eels examined	Infected eels	Prevalence (%)	Number of acanthocephalans	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	2	7.7	11	0.4±1.8	5.5±4.9	2-9
20.5-25	32	5	15.6	24	0.75±2.4	4.8±4.5	1-12
25.5-30	32	10	31.3	21	0.7±1.4	2.1±1.9	1-7
30.5-35	22	9	40.9	51	2.3±4.1	5.7±4.7	1-15
35.5-86	28	16	57.1	362	12.9±21.0	22.6±23.7	1-81

With respect to eel condition factor, there was significant difference (p= 0.0015-0.0201) in the prevalence of acanthocephalan infection between the largest category of condition factor and the two smallest categories of condition factor (Table 4.6). No other significant differences in acanthocephalan prevalence occurred between the remaining eel condition factor categories (p: 0.1323 - 0.3849). There was also no significant difference in acanthocephalan intensity between the different categories of eel condition factor (p: 0.17 – 0.75).

Table 4.6 Acanthocephalan infection data with respect to eel condition factor category.

Condition factor range	Eels examined	Infected eels	Prevalence (%)	Number of acanthocephalans	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.04-0.12	34	4	11.8	17	0.5±2.1	4.3±5.3	1-12
0.13-0.14	38	9	23.7	59	1.6±5.1	6.6±9.1	1-30
0.15-0.16	28	9	32.1	35	1.3±2.5	3.9±3.0	1-10
0.17-0.29	40	20	50	358	8.9±18.1	17.9±22.5	1-81

4.4.2 *Pomphorhynchus laevis* (Muller, 1776)

4.4.2.1 Morphological examination

P. laevis parasites were identified based upon key morphological features, not least of which were the presence of a characteristic proboscis bulb that was localised to the base of the proboscis and a long neck (Figure 4.13).

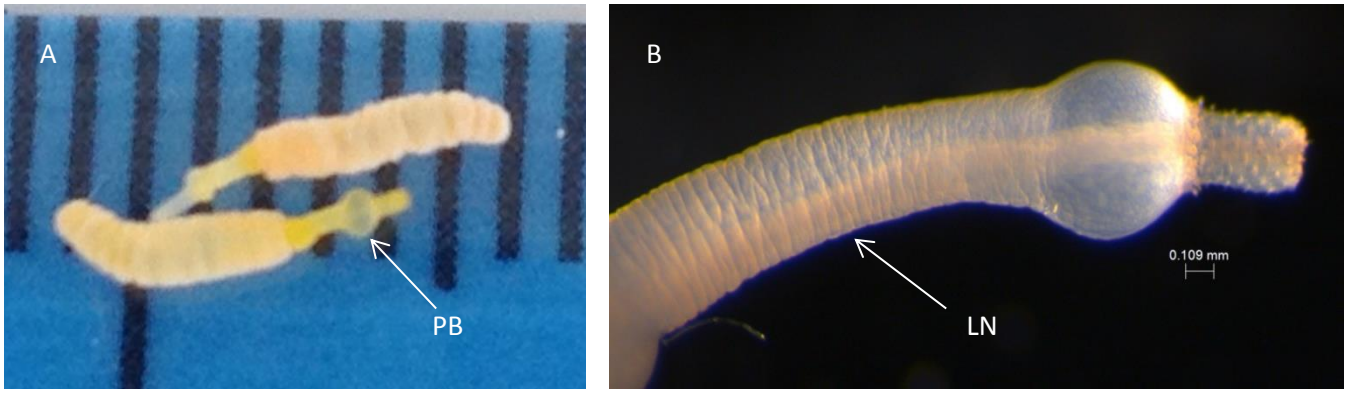

Figure 4.13 Representative images of *P. laevis* showing the presence of the characteristic proboscis bulb (PB) and the long neck (LN) (specimen number R3/2009/03) extracted from an eel (R3) sampled from the River Rhymney.

4.4.2.2 Molecular characterisation

Genomic DNA was extracted and purified from a representative acanthocephalan worm (specimen number C71/2009/02; extracted from eel C71) identified as *P. laevis* based upon morphology. The 18S rRNA gene was PCR amplified and the resulting 652 bp product was subjected to DNA sequencing. BlastN analysis of the resulting sequence data showed that it was identical to the 18S rRNA gene sequences of two *P. laevis* sequence deposits in GenBank and hence confirms the above morphological identification of this parasite (Table 4.7 and Figure 4.14). Nonetheless, the sequence was also identical to a fragment of the 18S rRNA gene of *P. tereticollis* and hence it was not possible to confirm with absolute certainty the identity of the parasite.

Table 4.7 BlastN analysis of the 18S RNA gene fragment derived from an acanthocephalan isolated from eel specimen C71 (specimen number C71/2009/02).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pomphorhynchus laevis haplotype Hum10 18S ribosomal RNA gene, partial sequence	652	652	100%	0.0	100%	KF559309.1
Pomphorhynchus laevis 18S ribosomal RNA gene, partial sequence	652	652	100%	0.0	100%	AY423346.1
Pomphorhynchus tereticollis 18S ribosomal RNA gene, partial sequence	652	652	100%	0.0	100%	AY423347.1

```
gi|540074015|gb|KF559309.1|      AGTTAGAGGATCGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAAC 950
413754701_Acan1_For_C09         ----------------------------------------CTAACTGTAAAC 12
                                                                         ************

gi|540074015|gb|KF559309.1|      TATGCCGACTGGGGATTCGCCAGTGCAATTTAGCTTGGCGAGCACCCTCC 1000
413754701_Acan1_For_C09         TATGCCGACTGGGGATTCGCCAGTGCAATTTAGCTTGGCGAGCACCCTCC 62
                                ************************************************

gi|540074015|gb|KF559309.1|      GGGAAACCAAAGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAA 1050
413754701_Acan1_For_C09         GGGAAACCAAAGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAA 112
                                ************************************************

gi|540074015|gb|KF559309.1|      ACTTAAAGGAATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTTA 1100
413754701_Acan1_For_C09         ACTTAAAGGAATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTTA 162
                                ************************************************

gi|540074015|gb|KF559309.1|      ATTTGACTCAACGCGCGAAAGCTTACCCGGTCCGAACACCGTGAGGATTG 1150
413754701_Acan1_For_C09         ATTTGACTCAACGCGCGAAAGCTTACCCGGTCCGAACACCGTGAGGATTG 212
                                ************************************************

gi|540074015|gb|KF559309.1|      ACAGGTTGAAAGCTCTTTCTTGATCCGGTGGGTAGCGGTGCATGGCCGTT 1200
413754701_Acan1_For_C09         ACAGGTTGAAAGCTCTTTCTTGATCCGGTGGGTAGCGGTGCATGGCCGTT 262
                                ************************************************

gi|540074015|gb|KF559309.1|      CGTAGTTGGTGAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACT 1250
413754701_Acan1_For_C09         CGTAGTTGGTGAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACT 312
                                ************************************************

gi|540074015|gb|KF559309.1|      CTAGCCTACTAATTAGCGTAGCGATTGTTCGTCGTTACAATGCTTCTTAG 1300
413754701_Acan1_For_C09         CTAGCCTACTAATTAGCGTAGCGATTGTTCGTCGTTACAATGCTTCTT-- 360
                                *********************************************

gi|540074015|gb|KF559309.1|      AGGGACAGGTGTTGCTTAAGCACACGAAGTAGAGCAATAACAGGTCTGTG 1350
413754701_Acan1_For_C09         ------------------------------------------------
```

Figure 4.14 The 18S rRNA DNA sequence (413754701_Acan1_For_C09) from the acanthocephalan (specimen number C71/2009/02; extracted from eel C71) aligned with the *P. laevis* 18S rRNA sequence deposited in GenBank (gi: 540074015).

4.4.2.3 Primary infection data for *P. laevis*

Examination of eel gastrointestinal tracts confirmed the presence of *P. laevis* in 23 of the 140 European eel specimens (16.4%). The positive infections were observed in eels sampled from the following four English and two Welsh rivers (prevalence in parenthesis): Crouch (50%), Petteril (20%), Leven (10%), Bela (10%), Rhymney (90%) and Taff (50%) (Table 4.8).

Table 4.8 Primary infection data for *P. laevis* parasites in European eels sampled from the six positive catchment sites across England and Wales. Ten eels were examined at each site.

Catchment site	Prevalence (%)	Total number of *P. laevis*	Mean abundance (±sd)	Mean intensity (±sd)
Taff, TB: Wales	50	18	1.8±2.9	3.6±3.4
Rhymney: Wales	90	50	5±4.3	5.5±4.1
Bela: England	10	4	0.4±1.3	4
Petteril: England	20	3	0.3±0.7	1.5±0.7
Leven: England	10	1	0.1±0.3	1
Crouch: England	50	139	13.9±26.7	27.8±33.4
	38.3% (23/60)	215	1.5±7.8	9.3±17.7

4.4.2.3.1 Primary infection data: the environment

At these six positive catchment sites, the total number of *P. laevis* collected was 215 parasites (mean intensity of infection = 9.3±17.7 and mean abundance = 1.5±7.8) (Table 4.8). The number of *P. laevis* isolated from individual hosts ranged from 1 (eel specimens RP8, RL5, R1, R8, TB2 and TB6) to 81 (eel specimen C131) (Table 4.9). The majority (18/23, 78%) of the infected eels harboured less than 10 *P. laevis*; indeed, 26% (6/23) of the hosts were infected with just a single parasite (Table 4.9). Overall, the parasites were over-dispersed (dispersion index = 41) (Figure 4.15).

Table 4.9 Eel morphometric and associated *P. laevis* infection data for the six positive catchment sites in England and Wales. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of *P. laevis*
Crouch: C England	C157	33	65.8	0.18	2
	C74	50	209.8	0.17	40
	C71	46	198.2	0.19	12
	C131	39	112	0.19	81
	C48	31	44.7	0.15	4
Petteril: RP England	RP6	27.5	38.2	0.18	2
	RP8	43	101.1	0.07	1
Leven: RL England	RL5	34	55.5	0.14	1
Bela: B England	B8	48	24.5	0.18	4
Rhymney: R Wales	R1	32	48.9	0.15	1
	R2	31	48.6	0.16	10
	R3	30	42.3	0.16	3
	R4	31.5	67.2	0.22	9
	R5	27	29.3	0.15	2
	R7	29	53.5	0.22	1
	R8	30	43.9	0.16	7
	R9	23.5	12.7	0.10	12
	R10	23.5	18.5	0.14	5
Taff: TB Wales	TB1	20	10.2	0.13	9
	TB2	25	33.5	0.21	1
	TB6	24	24.4	o.18	1
	TB7	25	21.6	0.14	5
	TB9	19	10.6	0.15	2

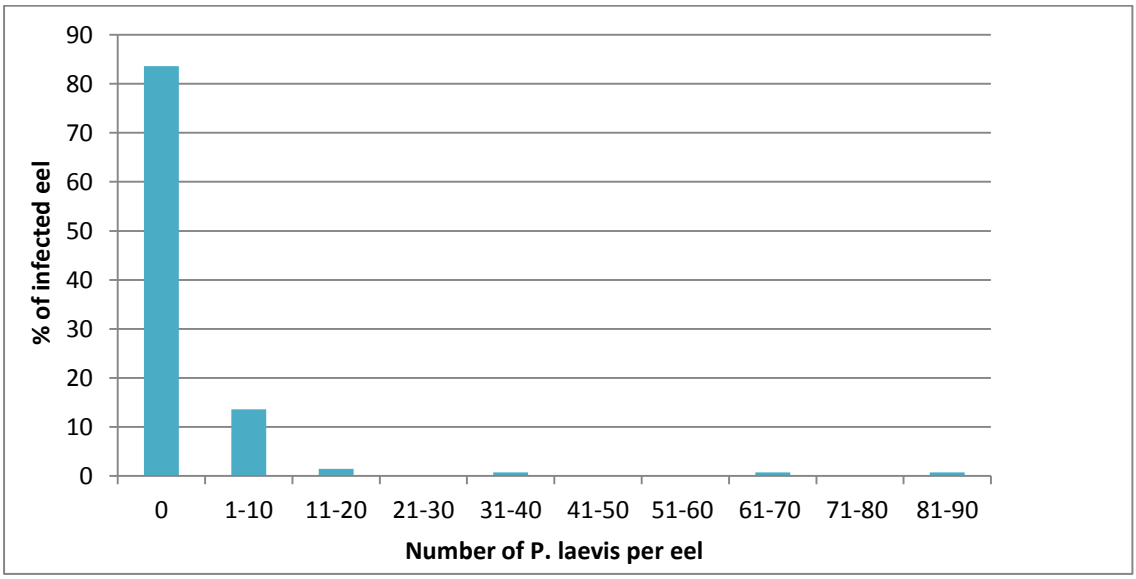

Figure 4.15 Summary of the dispersion of *P. laevis* within the sampled eel population.

P. laevis was isolated as the only acanthocephalan species present in European eel at 3 catchment sites: the River Bela in England and the Rivers Taff and Rhymney in Wales. At the other three catchment sites (Petteril, Leven and Crouch), *P. laevis* was isolated as a mixed infection with other non-bulb acanthocephalans.

At the regional level, eel specimens from rivers in North Wales were found to be free of *P. laevis* and this contrasted sharply with data from South Wales as eel from this region had the greatest prevalence of *P. laevis*. Indeed, there was a significant difference in the *P. laevis* prevalence data between South Wales and the other examined regions with the exception of South East England (p: 0.001 - 10^{-4}).

With respect to abundance data, there were significant differences between rivers in North West England and both South East England ($p = 0.039$) and South Wales ($p = 10^{-4}$). There were no significant differences in intensity of infection in eels sampled from the different regions that were positive for *P. laevis* (p: 0.063 – 0.212).

Table 4.10 Regional infection data for *P. laevis* isolated from European eel sampled across England and Wales.

Regions	Number of examined river sites	Number of *P. laevis* positive sites	Number of examined eel	Number of infected eel	Prevalence (%)	Number of *P. laevis*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	3	50	4	8	8	0.2±0.7	2±1.4	1-4
South East England	2	1	20	5	25	139	6.9±19.7	27.9±33.4	2-81
North Wales	4	0	40	0	0	0	0	0	0
South Wales	3	2	30	14	46.7	68	2.3±3.6	4.9±3.9	1-12
Total	14	6	140	23	16.4	215	1.5±7.8	9.3±17.7	1-81

4.4.2.3.2 Primary infection data: the host

For the *P. laevis* infected eels, host body length ranged from 19 to 50 cm (mean = 31.4 ±8.7 cm) and body weight ranged from 10.2 to 204.5 g (mean = 50.8±51.8 g). For the uninfected eels, the body length ranged from 10 to 86 cm (mean = 30.9±15.9 cm) and body weight ranged from 1.2g to 1380 g (mean = 103.8±230.4 g). These differences between the infected and uninfected hosts were not statistically significant (p = 0.60 and 0.36 respectively). The mean condition factor for *P. laevis* infected eels was 0.16±0.04, whilst the mean condition factor for the uninfected eels was 0.15±0.04 (Table 4.8) and this was a statistically significant difference (p = 0.012).

Upon analysis of the different categories of eel length it was apparent that there were no significant differences in *P. laevis* prevalence (p: 0.12 – 1.0), mean abundance (p: 0.25 – 0.71) or mean intensity (p: 0.17 – 0.56) (Table 4.11).

Table 4.11 *P. laevis* infection data with respect to eel body length category.

Body length range (cm)	Eels examined	Infected eels	Prevalence (%)	Number of *P. laevis*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	2	7.7	11	0.4±1.8	5.5±4.9	2 – 9
20.5-25	32	5	15.6	24	0.8±2.4	4.8±4.5	1 - 12
25.5-30	32	5	15.6	15	0.5±1.4	3±2.3	1 - 7
30.5-35	22	6	27.3	27	1.2±2.8	4.5±4.0	1 - 10
35.5-86	28	5	17.9	138	4.9±16.8	27.6±33.6	1 - 81

With respect to eel condition factor, there was a significant difference (p = 0.0307) in the prevalence of *P. laevis* infection between the largest category of condition factor and the smallest category of condition factor (Table 4.12). No other significant differences in *P. laevis* prevalence occurred between the remaining eel condition factor categories (p: 0.14 -

1.0). There was a significant difference (p= 0.0274) in the abundance of *P. laevis* between the largest and smallest categories of condition factor; there were no other significant differences in abundance between the remaining categories of condition factor (p: 0.10 - 0.98). There were also no significant differences in *P. laevis* intensity between the different categories of eel condition factor (p: 0.88 – 1.0).

Table 4.12 *P. laevis* infection data with respect to eel condition factor category.

Condition factor range	Eels examined	Infected eels	Prevalence (%)	Number of *P. laevis*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.04-0.12	34	2	5.9	13	0.4±2.1	6.5±7.8	1 - 12
0.13-0.14	38	4	10.5	20	0.5±1.8	5±3.3	1 - 9
0.15-0.16	28	7	25	29	1.0±2.4	4.1±3.2	1 - 10
0.17-0.29	40	10	25	153	3.8±14.2	15.3±25.9	1 - 81

4.4.3 Non-bulb acanthocephalans

4.4.3.1 Morphological examination

The non-bulb acanthocephalans were characterised by the absence of a proboscis bulb as shown in the images below (Figures 4.16 and 4.17).

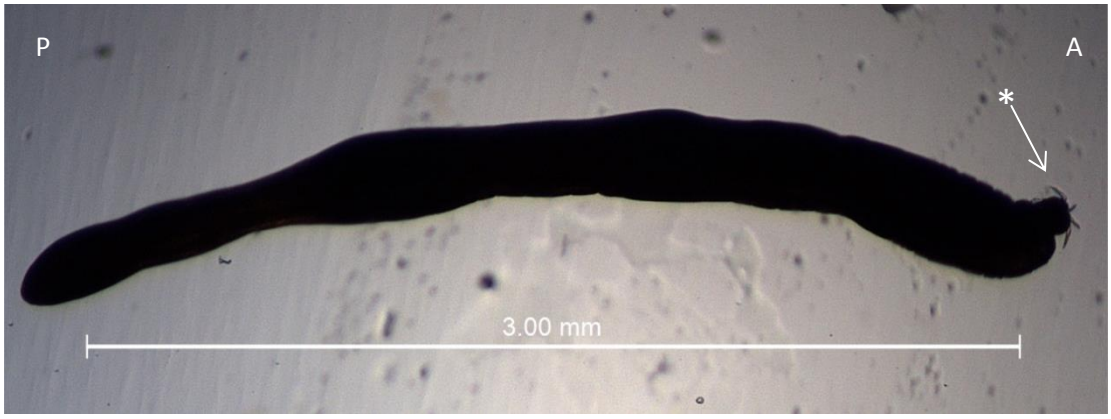

Figure 4.16 Representative image of an acanthocephalan (specimen number CN10/2012/05) without a proboscis bulb (extracted from eel CN10) that highlights the small proboscis (*) with few rows of hooks. A = anterior; P = posterior.

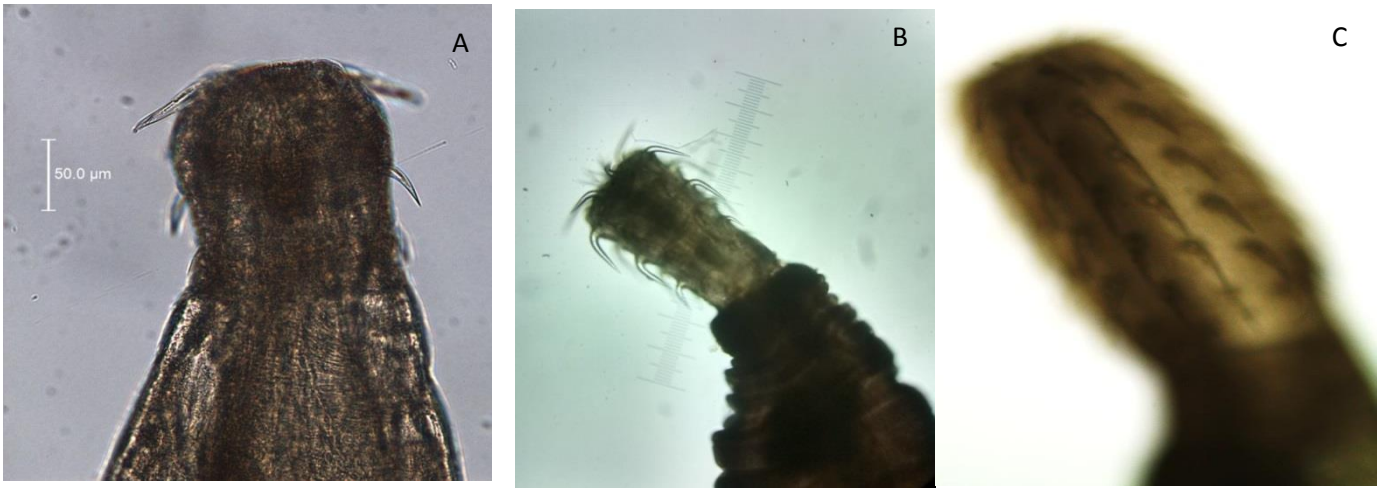

Figure 4.17 Representative images of the different types of proboscis associated with the non-bulb acanthocephalans observed in the samples. A: small proboscis with few rows of hooks (specimen number CN10/2012/05; extracted from eel CN10), B: long proboscis with three rows of prominent hooks (specimen number C16/2009/01; extracted from eel C116), C: long proboscis with multiple rows of discrete hooks (specimen number C17/2009/02; extracted from eel C117).

4.4.3.2 Primary infection data for acanthocephalans without a proboscis bulb

Examination of eel gastrointestinal tracts confirmed the presence of acanthocephalans lacking a proboscis bulb in 22 of the 140 (15.7%) European eel specimens. The positive infections were observed in eel sampled from five English rivers (prevalence in parenthesis): Crane (100%), Crouch (70%), Gowy (20%), Leven (20%) and Petteril (10%) (Table 4.13). There were no non-bulb acanthocephalans infecting eels sampled from Welsh rivers.

Table 4.13 Primary infection data for acanthocephalans without a proboscis bulb in European eels sampled from 7 catchment sites across England. Ten eels were examined from each site.

Eel catchment sites	Prevalence (%)	Number of non-bulb acanthocephalans	Mean parasitic abundance (± sd)	Mean infection intensity (± sd)
Crane: CN England	100	208	20.8±17.5	20.8±17.5
Crouch: C England	70	38	3.8±5.1	5.4±5.3
Petteril: RP England	10	1	0.1±0.3	1
Leven: RL England	20	3	0.3±0.9	3
Gowy: RG England	20	4	0.4±0.9	2.0±1.4
Bela: B England	0	0	0	0
Hether Burn: HB England	0	0	0	0
	15.7	254	1.8±7.1	11.6±14.7

4.4.3.2.1 Primary infection data: the environment

At the positive catchment sites, the total number of acanthocephalans without a proboscis bulb was 254 parasites (mean intensity of infection = 11.5±14.7 and mean abundance = 1.8±7.1) (Table 4.13). The number of acanthocephalans per eel ranged from 1 (specimens CN8, CN9, C116, RP10, RL3 and RG10) to 56 (specimen CN1) (Table 4.14). The majority (13/22, 59%) of the infected eels harboured less than 10 acanthocephalans; indeed, 27.3% (6/22) of the hosts harboured a single acanthocephalan (Table 4.14). Overall, the acanthocephalans lacking a proboscis bulb were over-dispersed (dispersion index = 28) (Figure 4.18).

Acanthocephalans without a proboscis bulb were isolated as the only acanthocephalan type present in European eel at 2 catchment sites: the Rivers Crane and Gowy. At the other three catchment sites (Petteril, Leven and Crouch), the non-bulb acanthocephalans were isolated as a mixed infection with *P. laevis*.

Table 4.14 Eel morphometric and associated non-bulb acanthocephalan infection data for sampling sites across England. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Number of acanthocephalans
Crane: CN England	CN1	70	682.3	0.21	56
	CN2	74	702.5	0.17	24
	CN3	86	1380.3	0.22	19
	CN4	77	1058.8	0.23	39
	CN5	70	668.6	0.19	6
	CN6	74	839.7	0.21	20
	CN7	86	805.7	0.13	30
	CN8	77	661.9	0.14	1
	CN9	69	563.5	0.17	1
	CN10	74	743.4	0.18	12
Crouch: C England	C157	33	65.8	0.18	13
	C107	32	47	0.14	2
	C117	28	27.5	0.13	2
	C59	32	50.1	0.15	5
	C74	50	209.8	0.17	13
	C116	29	36.4	0.15	1
	C40	32	43.9	0.13	2
Petteril: RP England	RP10	26	29.7	0.17	1
Leven: RL England	RL3	27	35.8	0.18	1
	RL5	34	55.5	0.14	2
Gowy: RG England	RG5	40	65.7	0.10	3
	RG10	27	24.4	0.07	1

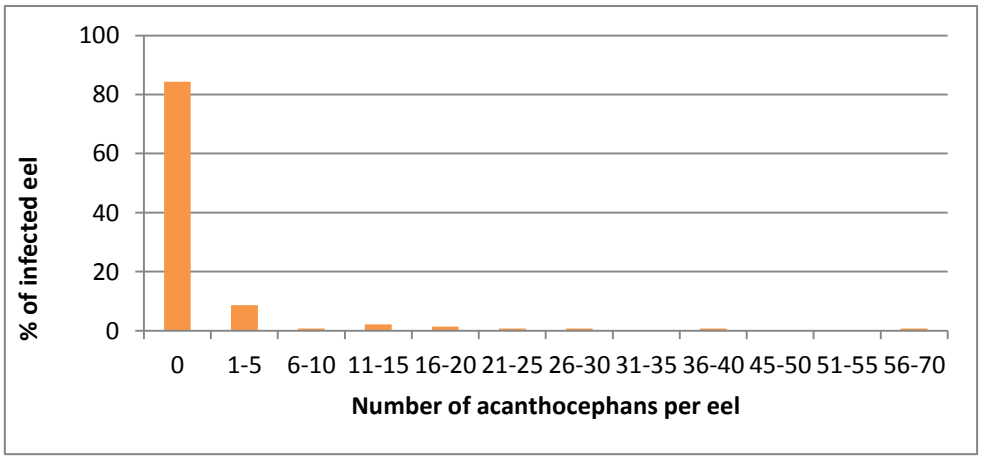

Figure 4.18 Summary of the dispersion of non-bulb acanthocephalans within the sampled eel population.

As stated above, at the regional level, there were no non-bulb acanthocephalan infections detected in eels examined from Welsh rivers. In contrast, both rivers sampled from South East England and the majority from North West England harboured eels with non-bulb acanthocephalan infections (Table 4.15). Indeed, there was a significant difference in non-bulb acanthocephalan prevalence data between eels sampled from South East England and North West England ($p = 10^{-4}$). The mean abundance and mean intensity data for non-bulb acanthocephalan infections was also significantly different between eels sampled from South East England and North West England ($p = 10^{-4}$ and $p = 0.032$ respectively).

Table 4.15 Regional infection data for non-bulb acanthocephalans isolated from European eel sampled across England and Wales.

Regions	Number of examined river sites	Number of non-bulb acanthocephalan positive sites	Number of examined eel	Number of infected eel	Prevalence (%)	Number of non-bulb acanthocephalan	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	3	50	5	10	8	0.2±0.5	1.6±0.9	1 - 3
South East England	2	2	20	17	85	246	12.3±15.3	14.5±15.6	1 - 56
North Wales	4	0	40	0	0	0	0	0	0
South Wales	3	0	30	0	0	0	0	0	0
Total	14	5	140	22	15.7	254	1.8±7.1	11.5±14.7	1 - 56

4.4.3.2.2 Primary infection data: the host

For the non-bulb acanthocephalan infected eels, host body length ranged from 26 to 86 cm (mean = 52.1 ±22.9 cm) and body weight ranged from 22.4 to 1380 g (mean = 399.9±416.5 g). For the uninfected eels, the body length ranged from 10 to 52 cm (mean = 26.7±8.2 cm) and body weight ranged from 1.2g to 208 g (mean = 38.2±41.5 g). These differences in length and weight between the infected and uninfected eels were highly significant ($p = 10^{-4}$). The mean condition factor for infected eels was 0.16±0.03, whilst the mean condition factor

for the uninfected eel was 0.15±0.04 (Table 4.16); this difference was also statistically significant (p = 0.015).

Upon analysis of different categories of eel length it was apparent that non-bulb acanthocephalan infections were not found in eels less than 25.5 cm body length (Table 4.16). It was also apparent that non-bulb acanthocephalan prevalence, as well as mean intensity, generally increased as the eel body length category increased.

With respect to prevalence of non-bulb acanthocephalans, there were significant differences in the data between the largest length category and three smallest length categories (*p*: 0.024 – 10^{-4}). The intensity and abundance data were also significantly different between the largest length category of eel and the smallest infected category of eel (25.5- 30 cm) (*p*= 0.0113 and *p* = 0.0066 respectively).

Table 4.16 Non-bulb acanthocephalan infection data with respect to eel body length category

Body length range (cm)	Eels examined	Infected eels	Prevalence (%)	Number of Non-bulb acanthocephalans	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	0	0	0	0	0	0
20.5-25	32	0	0	0	0	0	0
25.5-30	32	5	15.6	6	1.9±0.5	1.2±0.4	1 – 2
30.5-35	22	5	22.7	24	1.1±2.9	4.8±4.8	1 - 13
35.5-86	28	12	42.9	224	8±14.2	18.7±16.7	1 - 56

Upon analysis of the different categories of eel condition factor it was apparent that the greatest non-bulb acanthocephalan prevalence, as well as mean abundance and intensity, were found in the largest category of eel condition factor (Table 4.17). In general, non-bulb acanthocephalan infection prevalence, mean abundance and mean intensity increased as the eel condition factor category increased.

With respect to non-bulb acanthocephalan prevalence, there were significant differences in the data between the largest category of condition factor and both the smallest (p = 0.015) and second largest (k= 0.15-0.16) (p = 0.0317) categories of condition factor.

There was also a significant difference in abundance of the non-bulb acanthocephalans between the largest category of condition factor and both the smallest (p= 0.0150) and second largest (p= 0.0374) categories of condition factor.

No significant differences in non-bulb acanthocephalan intensity data were observed between the different categories of eel condition factor (p: 0.20 – 1.0).

Table 4.17 Non-bulb acanthocephalan infection data with respect to eel condition factor category.

Condition factor range	Eels examined	Infected eels	Prevalence (%)	Number of Non-bulb acanthocephalan	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.04-0.12	34	2	5.9	4	0.1±0.5	2±1.4	1 - 3
0.13-0.14	38	6	15.8	39	1.0±4.9	6.5±11.5	1 - 30
0.15-0.16	28	2	7.1	6	0.2±0.9	3±2.8	1 - 5
0.17-0.29	40	12	30	205	5.1±11.8	17.1±16.5	1 - 56

4.4.3.3 Molecular characterisation of the non-bulb acanthocephalans

Genomic DNA was extracted and purified from 4 non-bulb acanthocephalan worms; all isolated from eel specimen CN1and including the different morphological types shown in Figure 4.6. The acanthocephalan 18S rRNA gene was PCR amplified and the resulting 652 bp products were subjected to DNA sequencing. BlastN analysis of the data showed that one sequence was identical to the 18S rRNA gene of *A. lucii* (Table 4.18), as confirmed by sequence alignment (Figure 4.19).

Table 4.18 BlastN analysis of the 18S RNA gene fragment derived from an acanthocephalan isolated from eel specimen (specimen number CN1/2012/06).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Acanthocephalus lucii 18S ribosomal RNA gene, complete sequence	706	706	100%	0.0	100%	AY830152.1
Acanthocephalus dirus 18S ribosomal RNA gene, complete sequence	695	695	100%	0.0	99%	AY830151.1
Acanthocephalus anguillae 18S ribosomal RNA gene, partial sequence	689	689	100%	0.0	99%	Af469413.1

```
414629901_Acantho6_For_C04      ---------------------------------------AGTTAGAGGAT 11
gi|61200890|gb|AY830152.1|      AAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGAT 950
                                                                      ***********

414629901_Acantho6_For_C04      CGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACTG 61
gi|61200890|gb|AY830152.1|      CGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACTG 1000
                                **************************************************

414629901_Acantho6_For_C04      GGGATTCGCCAGTGTCAACAAACTTGGCGAGCACCCTCCGGGAAACCAAA 111
gi|61200890|gb|AY830152.1|      GGGATTCGCCAGTGTCAACAAACTTGGCGAGCACCCTCCGGGAAACCAAA 1050
                                **************************************************

414629901_Acantho6_For_C04      GTGATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAA 161
gi|61200890|gb|AY830152.1|      GTGATTGGGTTCCGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGAA 1100
                                **************************************************

414629901_Acantho6_For_C04      TTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCAA 211
gi|61200890|gb|AY830152.1|      TTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCAA 1150
                                **************************************************

414629901_Acantho6_For_C04      CGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAAA 261
gi|61200890|gb|AY830152.1|      CGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAAA 1200
                                **************************************************

414629901_Acantho6_For_C04      GCTCTTTCTTGATCCGGTGGGTAGCGGTGCATGGCCGTTCGTAGTTGGTG 311
gi|61200890|gb|AY830152.1|      GCTCTTTCTTGATCCGGTGGGTAGCGGTGCATGGCCGTTCGTAGTTGGTG 1250
                                **************************************************

414629901_Acantho6_For_C04      AAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTGGCCTACTA 361
gi|61200890|gb|AY830152.1|      AAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTGGCCTACTA 1300
                                **************************************************

414629901_Acantho6_For_C04      ATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGCG 411
gi|61200890|gb|AY830152.1|      ATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGCG 1350
                                **************************************************

414629901_Acantho6_For_C04      CAATGAAAGGCGCACGAAGTA---------------------------- 432
gi|61200890|gb|AY830152.1|      CAATGAAAGGCGCACGAAGTAGAGCAATAACAGGTCTGTGATGCCCTTCG 1400
                                ********************
```

Figure 4.19 The sequence of the 18S rRNA gene fragment from one acanthocephalan (414629901_Acantho6_For_C04) isolated from eel sample CN1 (specimen number CN1/2012/06), aligned with the *A. lucii* (gi|61200890) 18S rRNA gene sequence.

The remaining 3 sequences were identical to each other; however, there was not an exact match to any sequence deposited in the GenBank database (Table 4.19). The two highest scoring matches (99% identity) were to the 18S rRNA gene sequences from *A. anguillae* (gi: 23307641) and *A. dirus* (gi: 61200887) (Figure 4.20).

Table 4.19 BlastN analysis of the 18S RNA gene fragment derived from an acanthocephalan isolated from eel specimen CN1 (specimen number CN1/2012/01).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Acanthocephalus anguillae 18S ribosomal RNA gene, partial sequence	1029	1029	98%	0.0	99%	AF469413.1
Acanthocephalus dirus 18S ribosomal RNA gene, complete sequence	1029	1029	98%	0.0	99%	AY830151.1
Acanthocephalus lucii 18S ribosomal RNA gene, complete sequence	1024	1024	98%	0.0	99%	AY830152.1

```
gi|23307641|gb|AF469413.1|      TCGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACT 412
gi|61200887|gb|AY830151.1|      TCGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACT 998
414629901_Acantho1_For_A03      ----------TTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACT 40
gi|61200890|gb|AY830152.1|      TCGAAGACGATTAGATACCGTCCTAGTTCTAACTGTAAACTATGCCGACT 999
                                ************************************

gi|23307641|gb|AF469413.1|      GGGGATTCGCCAGTGTCAACAAACTTGGCGAGCCCCCTCCGGGAAACCAA 462
gi|61200887|gb|AY830151.1|      GGGGATTCGCCAGTGTCAACAAACTTGGCGAGCACCCTCCGGGAAACCAA 1048
414629901_Acantho1_For_A03      GGGGATTCGCCAGTGTCAACAAACTTGGCGAGCACCCTCCGGGAAACCAA 90
gi|61200890|gb|AY830152.1|      GGGGATTCGCCAGTGTCAACAAACTTGGCGAGCACCCTCCGGGAAACCAA 1049
                                ********************************* ***************

gi|23307641|gb|AF469413.1|      AGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGA 512
gi|61200887|gb|AY830151.1|      AGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGA 1098
414629901_Acantho1_For_A03      AGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGA 140
gi|61200890|gb|AY830152.1|      AGTGATTGGGTTCCGGGGGGGAGTATGGTTGCAAAATCGAAACTTAAAGGA 1099
                                ************************************************

gi|23307641|gb|AF469413.1|      ATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCA 562
gi|61200887|gb|AY830151.1|      ATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCA 1148
414629901_Acantho1_For_A03      ATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCA 190
gi|61200890|gb|AY830152.1|      ATTGACGGAGGGGCACACCAGAAGTGGAGCCTGCGGCTCAATTTGACTCA 1149
                                ************************************************

gi|23307641|gb|AF469413.1|      ACGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAA 612
gi|61200887|gb|AY830151.1|      ACGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAA 1198
414629901_Acantho1_For_A03      ACGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAA 240
gi|61200890|gb|AY830152.1|      ACGCACGAAAGCTTACTCGGTCCGAACACCGTGAGGATTGACAGGTTGAA 1199
                                ************************************************

gi|23307641|gb|AF469413.1|      AGCTCTTTCTTGATCCGGTGGGTAGTGGTGCATGGCCGTTCGTAGTTGGT 662
gi|61200887|gb|AY830151.1|      AGCTCTTTCTTGATCCGGTGGGTAGTGGTGCATGGCCGTTCGTAGTTGGT 1248
414629901_Acantho1_For_A03      AGCTCTTTCTTGATCCGGTGGGTAGTGGTGCATGGCCGTTCGTAGTTGGT 290
gi|61200890|gb|AY830152.1|      AGCTCTTTCTTGATCCGGTGGGTAGCGGTGCATGGCCGTTCGTAGTTGGT 1249
                                ************************ ************************

gi|23307641|gb|AF469413.1|      GAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTAGCCTACT 712
gi|61200887|gb|AY830151.1|      GAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTAGCCTACT 1298
414629901_Acantho1_For_A03      GAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTAGCCTACT 340
gi|61200890|gb|AY830152.1|      GAAGTGATTTGTCTGGTTTATTCCGATAACGAACGAGACTCTGGCCTACT 1299
                                ****************************************** *******

gi|23307641|gb|AF469413.1|      AATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGC 762
gi|61200887|gb|AY830151.1|      AATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGC 1348
414629901_Acantho1_For_A03      AATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGC 390
gi|61200890|gb|AY830152.1|      AATTAGCGTAGTGATCTCATGTCGCTATAATGCTTCTTAGAGGGACAGGC 1349
                                ************************************************

gi|23307641|gb|AF469413.1|      GCAATGAAAGGCGCACGAAGTAGAGCAATAACAGGTCTGTGATGCCCTTC 812
gi|61200887|gb|AY830151.1|      GCAATGAAAGGCGCACGAAGTAGAGCAATAACAGGTCTGTGATGCCCTTC 1398
414629901_Acantho1_For_A03      GCAATGAAAGGCGCACGAAGTAGAGCAATAACAGGTCTGTGATGCCCTTC 440
gi|61200890|gb|AY830152.1|      GCAATGAAAGGCGCACGAAGTAGAGCAATAACAGGTCTGTGATGCCCTTC 1399
                                ************************************************

gi|23307641|gb|AF469413.1|      GATGTTCGAGGCTGCACGCGCGCTACAATGGAGGGCGCAAAGCGCATGTT 862
gi|61200887|gb|AY830151.1|      GATGTTCGAGGCTGCACGCGCGCTACAATGGAGGGCGCAAAGCGCATGTT 1448
414629901_Acantho1_For_A03      GATGTTCGAGGCTGCACGCGCGCTACAATGGAGGGCGCAAAGCGCATGTT 490
gi|61200890|gb|AY830152.1|      GATGTTCGAGGCTGCACGCGCGCTACAATGGAGGGCGCAAAGCGCATGTT 1449
                                ************************************************

gi|23307641|gb|AF469413.1|      GCCTCTTGAAAGAGAGTTGCAGAATCGTAAATAGCCTTCATGACTGGGAT 912
gi|61200887|gb|AY830151.1|      GCCTCTTGAAAGAGAGTTGCAGAATCGTAAATAGCCCTCATGACTGGGAT 1498
414629901_Acantho1_For_A03      GCCTCTTGAAAGAGAGTTGCAGAATCGTAAATAGCCTTCATGACTGGGAT 540
gi|61200890|gb|AY830152.1|      GCCTCTTGAAAGAGAGTTGCAGAATCGTAAATAGCCTTCATGACTGGGAT 1499
                                ********************************** *************

gi|23307641|gb|AF469413.1|      CGGAGATTGAAATTATTCTTCGTGAACGAGGAATTCCTAGTAAGCGCGAA 962
gi|61200887|gb|AY830151.1|      CGGAGATTGAAATTATTCTTCGTGAACGAGGAATTCCTAGTAAGCGCGAA 1548
414629901_Acantho1_For_A03      CGGAGATTGAAATTATTCTTC---------------------------- 561
gi|61200890|gb|AY830152.1|      CGGAGATTGAAATTATTCTTCGTGAACGAGGAATTCCTAGTAAGCGCGAA 1549
                                ********************
```

Figure 4.20 The sequence of the 18S rRNA gene fragment (414629901_Acantho1_For_A03) from one acanthocephalan (specimen CN1/2012/01) isolated from eel sample CN1 aligned with *A. anguillae* (gi|23307641), *A. lucii* (gi|61200890) and *A. dirus* (gi|61200887) 18S rRNA gene sequences.

The novel sequence was further analysed phylogenetically (Figure 4.21). The 18S rRNA gene sequence from the acanthocephalan specimen CN1/2012/01 was positioned in the phylogram within the *Acanthocephalus* spp. group and it appeared most related to *A. anguillae*. Consequently, it is reasonable to propose that the novel 18S rRNA sequence derived from the acanthocephalan CN1/2012/01 was representative of *A. clavula*. However, this remains questionable on the basis that currently there is no 18S rRNA sequence deposited in GenBank for *A. clavula*.

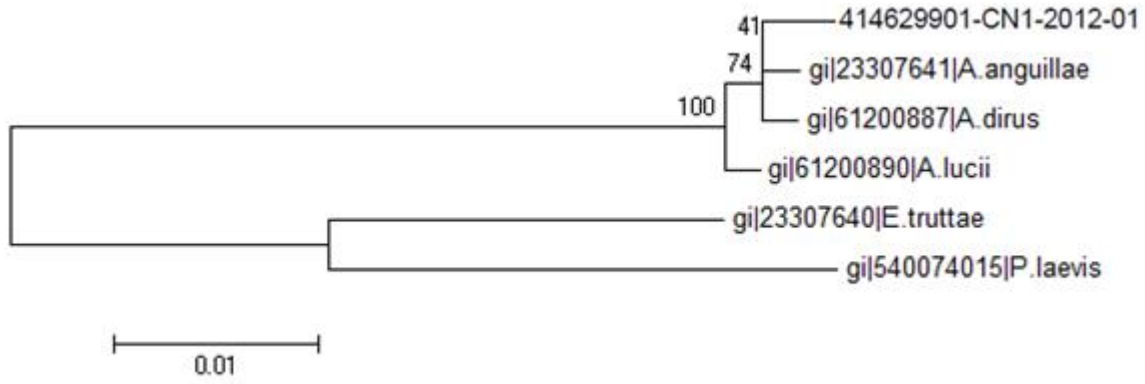

Figure 4.21 A phylogram constructed using MEGA 6.0 of 18S rRNA gene fragments from acanthocephalans and including the worm extracted from eel CN1 (specimen number CN1/2012/01). The sequences extracted from GenBank are as follows: *A. lucii* (gi|61200890), *P. laevis* (gi|540074015), *E. truttae* (gi|23307640), *A. dirus* (gi|61200887) and *A. anguillae* (gi|23307641).

4.4.4 Gastrointestinal pathology

Prior to dissection of the gastrointestinal tract, a number of the European eel specimens showed protrusions of acanthocephalan proboscis bulbs through the wall of the gastrointestinal tract (Figure 4.22). Consequently, this observation was utilised as a pathological indicator of acanthocephalan infection. In addition, upon dissection, it was noted that a large proportion of the acanthocephalans were found attached to, and penetrating through, the gut wall. For example, the gut of eel specimens C71and C74 had 33% (4/12) and 26% (14/53) respectively of their acanthocephalans penetrating the gut wall (Figure 4.23). Given the large numbers of acanthocephalans isolated from some European eel specimens (eg. C131=81, C74=53, CN1=56) then it may be possible for these parasites to cause severe pathological impact on the integrity of the gut wall and also, potential blockage of the gastrointestinal tract.

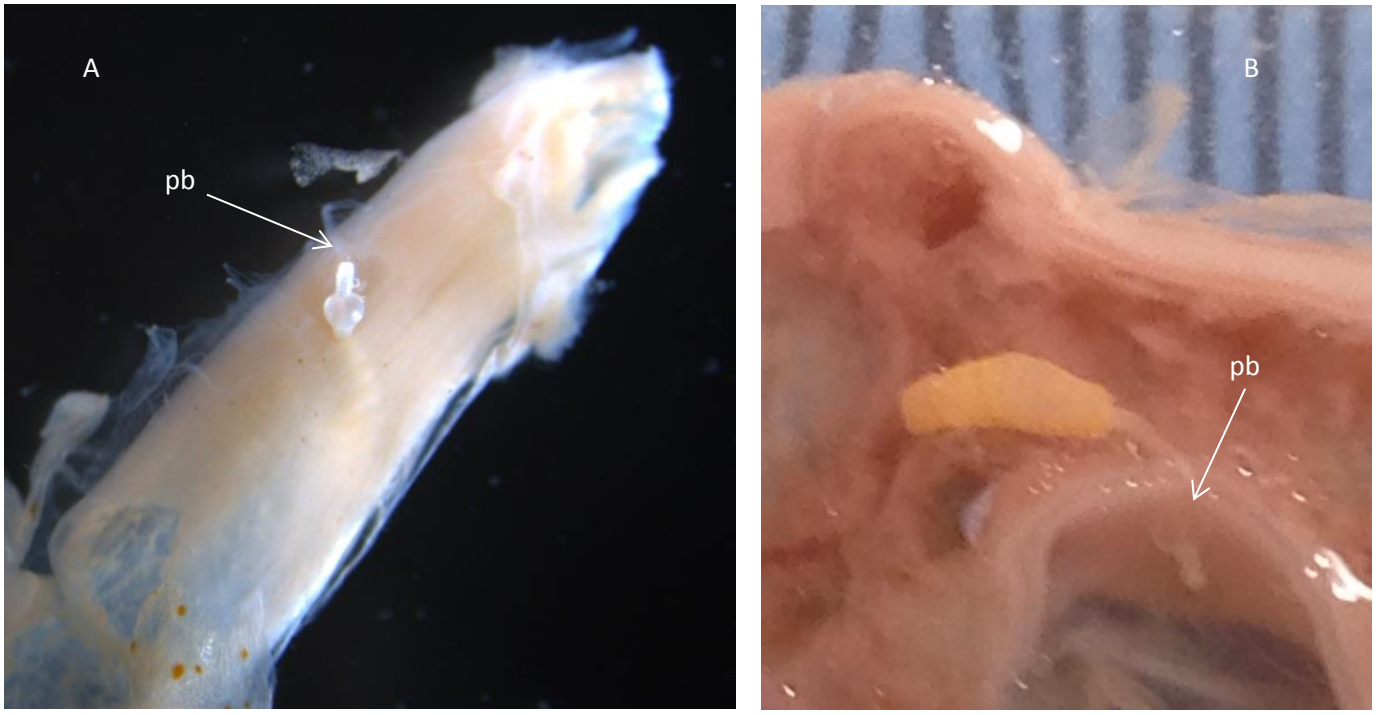

Figure 4.22 Representative images of *P. laevis* showing (A) the proboscis bulb (pb) protruding through the gut wall (eel R3) and (B) the pathological damage to the gut wall caused by penetration of the proboscis bulb (eel TB1).

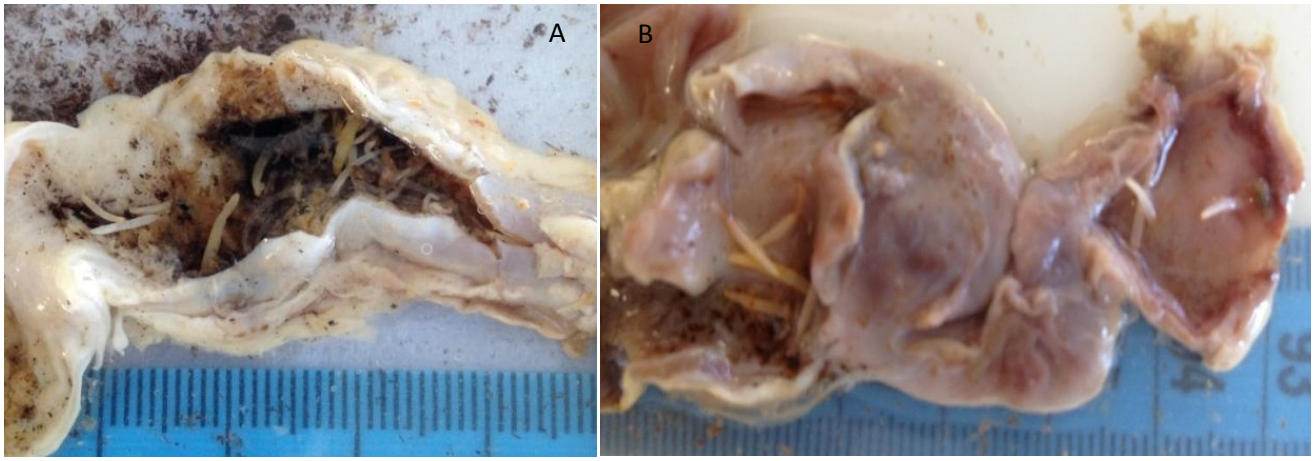

Figure 4.23 Representative images of *P. laevis* showing (A) large number of acanthocephalans occupying the gut lumen (eel C74) and (B) multiple gut penetration by the acanthocephalans (eel C131).

4.4.4.1 Tapeworms: Primary infection data

In total, 13 of the 140 (9.3%) European eel specimens contained tapeworms within their gastrointestinal tracts. The infected eels were sampled from 8 out of the 14 (57.1%) river systems surveyed across England and Wales (Table 4.20). No tapeworms were recovered from eels sampled from the River Leven in England and the Rivers Dee-Eitha, Mawddach-eden, Taff, Cadoxton and Rhymney from Wales. At sites positive for tapeworms, the prevalence ranged from 10 to 30%. The Rivers Crane and Clwyd-Meirchion showed the highest prevalence of infection (30%), while the lowest prevalence (10%) was observed in 5 of the remaining 6 infected catchment sites.

Table 4.20 Summary of the primary infection data for tapeworms isolated from European eel sampled across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence (%)	Number of tapeworms	Mean parasitic abundance ±(sd)	Mean infection intensity ±(sd)
Leven RL: England	0	0	0	0
Petteril RP: England	20	3	0.3±0. 7	1.5±0.7
Bela B: England	10	1	0.1±0.3	1
Crane CN: England	30	6	0.6±1.3	2±1.7
Hether Burn HB: England	10	2	0.2±0.6	2
Gowy RG: England	10	1	0.1±0.3	1
Crouch C: England	10	1	0.1±0.3	1
Clwyd-Meirchion MC: Wales	30	3	0.3±0.5	1
Clwyd-Elwy CE: Wales	10	1	0.1±0.3	1
Dee-Eitha D: Wales	0	0	0	0
Mawddach-eden M: Wales	0	0	0	0
Taff TB: Wales	0	0	0	0
Cadoxton CD: Wales	0	0	0	0
Rhymney R: Wales	0	0	0	0
	8.6%	18	0.1±0.5	1.4 ±0.9

4.4.4.1.1 Primary infection data: the environment

Overall, 18 tapeworms were collected from the eel specimens (mean abundance = 0.1±0.5; mean intensity = 1.4 ±0.9). The number of tapeworms extracted from individual eel ranged between 1 and 4; the latter being isolated from a fish sampled from the River Crane (specimen CN5). A single tapeworm was extracted from 76.9% of infected animals (10/13), (Table 4.21). With respect to the sampling sites, the River Crane yielded most tapeworm specimens (n =6). Overall, the parasites were over-dispersed (dispersion index = 2.5) (Figure 4.24).

Table 4.21 Eel morphometric and associated tapeworm infection data for the positive infections. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Number of tapeworms
Clwyd-Meirchion: Wales	MC1	42	104.4	0.14	1
	MC3	35	54.6	0.13	1
	MC4	25	6.8	0.17	1
Clwyd-Elwy: Wales	CE9	20	10.9	0.14	1
Gowy: RG England	RG6	32	41.8	0.13	1
Bela: B England	B8	48	204.5	0.18	1
Petteril: RP England	RP2	31.5	82.8	0.28	1
	RP5	35.5	74.9	0.17	2
Hether Burn HB: England	HB3	23	14.7	0.12	2
Crane: CN England	CN3	86	1380.3	0.22	1
	CN5	70	668.6	0.19	4
	CN8	77	661.9	0.14	1
Crouch: C England	C157	33	65.8	0.18	1

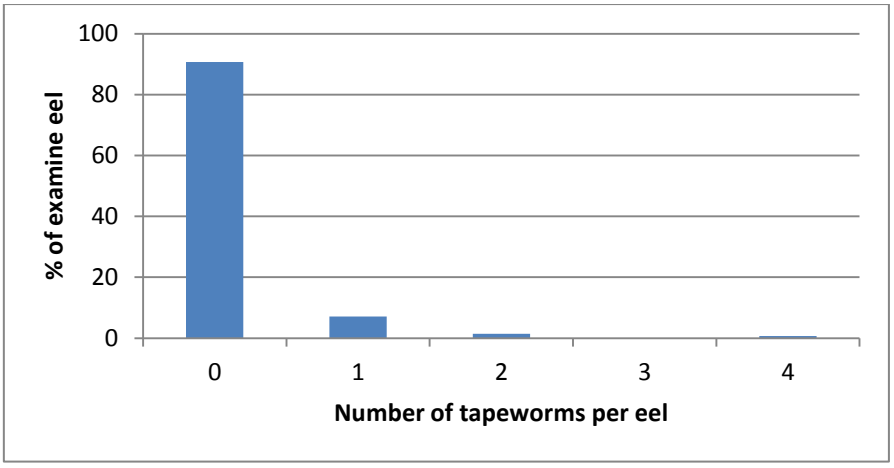

Figure 4.24 Summary of the dispersion of tape worms within the sampled eel population.

Upon analysis of the infection data at a regional level it was evident that all examined rivers in South East England contained eel infected with tapeworms (Table 4.22). This region also generated the greatest tapeworm mean abundance and mean intensity of infection data. In North West England, only the River Leven appeared to contain eels lacking a tapeworm infection. In South Wales the eels from all the examined rivers were lacking a tapeworm infection. The majority of rivers examined from North Wales also contained eels lacking a tapeworm infection.

Overall, there were no significant differences observed in the tapeworm prevalence between the different geographic regions (*p*: 0.151-1) with the exception of data observed between South East England and South Wales (*p*: 0.021).

Table 4.22 Regional infection data for tapeworms isolated from European eels sampled across England and Wales.

Regions	Number of examined river sites	Number of tapeworm positive sites	Number of examined eel	Number of infected eel	Prevalence (%)	Number of tapeworms	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	4	50	5	10	6	0.1±0.4	1.2±0.4	1 - 2
South East England	2	2	20	4	20	7	0.4±0.9	1.8±1.5	1 - 4
North Wales	4	2	40	4	10	4	0.1±0.3	1.0±0	1 – 1
South Wales	3	0	30	0	0	0	0	0	0
Total	14	8	140	13	9.3	17	1.4 ±0.9	0.1±0.5	1 – 4

4.4.4.1.2 Primary infection data: the host

For tapeworm infected eels, host body length ranged from 19 to 86 cm (mean = 42.5±21.9 cm) and body weight ranged from 6.8 to 1380.3 g (mean = 259.4±407.2 g). For uninfected eels, host body length ranged from 10 to 86 cm (mean = 29.5±13.6 cm) and body weight ranged from 1.2 g to 1058.8 g (mean = 78.3±175.8 g). These differences in length and weight between the infected and uninfected eels were statistically significant (p = 0.012 and 0.013 respectively). The mean condition factor for tapeworm infected eels was 0.17±0.04, whilst the mean condition factor for the uninfected eels was 0.15±0.04 and this difference was not statistically significant (p = 0.07).

Upon analysis of different categories of eel length it was found that the greatest tapeworm prevalence and intensity data were associated with the two largest length categories of eel (Table 4.23). Indeed, there were significant differences in the prevalence of tapeworm infection between the largest length category of eel and the two length categories between 20.5 cm and 30 cm (p: 0.0429 and 0.0075 respectively). There was also a significant difference in prevalence between the second largest length category of eel and the 25.5-30 cm length category (p = 0.023). There were no further significant differences between the eel length categories and tapeworm prevalence (p > 0.15). With respect to the tapeworm abundance data, the only significant difference occurred between the largest and the second smallest categories of eel length (p= 0.0412).

Table 4.23 Tapeworm infection data with respect to eel body length category.

Body length range (cm)	Number of examined eels	Number of infected eels	Prevalence (%)	Number of tapeworms	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	2	7.7	2	0.1±0.3	1	0-1
20.5-25	32	1	3.1	2	0.1±0.4	2	0-2
25.5-30	32	0	0	0	0	0	0
30.5-35	22	4	18.2	4	0.2±0.4	1	0-1
35.5-86	28	6	21.4	10	0.4±0.9	1.7±1.2	1-4

Table 4.24 Tapeworm infection data with respect to eel condition factor category.

Condition factor range	Number of eels examined	Number of infected eels	Prevalence (%)	Number of tapeworms	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.02 - 0.12	34	1	2.9	2	0.1±0.3	2	1 - 2
0.13-0.14	38	5	13.2	5	0.1±0.3	1	1 – 1
0.15-0.16	28	0	0	0	0	0	0
0.17-0.32	40	7	17.5	11	0.3±0.7	1.6±0.8	1 - 4

There was a general trend since as the eel condition factor category increased there was also an increase in tapeworm prevalence and abundance (Table 4.24). Interestingly, eels with a condition factor between 0.15 and 0.16 had no tapeworm infection. Indeed, there was a significant difference in tapeworm prevalence between the highest and second highest condition factor categories (p= 0.0359).

With respect to the tapeworm intensity data, there were significant differences between the smallest and both the second smallest and the largest condition factors (p = 0.0006 and 0.03 respectively). However, there were no significant abundance differences between the eel condition factor categories (p > 0.052).

4.4.4.2 Tapeworm morphology

Morphological examination of the extracted tapeworms showed that there were two distinct species infecting the eel; *B. claviceps* and *P. macrocephalus* (Figure 4.25). The carmine staining pattern was a distinctive feature that enabled species identification based upon morphology. Specifically, the staining for one parasite (specimen number B8/2009/01), showed the testes in the centre of proglottides (Figure 4.26).

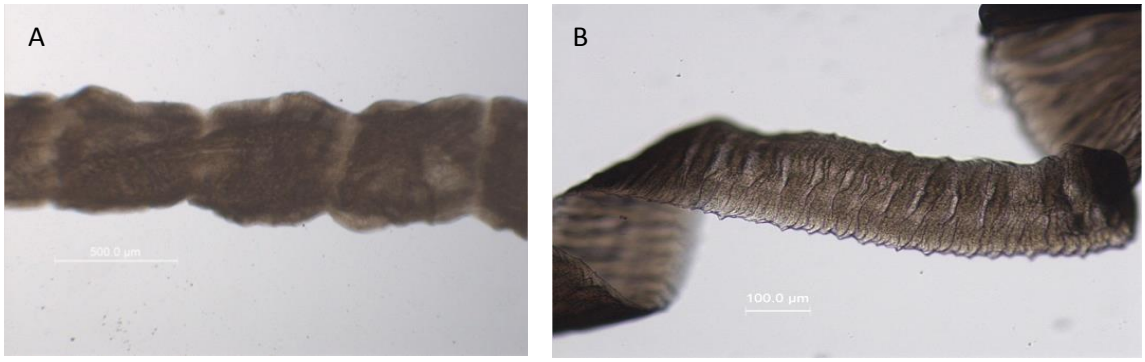

Figure 4.25 Representative images of the tapeworms recovered from European eel specimens. (A) Unstained large (length = 13 cm) *P. macrocephalus* (specimen number B8/2009/01) recovered from an eel sampled from the River Bela (B8). (B) Unstained small (length = 5cm) *B. claviceps* (specimen number RP2/2009/01) recovered from an eel sampled from the River Petteril (RP2).

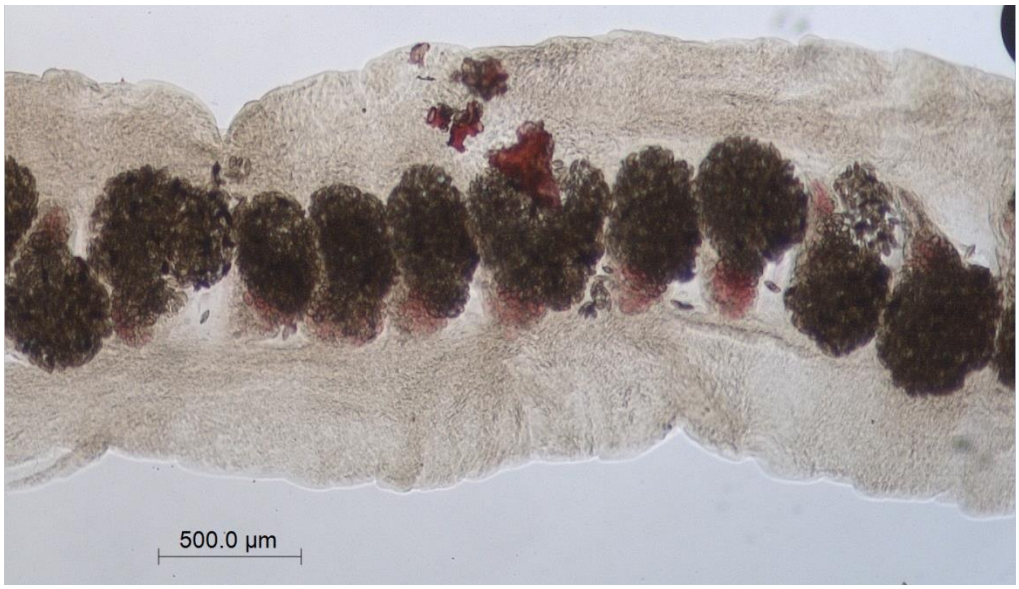

Figure 4.26 Representative image of *B. claviceps* recovered from the European eel specimens. The tapeworm (specimen number B8/2009/01) was stained with Carmine following isolation from an eel sampled from the River Bela (B8) and the image shows the testes in the centre of proglottides.

4.4.4.3 Molecular characterisation of tapeworms

Confirmation of the morphological identification of tapeworm species was provided by PCR amplification and DNA sequencing of the parasite 18S rRNA genes from representative samples. The resulting sequence data for tapeworms extracted from eel specimens B8, CN3 and HB3 showed that the PCR products were identical to each other and to the 18S rRNA

gene of *B. claviceps* (Table 4.25 and Figure 4.27). These parasites were indeed all classified as *B. claviceps* based upon their morphology (Figure 4.26).

Table 4.25 BlastN analysis of the 18S rRNA gene fragment derived from a tapeworm isolated from eel specimen CN3 (specimen number CN3/2012/01).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bothriocephalus claviceps isolate PBI_526 18S ribosomal RNA gene, partial sequence	883	883	100%	0.0	100%	KR780957.1
Bothriocephalus claviceps 18S ribosomal RNA gene, complete sequence	878	878	100%	0.0	99%	AF267288.1

```
420688201_TW4_F_G04                 ----------------------TGACGATCCGTGATGGTAGCATTTAAAC 28

gi|10312154|gb|AF267288.1|AF26      TCTGACCATAAACGATGCCAACTGACGATCCGTGATGGTAGCATTTAAAC 1200
                                                          ****************************

420688201_TW4_F_G04                 CTTCCTCACGGGCAGTCCCCGGGAAACCATTAAGTCTATGGGTTCCGGGG 78
gi|10312154|gb|AF267288.1|AF26      CTTCCTCACGGGCAGTCCCCGGGAAACCATTAAGTCTATGGGTTCCGGGG 1250
                                    **************************************************

420688201_TW4_F_G04                 GAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCA 128
gi|10312154|gb|AF267288.1|AF26      GAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCA 1300
                                    **************************************************

420688201_TW4_F_G04                 CCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACC 178
gi|10312154|gb|AF267288.1|AF26      CCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACC 1350
                                    **************************************************

420688201_TW4_F_G04                 CGGGCCGGACACTATGAGGATTGACAGATTGAAAGCTCTTTCTTGATTTG 228
gi|10312154|gb|AF267288.1|AF26      CGGGCCGGACACTATGAGGATTGACAGATTGAAAGCTCTTTCTTGATTTG 1400
                                    **************************************************

420688201_TW4_F_G04                 GTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGT 278
gi|10312154|gb|AF267288.1|AF26      GTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGT 1450
                                    **************************************************

420688201_TW4_F_G04                 TAATTCCGATAACGAACGAGACTCCAGCCTGCTAATTAGTTCTCCTGTCC 328
gi|10312154|gb|AF267288.1|AF26      TAATTCCGATAACGAACGAGACTCCAGCCTGCTAATTAGTTCTCCTGTCC 1500
                                    **************************************************

420688201_TW4_F_G04                 ACTGTACTTGTGCAGGCGGGCGCTTGCCAAATCTGCTCTTCGCGGTTGAC 378
gi|10312154|gb|AF267288.1|AF26      ACTGTACTTGTGCAGGCGGGCGCTTGCCAAATCTGCTCTTCGCGGTTGAC 1550
                                    **************************************************

420688201_TW4_F_G04                 CATCTGGTGGCGTTGTTGGTTGCCTAAAGTGCCGGCCGCAATGCTGGTGC 428
gi|10312154|gb|AF267288.1|AF26      CATCTGGTGGCGTTGTTGGTTGCCTAAAGTGCCGGCCGCAATGCTGGTGC 1600
                                    **************************************************

420688201_TW4_F_G04                 TTTT---------------------------------------------- 432
gi|10312154|gb|AF267288.1|AF26      TTTTTGTGTACTCGTGATGCATGTCCGGTGGGATGACTTGGGCGGATAGA 1650
                                    ****
```

Figure 4.27 The 18S rRNA DNA sequence (420688201_TW4_F_G04) from a tapeworm (specimen number CN3/2012/01), isolated from an eel sampled from the River Crane (CN3) aligned with a fragment of the *B. claviceps* 18S rRNA gene deposited in GenBank (gi: 10312154).

The 18S rRNA sequence data for the representative other tapeworms showed that the PCR product was identical to a fragment of the 18S rRNA gene from *P. macrocephalus* (Table 4.26 and Figure 4.28). This representative was a typical example of the tapeworm classified as *P. macrocephalus* based upon morphology.

Table 4.26 BlastN analysis of the 18S rRNA gene fragment derived from a tapeworm isolated from eel specimen RG6 (specimen number R/2010/01).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Proteocephalus macrocephalus 18S small subunit ribosomal RNA gene, complete sequence	904	904	100%	0.0	100%	EF095247.1
Proteocephalus sp. JDC-2005 18S ribosomal RNA gene, partial sequence	887	887	100%	0.0	100%	DQ181940.1

```
420976301_TW1_FBo_A03          ------------ACTGACGATCCGTGGTGGTAGTCCATCAACCTTCCCC 37
gi|126513420|gb|EF095247.1|    ATAAACGATGCCAACTGACGATCCGTGGTGGTAGTCCATCAACCTTCCCC 1150
                                           ************************************

420976301_TW1_FBo_A03          ACGGGCAGTCCCCGGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTAT 87
gi|126513420|gb|EF095247.1|    ACGGGCAGTCCCCGGGAAACCTTTAAGTCTTTGGGTTCCGGGGGAAGTAT 1200
                               **************************************************

420976301_TW1_FBo_A03          GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAG 137
gi|126513420|gb|EF095247.1|    GGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAG 1250
                               **************************************************

420976301_TW1_FBo_A03          TGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCCGGCCCG 187
gi|126513420|gb|EF095247.1|    TGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCCGGCCCG 1300
                               **************************************************

420976301_TW1_FBo_A03          GACACTATGAGGATTGACAGATTGATAGCTCTTTCTTGATTTGGTGGTTG 237
gi|126513420|gb|EF095247.1|    GACACTATGAGGATTGACAGATTGATAGCTCTTTCTTGATTTGGTGGTTG 1350
                               **************************************************

420976301_TW1_FBo_A03          GTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCC 287
gi|126513420|gb|EF095247.1|    GTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCC 1400
                               **************************************************

420976301_TW1_FBo_A03          GATAACGAACGAGACTCCTGCCTGCTAATTAGTGCATCTGTCTACTGTAC 337
gi|126513420|gb|EF095247.1|    GATAACGAACGAGACTCCTGCCTGCTAATTAGTGCATCTGTCTACTGTAC 1450
                               **************************************************

420976301_TW1_FBo_A03          CTGCGTAGGCGGTGTTTGGCGAGG------------------------- 361
gi|126513420|gb|EF095247.1|    CTGCGTAGGCGGTGTTTGGCGAGGTTGCTCCCGTACGCTGCCCTGGTGGC 1500
                               ************************
```

Figure 4.28 The 18S rRNA sequence (420976301_TW1_FBo_A03) from a tapeworm (specimen number RG6/2010/01), isolated from an eel sampled from the River Gowy (RG6), aligned with a fragment of the *P. macrocephalus* 18S rRNA gene deposited in GenBank (gi: 126513420).

4.4.4.4 Primary infection data for *B. claviceps*

In total, 12 of the 140 (8.6%) European eels examined contained the tapeworm *B. claviceps* within their gastrointestinal tracts. These infected eels were sampled from 4 out of the 14 (28.6%) river systems surveyed across England and Wales (Table 4.27). At sites positive for *B.claviceps*, the prevalence ranged from 10 to 30%. The Rivers Crane and Clwyd-Meirchion showed the highest prevalence of infection (30%), while the lowest prevalence (10%) was observed in the two remaining infected catchment sites. No mixed tapeworm infections were characterised in these eel specimens.

Table 4.27 Summary of the primary infection data for *B. claviceps* isolated from European eels sampled across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence (%)	Number of tapeworms	Mean parasitic abundance ±(sd)	Mean infection intensity ±(sd)
Bela B: England	10	1	0.1±0.3	1
Crane CN: England	30	6	0.6±1.3	2±1.7
Hether Burn HB: England	10	2	0.2±0.6	2
Clwyd-Meirchion MC: Wales	30	3	0.3±0.5	1
	8.6%	12	0.1±0.5	1.4 ±0.9

4.4.4.4.1 Primary infection data: the environment

Overall, 12 *B. claviceps* were collected from the eel specimens (mean abundance = 0.1±0.4; mean intensity = 1.5 ±1.1). The number of *B. claviceps* extracted from individual eels ranged between 1 and 4; the latter being isolated from a fish sampled from the River Crane (specimen CN5). A single tapeworm was extracted from 75% of the infected eels (6/8) (Table 4.28). With respect to the sampling sites, the River Crane yielded most tapeworm specimens (n =6). Overall, the parasites were over-dispersed (dispersion index = 2.5) (Figure 4.29).

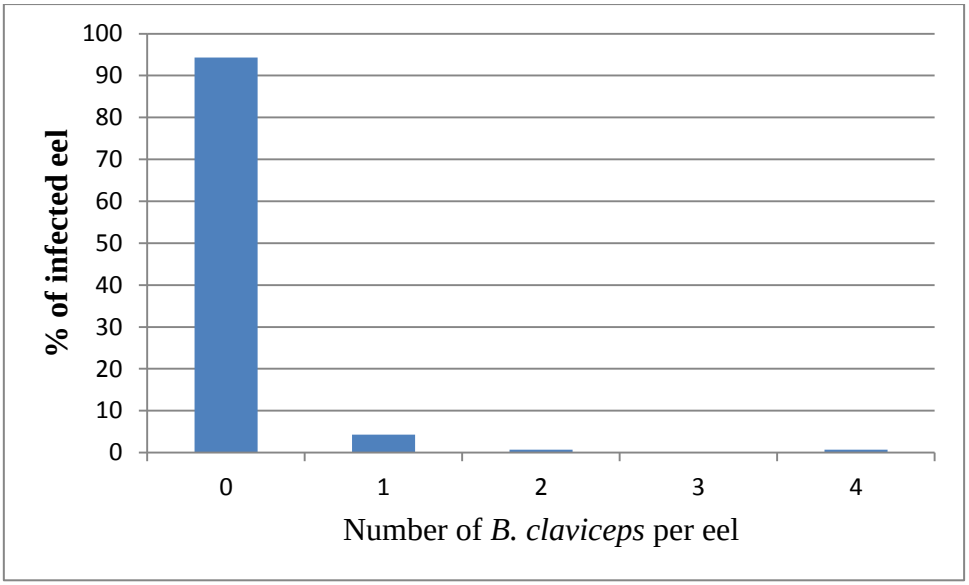

Figure 4.29 Summary of the dispersion of *B. claviceps* within the sampled eel population.

Table 4.28 Eel morphometric and associated *B. claviceps* infection data for the positive infections. Ten eels were sampled at each catchment site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of tapeworm
Clwyd-Meirchion: MC Wales	MC1	42	104.4	0.14	1
	MC3	35	54.6	0.13	1
	MC4	25	6.8	0.17	1
Bela: B England	B8	48	24.5	0.11	1
Hether Burn: HB England	HB3	23	14.7	0.12	2
Crane: CN England	CN3	86	1380.3	0.22	1
	CN5	70	668.6	0.19	4
	CN8	77	661.9	0.14	1

Upon analysis of the *B. claviceps* infection data at a regional level it was evident that eels sampled from South Wales lacked this tapeworm. In contrast, eels sampled from South East England showed the greatest prevalence and mean intensity of *B. claviceps* infection (Table 4.29). Overall, there were no significant differences in prevalence (p: 0.058 - 0.65), abundance (p: 0.1188 – 0.4961) and intensity of infection ($p > 1.0$) between the different sampling regions.

Table 4.29 Regional infection data for *B. claviceps* isolated from European eels sampled across England and Wales.

Regions	Number of examined river sites	Number of *B. claviceps* positive sites	Number of examined eel	Number of infected eel	Prevalence (%)	Number of *B. claviceps*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	2	50	2	4	3	0.1±0.3	1.5±0.7	1 - 2
South East England	2	1	20	3	15	6	0.3±0.9	2±1.7	1 - 4
North Wales	4	1	40	3	7.5	3	0.1±0.3	1	1
South Wales	3	0	30	0	0	0	0	0	0
Total	14	4	140	8	5.7	12	0.1±0.4	1.5±1.1	1 - 4

4.4.4.4.2 Primary infection data: the host

For *B. claviceps* infected eels, host body length ranged from 19 to 86 cm (mean = 50±25.1 cm) and body weight ranged from 6.8 to 1380 g (mean = 386.9±485.2 g). For uninfected eels, host body length ranged from 10 to 86 cm (mean = 29.5±13.3 cm) and body weight ranged from 1.2 g to 1058.8 g (mean = 77.4±172.5 g). These differences in eel length and weight were significantly different between the infected and uninfected eels (p = 0.017 and 0.031 respectively). The mean condition factor for *B. claviceps* infected eels was 0.16±0.03, whilst the mean condition factor for the uninfected eels was 0.15±0.04 and this was not statistically significant (p = 0.26).

Upon analysis of different categories of eel length it was found that the greatest *B. claviceps* prevalence and abundance data were associated with the largest length category of eel (Table 4.30). The only statistically significant data though was for the prevalence difference between the largest and the 25.5-30cm length categories (p = 0.018).

Table 4.30 *B. claviceps* infection data with respect to eel body length category.

Body length range (cm)	Number of examined eels	Number of infected eels	Prevalence (%)	Number of *B. claviceps*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	1	3.8	1	0.04±0.2	1	1
20.5-25	32	1	3.1	2	0.1±0.4	2	2
25.5-30	32	0	0	0	0	0	0
30.5-35	22	1	4.5	1	0.05±0.2	1	1
35.5-86	28	5	17.9	8	0.3±0.8	1.6±1.3	1 - 4

With respect to eel condition factor, the highest *B. claviceps* prevalence and mean abundance data were observed for eels with the greatest condition factor category (Table 4.31). However, there were no statistically significant differences between the condition factor categories and the *B. claviceps* infection data ($p > 0.14$).

Table 4.31 *B. claviceps* infection data with respect to eel condition factor category

Condition factor range	Number of eels examined	Number of infected eels	Prevalence (%)	Number of *B. claviceps*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.02 - 0.12	34	1	2.9	2	0.1±0.3	2	2
0.13-0.14	38	3	7.9	3	0.1±0.3	1	1
0.15-0.16	28	0	0	0	0	0	0
0.17-0.32	40	4	10	7	0.2±0.7	1.8±1.5	1 - 4

4.4.4.5 Primary infection data: *P. macrocephalus*

In total, 5 of the 140 (3.6%) European eel examined contained the tapeworm *P. macrocephalus* within their gastrointestinal tracts. The infected eels were sampled from 4 out of the 14 (28.6%) river systems surveyed across England and Wales (Table 4.32); these were different sites relative to the *B. claviceps* positive infection sites. At sites positive for *P. macrocephalus*, the prevalence ranged from 10 to 20%. The River Petteril showed the highest prevalence of infection (20%), while the lowest prevalence (10%) was observed in the 3 remaining infected catchment sites.

Table 4.32 Summary of the primary infection data for *P. macrocephalus* isolated from European eels sampled across England and Wales. Ten eels were examined from each site.

Eel catchment sites	Prevalence (%)	Number of tapeworms	Mean parasitic abundance ±(sd)	Mean infection intensity ±(sd)
Petteril RP: England	20	3	0.3±0. 7	1.5±0.7
Gowy RG: England	10	1	0.1±0.3	1
Crouch C: England	10	1	0.1±0.3	1
Clwyd-Elwy CE: Wales	10	1	0.1±0.3	1
	3.6%	6	0.04±0.2	1.2 ±0.4

4.4.4.5.1 Primary infection data: the environment

Overall, 6 *P. macrocephalus* were collected from the eel specimens (mean abundance = 0.04±0.2; mean intensity = 1.2 ±0.4). The number of *P. macrocephalus* extracted from individual eel was either 1, or 2; the latter being isolated from a fish sampled from the River Petteril (specimen RP5). A single *P. macrocephalus* was extracted from the remaining 4 infected animals (Table 4.33). With respect to the sampling sites, the River Petteril yielded most tapeworm specimens (n =3). Overall, the parasites were over-dispersed (dispersion index = 2.5).

Table 4.33 Eel morphometric and associated *P. macrocephalus* infection data for the positive infections. Ten eel were sampled at each catchment site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of tapeworm
Clwyd-Elwy: Wales	CE9	20	10.9	0.14	1
Gowy: RG England	RG6	32	41.8	0.13	1
Petteril: RP England	RP2	31.5	82.8	0.28	1
	RP5	35.5	74.9	0.17	2
Crouch: C England	C157	33	65.8	0.18	1

Upon analysis of the infection data at a regional level, it was evident that eels sampled from rivers in South Wales were lacking a *P. macrocephalus* infection (Table 4.34). Two rivers in North West England contained eel infected with *P. macrocephalus;* this region generated the greatest *P. macrocephalus* prevalence and mean intensity of infection data. However, overall there were no significant differences observed in the *P. macrocephalus* prevalence data between the different geographic regions (*p*: 0.2879 - 1). There was also no significant differences in the abundance data between the different regions (*p*: 0.426 – 0.871).

Table 4.34 Regional infection data for *P. macrocephalus* isolated from European eels sampled across England and Wales.

Regions	Number of examined river sites	Number of *P. macrocephalus* positive sites	Number of examined eel	Number of infected eel	Prevalence (%)	Number of *P. macrocephalus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	2	50	3	6	4	0.1±0.3	1.3±0.6	1 - 2
South East England	2	1	20	1	5	1	0.1±0.2	1	1
North Wales	4	1	40	1	2.5	1	0.03±0.2	1	1
South Wales	3	0	30	0	0	0	0	0	0
Total	14	4	140	5	3.6	6	0.03±0.2	1.2 ±0.2	1-2

4.4.4.5.2 Primary infection data: the host

For *P. macrocephalus* infected eels, host body length ranged from 20 to 35.5 cm (mean = 30.4±6.0 cm) and body weight ranged from 10.9 to 82.8 g (mean = 55.2±29.2 g). For uninfected eels, host body length ranged from 10 to 86 cm (mean = 30.7±15.1 cm) and body weight ranged from 1.2 g to 1380 g (mean = 95.5±216.1 g). The mean condition factor for tapeworm infected eels was 0.18±0.1, whilst the mean condition factor for the uninfected eels was 0.15±0.04. There were no statistically significant differences between body length (p = 0.35), weight (p = 0.24), or condition factor (p = 0.16), for the *P. macrocephalus* infected and uninfected eels.

Upon analysis of different categories of eel length it was found that the greatest *P. macrocephalus* prevalence and abundance data were associated with the largest length categories of eel (Table 4.35). No *P. macrocephalus* infection was detected in eels from the median size length category (25.5-30 cm) and this was significant when compared to the largest category of eel length (p = 0.018). There were no further significant differences between the different length categories of eel with respect to prevalence (p: 0.19-1.0), or abundance (p: 0.07-0.93).

Table 4.35 *P. macrocephalus* infection data with respect to eel body length category

Body length range (cm)	Number of eels examined	Number of infected eels	Prevalence (%)	Number of *P. macrocephalus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	1	3.8	1	0.03±0.2	1	1
20.5-25	32	1	3.1	2	0.1±0.4	2	2
25.5-30	32	0	0	0	0	0	0
30.5-35	22	1	4.5	1	0.05±0.2	1	1
35.5-86	28	5	17.9	8	0.3	1.6±1.3	1 - 4

With respect to condition factor, the highest *P. macrocephalus* prevalence and mean intensity data were associated with eels from the greatest condition factor category (Table 4.36). Eels with a condition factor between 0.02-0.12 and also, 0.15-0.16, had no *P. macrocephalus* infection. However, there were no significant differences in *P. macrocephalus* prevalence (p: 0.244 - 1) or abundance (p = 0.680) between the different condition factor categories.

Table 4.36 *P. macrocephalus* infection data with respect to eel condition factor category

Condition factor range	Number of eels examined	Number of infected eels	Prevalence (%)	Number of *P. macrocephalus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.02 - 0.12	34	0	0	0	0	0	0
0.13-0.14	38	2	5.3	2	0.1±0.2	1	1
0.15-0.16	28	0	0	0	0	0	0
0.17-0.32	40	3	7.5	4	0.1±0.4	1.3±0.6	1 – 2

4.5 Discussion

Work described in this chapter is focussed upon describing infection of European eels sampled from sites across England and Wales with acanthocephalans and tapeworms. To this end, the data produced adds knowledge to the infection status of *A. anguilla* stocks in UK rivers.

4.5.1 Acanthocephalans

The primary infection data generated for acanthocephalans in this Chapter shows that these worms were present in eels sampled from multiple sites across England and Wales. Indeed, as the sampling sites have not been recorded in previous studies examining acanthocephalans in European eels, the data informs with respect to the geographic spread of these parasites. As expected, the parasites were over-dispersed since most of the eels lacked acanthocephalans whereas a small number of the hosts carried large numbers of these worms.

A number of the sampling sites appeared to be free of acanthocephalan infections. However, there remains the possibility that the prevalence of infection at these sites may be very low and hence sampling of further eel specimens may in fact confirm the presence of acanthocephalans. For example, an extensive survey carried out on a small trout stream, the Afon Terrig, in North Wales, confirmed the presence of *E. truttae* (Awachie, 1965). Nonetheless, at the regional level, a total of 40 eels were examined in this study from North Wales and none of these were infected with acanthocephalans. In contrast, every eel examined from the sampling sites in South East England were infected with acanthocephalans. This regional difference was highly significant and is possibly reflective of a contrast in the distribution of intermediate hosts. Indeed, changes in the composition of the crustacean fauna have been documented to correlate with changes to the eel intestinal parasite fauna (Thielen, Münderle, *et al.*, 2007). Given that the appearance of acanthocephalans in the

amphipods has been shown to be seasonal (Awachie, 1965; Brattey, 1986), it is also possible that the regional infection differences observed may be partly explained by different sampling times. However, perhaps a more plausible explanation of the observed regional differences may be due to the age of the hosts; eels sampled from rivers in South East England were the largest in the study and hence most likely to encounter and prey upon the infected intermediate hosts. Of course, a combination of the above explanations may account for the observed regional differences.

With respect to the category 2 pathogen, *P. laevis*, the data in this chapter confirms that eels in England and Wales are exposed to infection risk. Interestingly, it appears that the sampling sites in South Wales were more likely than elsewhere to harbour *P. laevis* infected eels. Since eels from South Wales were of average size then one explanation for this data is that the availability of infected intermediate hosts is greater than in other areas of the country. In addition, the absence, or presence, of preferred definitive hosts, such as trout, chub and barbel, are likely to influence the *P. laevis* infection data (Kennedy *et al.*, 1989). Some of the eels were infected with relatively large numbers of *P. laevis*, particularly specimens from the river Crouch. It was apparent that eels infected with *P. laevis* exhibited a pathology associated with gut penetration by the proboscis, as has been noted elsewhere (Hine & Kennedy, 1974a; Dezfuli *et al.*, 2002). The immune response to *P. laevis* has been studied in barbel (*B. barbus*), sheat fish (*S. glanis*) and chub (*S. cephalus*), and in three-spined sticklebacks, (*G. aculeatus*); (Dezfuli *et al.*, 2011; Bosi & Dezfuli, 2015; Dezfuli *et al.*, 2015). As such, the immediate and broader impacts of *P. laevis* upon the European eel are areas worthy of further investigation.

The molecular analysis of representative *P. laevis* specimens in this study showed that they were identical to a haplotype of the parasite, Hum 10, isolated from chub sampled from a Croatian river (unpublished data) and also to a specimen extracted from the intermediate host

G. pulex, sampled in the vicinity of Dijon, France (Perrot-Minnot, 2004). However, as the sequences were also identical to a deposit of the 18S rRNA gene from *P. tereticollis*, again isolated from *G. pulex* sampled in eastern France (Perrot-Minnot, 2004), then it remains a possibility that one, or more of the parasites collected in this study were *P. tereticollis*. Indeed, there has been much debate about the taxonomy of these two species (Perrot-Minnot, 2004; Emde *et al.*, 2012); however, as more recently argued Emde *et al*. (2012), the validity of the classification is justified. Indeed, Emde et al. (2012) highlight minor morphological differences between the larval stages of the two species and suggest that misidentification may have occurred in earlier studies (Emde *et al.*, 2012). Based upon the mode of sample acquisition in this study, it would not be possible to morphologically examine the acanthocephalans in greater detail in order to assign species based upon the characteristics highlighted by Emde *et al.* (2012). As such, one alternative approach would be to PCR amplify a larger fragment of the 18S rRNA gene of the acanthocephalan samples and subject the resulting products to DNA sequencing. Should this additional data confirm the presence of *P. tereticollis*, this would be the first confirmed report of this parasite in UK fish since published reports document only the three strains of *P. laevis* (Kennedy *et al.*, 1989; O'mahony, Bradley, *et al.*, 2004; O'Mahony, Kennedy, *et al.*, 2004; Smrzlić *et al.*, 2015).

With respect to the non-bulbed acanthocephalans, the data presented in this Chapter has confirmed the presence of these parasites in *A. anguilla* sampled from a number of the English rivers but interestingly, not from the Welsh catchment sites. Eels sampled from the rivers Crouch and Crane provided almost 97% of these parasites and hence not surprisingly, regional infection differences were noted as highly significant. Moreover, these parasites have also been reported to be common in several studies, most of which are focussed upon catchment sites in the South of the UK (Kennedy *et al.*, 1978; Kennedy, 1996). However, there remains the possibility that regional differences are less pronounced than the data

suggests since as noted previously, the eels studied from the river Crane are significantly larger than all the other specimens. As such, the age of the eels and hence the length of time that they have been preying upon intermediate, or paratenic hosts, is likely to be a significant factor in explaining the data (Smrzlić *et al.*, 2015).

The large number of acanthocephalans isolated from eels sampled from the river Crane was also notable for not including any parasites with a proboscis bulb. In contrast, three catchment sites, the rivers Petteril, Leven and Crouch contained eels that possessed mixed (non-bulbed and bulbed) types of acanthocephalan. Although stated in the literature that such mixed infections are rare within UK rivers (Kennedy & Moriarty, 1987), the data in this Chapter clearly indicates that this is no longer the case. On closer examination of the data from the river Crouch, it was evident that when mixed infections occurred, the number of parasites of one type was much greater than the other type. However, there were examples of both non-bulbed acanthocephalans being dominant and also, bulbed parasites being dominant. However, the dataset of mixed infections described in this Chapter was small and hence to make firm conclusions, a greater number of hosts with mixed-type infections would need to be analysed. Nonetheless, it could be argued that acanthocephalans have become more widespread and also, that the non-bulb and bulbed species have adapted to co-exist within the host gastrointestinal tract of eels; albeit, the factors that may contribute to the relative numbers of both types remain unknown.

Species identification of the non-bulb acanthocephalans based upon morphology was difficult as specimens were not freshly obtained and morphological differences were often minimal. As such, a representative sample of parasites was subjected to DNA extraction and PCR amplification of the 18S rRNA gene. This molecular data confirmed that *A. lucii* was present within the examined eel since there was an exact match to the 18S rRNA sequence deposited in GenBank for this parasite (García-Varela & Nadler, 2005). Confirmation of *A. lucii* within

the eel population sampled for this study is not surprising given that this parasite is commonly encountered within UK freshwater fish, including eels (Kennedy, 1985). In addition, a novel 18S rRNA sequence was generated for several acanthocephalan specimens that upon phylogenetic analysis was most closely related to *A. anguillae*. As such, it seems reasonable to propose that the specimens from which this novel sequence was generated were most likely to be *A. clavula* as this parasite lacks an 18S rRNA sequence deposit. Indeed, *A. clavula* is known to be commonly found within eels sampled from UK rivers (Kennedy & Lord, 1982). Confirmation of the species would necessitate either an attempt to characterise these parasites morphologically, or, given the aforementioned difficulties associated with this approach, an alternative molecular strategy could be utilised. Inspection of the NCBI GenBank database shows only one deposit for *A. clavula* and this corresponds to the sequence encoding the cytochrome oxidase gene (Benesh *et al.*, 2006). Specific PCR primers could therefore be designed to amplify the suspected *A. clavula* cytochrome oxidase gene and subsequent sequence analysis would either support, or reject, the above species proposal. Although the molecular strategy described in this Chapter was a useful means of assisting species identification, a more rigorous analysis, requiring multiple specimens from different localities, would be needed to gain a more confident overview of the non-bulb acanthocephalan community composition within the eels.

4.5.2 Cestodes

Previous surveys have reported that the *A. anguilla* is infected with two tapeworm species, *B. claviceps* and *P. macrocephalus*, and that these parasites are distributed across Europe, including the UK (Nie & Kennedy, 1991b; Kennedy, Nie, Kaspers, *et al.*, 1992; Nie & Kennedy, 1992b; Scholz *et al.*, 2004; Kuchta *et al.*, 2012).

Morphological and molecular data in this chapter confirms that both tapeworm species were present in the sampled eel population and hence it adds to the geographic knowledge of the spread of these parasites. Indeed, tapeworms were isolated from eels sampled from all regions with the exception being the catchment sites in South Wales. Given that overall prevalence of the tapeworms was relatively low and that a total of only 30 eels were examined from 3 rivers in South Wales, it remains a possibility that *B. claviceps* and/or *P. macrocephalus*, may be present in this region; albeit at very low prevalence. Moreover, data is this study corroborates earlier findings (Conneely & McCarthy, 1986), that eel length is a significant factor when considering the likelihood of the fish becoming infected with tapeworms. The most likely explanation for this finding is that the larger eels have been subject to a greater exposure time with regard to ingestion of infected intermediate hosts. Since eels sampled from South Wales are below average length it is likely that they have been exposed to any infected intermediate hosts for a shorter period than those eels sampled from other regions and hence they are less likely to become infected.

With regard to the two tapeworm species, *B. claviceps* was found to be more common than *P. macrocephalus*. Both species were more commonly encountered in the English rivers than the Welsh rivers. Moreover, *P. macrocephalus* was absent from sites that were positive for *B. claviceps* and hence these tapeworm species were not found as a co-infection.

As noted in previous studies, eel tapeworms are essentially non-pathogenic to the host (Abdelmonem *et al.*, 2010). However, these parasites are likely to influence immune outcomes to other infections via immunosuppression and interestingly, they have recently been reported, along with other helminths, as being associated with red anus syndrome in the European eel (Tamam, 2014). Unfortunately, due to the mode of acquisition of the eel specimens in this study, it was impossible to comment further on whether, or not, red anus syndrome is an indicator of eel tapeworm infection.

CHAPTER FIVE

Helminth community structure

5.1 Introduction

Previous Chapters have presented data on specific helminth infections in the European eels sampled from rivers in England and Wales. However, to fully appreciate the role(s) of helminths in the biology of the European eel, it is necessary to consider the individual parasite infection profiles as a component of an overall helminth community structure.

In the natural environment, animals may often act as host to multiple different parasitic organisms and the outcomes, both for the host and the parasites, may be profound (Borgsteede, 1996; Pedersen & Fenton, 2007; Khan, 2012). For example, competition between multiple co-infecting parasites may increase the likelihood of genetic variation, which in turn may enhance parasite virulence and have negative health consequences for the host (Rigaud *et al.*, 2010; Marcogliese & Pietrock, 2011). In contrast, an alternative outcome of co-infection might also be considered. For example, if parasitic infections lead to reduced growth and development of the host then this may limit resources for the parasite and ultimately, influence genetic change within the parasite so that it becomes less virulent (Bremermann & Pickering, 1983; Combes, 1997; Schjørring & Koella, 2003). In both cases, a clear impact on transmission of the parasite to the next host is apparent.

As the individual parasite species within a community may differ from one host to another, outcomes for both the parasites and the host will potentially vary. At the forefront of this community structure are the interactions that occur between parasites and the host immune system and also, potential interactions between the parasites (Pedersen & Fenton, 2007). As such, parasite community structures are shaped by host factors (Conneely & McCarthy, 1986; Han *et al.*, 2008; Schneebauer *et al.*, 2016), parasite factors (Boon *et al.*, 1990; Ashworth &

Kennedy, 1999; Nielsen, 1999; Buchmann & Lindenstrøm, 2002; Smrzlić *et al.*, 2015) and environmental factors (Kennedy, 1993a; Lafferty & Kuris, 1999; Galli *et al.*, 2001; Lefebvre *et al.*, 2002; Schabuss *et al.*, 2005; Thielen, Muenderle, *et al.*, 2007; Jakob, Hanel, *et al.*, 2009a; Marcogliese & Pietrock, 2011; Filippi *et al.*, 2013). Understanding these interplays is of importance; not least because it allows predictive models to be developed that assist further the understanding of parasite transmission and disease outcomes.

The parasite fauna of *A. anguilla* have been well investigated throughout continental Europe and component helminth communities are described as comprising of acanthocephalans, cestodes, nematodes and trematodes. Within these classes, generalist fish helminths are commonly found in addition to dominant eel specialist parasites (Kennedy *et al.*, 1997). Furthermore, it is also known that the European eel is host to accidental helminth species (Kennedy & Guégan, 1996; Kennedy *et al.*, 1997; Kennedy *et al.*, 1998; Borgsteede, Haenen, Bree, *et al.*, 1999; Sures, Knopf, Wurtz, *et al.*, 1999; Di Cave *et al.*, 2001; Sures & Streit, 2001; Kennedy & Moriarty, 2002; Aguilar, Alvarez, *et al.*, 2005b; Schabuss *et al.*, 2005; Kristmundsson & Helgason, 2007; Sasal *et al.*, 2008; Moravec & Scholz, 2015).

Within the UK, studies on European eel helminths have been conducted primarily in the South of England, including the river Thames at Windsor, the river Test in Hampshire and at several rivers (Exe, Clyst and Otter) and a lake (Shobrooke) in Devon (Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004). These studies, as for the continental European studies, confirm that *A. anguilla* is host to multiple different classes of helminths. Indeed, eel helminth fauna similarities have been reported between the UK and continental Europe (Kennedy *et al.*, 1997; Kennedy *et al.*, 1998; Kennedy & Moriarty, 2002; Schabuss *et al.*, 2005); albeit the component community structure within UK eels has also been documented as generally being less diverse than that observed for eels from mainland Europe (Norton *et al.*, 2003). The format of data publication

for the aforementioned UK studies generally describes the eel parasite species catalogued to the study location. To this end, it is unfortunately not possible to discern precise information about the important helminth coinfections within individual eel specimens from UK localities.

More recently, a study of eels from a Spanish saltwater lagoon examined individual eel physiology and fitness and concluded that the European eel may be able to tolerate co-infecting parasites (Mayo-Hernandez, Serrano, Penalver, *et al.*, 2015). The major influences upon the health of the individual eel appeared to be infections with an anisakid, *Contracaecum* sp. and a digenean, *Bucephalus anguillae* (Mayo-Hernandez, Serrano, Penalver, *et al.*, 2015). Also considered potential influences upon the health of these eels were overall parasite richness and infections with the digenean *Deropristis inflata* (Mayo-Hernandez, Serrano, Penalver, *et al.*, 2015). However, this study highlighted that *A. crassus* was present in the eels at very low prevalence and there was also no indication of any pseudodactylid, or *Pomphorhynchus laevis* infections within the population (Mayo-Hernandez, Serrano, Penalver, *et al.*, 2015). As such, it remains untested as to exactly how helminth coinfections, particularly those of known pathogenic impact, influence the overall health of eel populations and importantly, the ability of these infected fish to successfully migrate to the spawning grounds in the Sargasso Sea (Kirk, 2003; Schneebauer *et al.*, 2016).

5.2 Objectives

The primary aim of this Chapter was to analyse the infection data presented in earlier sections of the thesis in order to establish an overview of helminth community structure in the European eel across the sampling sites in England and Wales. In addition, data on the pathogenic swim bladder nematode *A. crassus* will be presented and analysed with respect to other co-infecting helminths.

5.3 Materials & methods

5.3.1. Sampling

Eel samples were acquired according to Chapter 1 (1.3). For *A. crassus* analysis, thirty European eels were examined from the rivers Leven, Crane and Petteril and this data was analysed in conjunction with *A. crassus* infections determined from the remaining 11 catchment sites (Ab Aziz, 2012).

5.3.2 Processing

Eel specimens were thawed and using a dissection kit a longitudinal incision was made along the ventral surface, allowing removal of the swim bladder. Following excision, the swim bladder was placed into a petri dish that contained distilled water and using a Wild Heerbrugg, M3B (Switzerland) dissecting microscope (x 100), it was opened with scissors and forceps and any *A. crassus* removed into an eppendorf tube. The number of *A. crassus* in each eel swim bladder was recorded. For longer term storage at -20C^{o}, the nematodes were preserved within eppendorf tubes containing 70% (v/v) ethanol and labelled with sampling details.

5.3.3 Imaging

Images of the *A. crassus* were acquired according to Chapter 2 (2.3.3).

5.3.4 Statistical analyses

Statistical analyses were carried out according to Chapter 2 (2.3.10).

5.4 Results

5.4.1 *A. crassus* infection analysis

For most of the catchment sites examined in this thesis, infection data for *A. crassus* within European eels was produced in an earlier study (Ab Aziz, 2012). However, additional *A. crassus* infection data has been independently produced in this thesis work at three of the catchment sites, the rivers Leven, Crane and Petteril, thereby now allowing a complete swim bladder nematode data analysis within the European eel at all of the 14 sites. As such, the *A. crassus* data will be presented below as a single infection analysis. Subsequently, a more comprehensive helminth community structure analysis, that includes the *A. crassus* data, will be presented.

5.4.1.1 *A. crassus* primary infection data: the environment

In total, 35 of the 140 (25%) European eels examined contained the pathogenic nematode *A. crassus* within their swim bladder. Infections involving relatively large numbers of *A. crassus* resulted in alterations to the swim bladder appearance as it became distinctly red-brown in colour due to haemorrhaging caused by the nematodes (Figure 5.1). These *A. crassus* infected eels were sampled from 7 (50%) of the river sites surveyed across England and Wales (Table 5.1). At sites positive for *A. crassus*, the prevalence ranged from 10 to 70%. Eels sampled from the rivers Leven and Cadoxton showed the highest prevalence of infection (70%), while the lowest prevalence (10%) was observed in specimens from the river Taff.

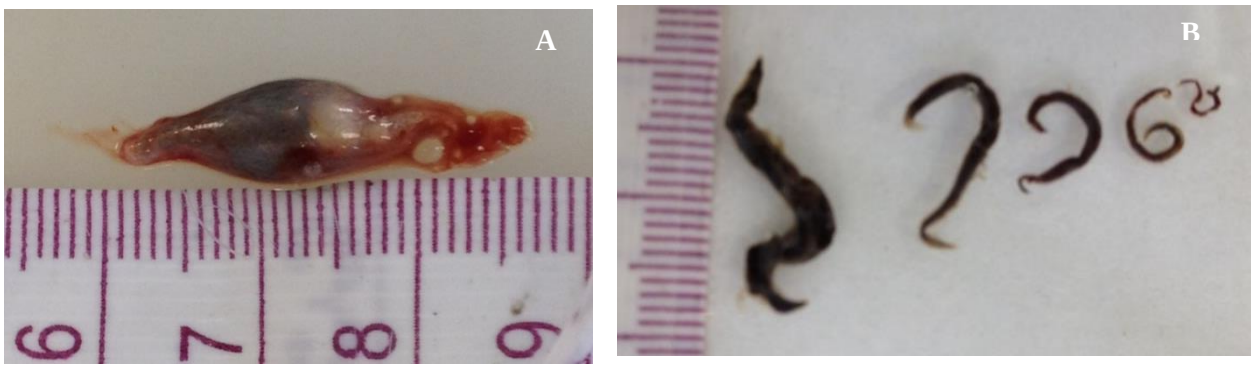

Figure 5.1 A swim bladder infected with *A. crassus* isolated from an eel (specimen CN6) collected from the river Crane (A). Adult and larval stage *A. crassus* nematodes following removal from the swim bladder (B).

Table 5.1 Primary infection data for *A. crassus* nematodes in European eels sampled from 14 catchment sites across England and Wales. Ten eels were examined from each site. * = data extracted from Ab Aziz (2012).

Eel catchment sites	Prevalence (%)	Total number of *A. crassus*	Mean abundance (± sd)	Mean infection intensity (± sd)	Intensity range
Leven: RL England	70	34	3.4±3.4	4.9±3.1	1 - 9
Crouch: C England*	60	29	2.9±3.7	4.8±3.7	1 - 10
Crane: CN England	40	15	1.5±3.4	3.8±4.9	1 - 11
Gowy: RG England*	40	16	1.6±2.2	4±1.2	3 - 5
Bela: B England*	0	0	0	0	0
Petteril: RP England	0	0	0	0	0
Hether Burn: HB England*	0	0	0	0	0
Cadoxton: CD Wales*	70	18	1.8±1.8	2.6±1.5	1 - 5
Rhymney: R Wales*	60	21	2.1±2.1	3.5±1.5	2 - 6
Taff: TB Wales*	10	2	0.2±0.6	2	2
Clwyd-Meirchion: MC Wales*	0	0	0	0	0
Dee-Eitha: D Wales*	0	0	0	0	0
Clwyd-Elwy: CE Wales*	0	0	0	0	0
Mawddach-eden: M Wales*	0	0	0	0	0
	25%	135	0.96±2.2	3.9±2.7	1 - 11

Overall, 135 *A. crassus* were collected from the sampled population of eels (mean abundance = 0.96 sd±2.2; mean intensity =3.9 sd±2.7) and the parasites were over-dispersed (dispersion index = 4.6) (Figure 5.2). The number of *A. crassus* extracted from individual eels ranged between 1 and 11; the latter being isolated from a fish sampled from the river Crane (specimen CN6). A single *A. crassus* was extracted from 17.1% (6/35) of the infected eels. The majority, 94.3% (33/35), of the infected eels carried less than 10 *A. crassus* and hence only 5.7% (2/35) of the hosts harboured 10+ swim bladder nematodes (Table 5.2). With respect to the sampling sites, the River Leven yielded most *A. crassus* (n = 34) and the lowest number was recovered from the River Taff (n = 2).

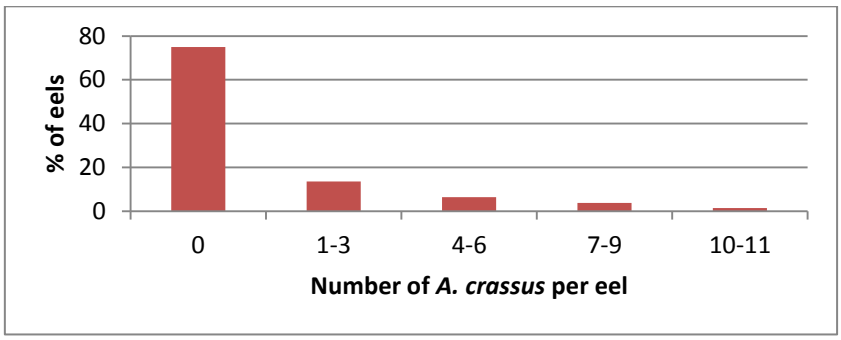

Figure 5.2 Dispersion of *A. crassus* within the sampled eel population.

Table 5.2 Eel morphometric and associated *A. crassus* infection data for positive sampling sites in England and Wales. Ten eels were examined from each site.

Eel catchment sites	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of *A. crassus*
Leven: RL England	RL1	21	13.9	0.16	4
	RL2	52	18.7	0.13	9
	RL3	27	35.8	0.18	7
	RL6	35	71.3	0.17	1
	RL7	27	26.3	0.13	7
	RL9	30	38.1	0.14	1
	RL10	26	22.3	0.13	5
Crouch: C England	C157	33	65.8	0.18	7
	C107	32	47	0.14	7
	C117	28	27.5	0.13	1
	C59	32	50.1	0.15	10
	C71	46	198.2	0.19	2
	C48	31	44.7	0.15	2
Crane: CN England	CN5	70	668.6	0.19	1
	CN6	74	839.7	0.21	11
	CN9	69	563.5	0.17	2
	CN10	74	743.4	0.18	1
Gowy: RG England	RG1	39	73.6	0.12	3
	RG2	37	61.7	0.12	3
	RG6	32	41.8	0.13	5
	RG8	33	62.2	0.17	5
River Cadoxton: CD Wales	CD1	33	59.9	0.17	4
	CD2	27	30.4	0.15	1
	CD3	24	21.4	0.06	3
	CD4	28	28.3	0.13	5
	CD5	28.5	32.6	0.05	2
	CD8	20	11	0.27	2
	CD9	21	8.8	0.09	1
River Rhymney: R Wales	R1	32	48.9	0.15	4
	R3	30	42.3	0.16	6
	R4	31.5	67.2	0.22	3
	R6	30	39.7	0.15	2
	R8	30	43.9	0.16	4
	R10	23.5	18.5	0.14	2
River Taff: TB Wales	TB1	20	10.2	0.13	2

Upon analysis of the *A. crassus* infection data at the regional level, it was apparent that all the catchment sites in North Wales appeared to contain European eels that were free of the swim bladder nematode (Table 5.3). In contrast, the greatest prevalence, and also the greatest mean abundance, and intensity of *A. crassus* infection, was observed in eels sampled from rivers in South East England. In addition, all catchment sites in South Wales were positive for *A. crassus* infections. In contrast, the majority of the catchment sites in North West England (rivers Bela, Petteril and Hether Burn) contained eels that appeared to be free of *A. crassus* infections.

Statistically, there was a significant difference in the *A. crassus* prevalence data between all the examined regions with the exception of South East England and South Wales (*p:* 10^{-7}-0.0141). The *A. crassus* abundance data was also significantly different between North West England and South East England ($p = 0.032$). However, there was no significant difference between the *A. crassus* abundance data from South Wales and either region in England (*p*: 0.06 – 0.8). With respect to mean intensity, there was no significant difference between any of the regional data ($p > 0.09$).

Table 5.3 Regional infection data for *A. crassus* isolated from European eel sampled across England and Wales.

Regions	Number of river sites examined	Number of *A. crassus* positive sites	Number of examined eels	Number of infected eels	Prevalence (%)	Number of *A. crassus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	2	50	11	22%	50	1±2.2	4.5±2.5	1 - 9
South East England	2	2	20	10	50%	44	2.2±3.5	4.4±3.9	1 - 11
North Wales	4	0	40	0	0	0	0	0	0
South Wales	3	3	30	14	46.7%	41	1.4±1.8	2.9±1.5	1 - 6
Total	14	7	140	35	25%	135	0.9±2.2	3.9±2.7	1 - 11

5.4.1.2 *A. crassus* primary infection data: the host

For the *A. crassus* infected eels, the body length ranged from 20 to 74 cm (mean = 35.1 ±14.9 cm) and body weight ranged from 8.8 to 839.9 g (mean = 124.1±217.8 g). For uninfected eels, the body length ranged from 10 to 86 cm (mean = 29.3±14.7 cm) and body weight ranged from 1.2g to 1380.3 g (mean = 84.0±210.4 g). These differences in body length and weight between the infected and uninfected eels were highly significant ($p = 0.003$ and $p = 0.004$ respectively). The mean condition factor for *A. crassus* infected eels was 0.14±0.04, whilst the mean condition factor for the uninfected eels was 0.15±0.04; this was not significant ($p = 0.81$).

Upon analysis of different categories of eel length it was apparent that prevalence, as well as mean *A. crassus* abundance and intensity, generally increased as the eel body length category increased; however, this trend was not observed with the largest length category of eel (Table 5.4). Indeed, the second largest length category of eel had a significant difference in prevalence of *A. crassus* when compared to the two smallest length categories (*p*: 0.006-0.011). There was also a significant difference in *A. crassus* prevalence between eels of length category 25.5-30 cm and the smallest length category ($p = 0.025$).

Table 5.4 *A. crassus* infection data with respect to eel body length category.

Body length range (cm)	Eels examined	Infected eels	Prevalence (%)	Number of *A. crassus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	2	7.7	4	0.2±10.5	2±0	2
20.5-25	32	4	12.5	10	0.3±0.9	2.5±1.3	1-4
25.5-30	32	11	34.4	41	1.3±2.3	3.7±2.4	1-7
30.5-35	22	10	45.5	48	2.2±3.0	4.8±2.7	1-10
35.5-86	28	8	28.6	32	1.1±2.7	4.0±3.8	1-11

With respect to intensity of *A. crassus* infection, there was no significant difference (*p*: 0.12 – 0.93), between any of the different length categories of eel.

Upon analysis of different categories of eel condition factor it was apparent that prevalence, as well as mean *A. crassus* abundance and intensity, generally increased as the eel condition factor increased (Table 5.5). However, there was no significant differences in either the prevalence data (*p*: 0.53 - 1), or the intensity data (*p*: 0.19 – 0.86), between the different condition factor categories.

Table 5.5 *A. crassus* infection data with respect to eel condition factor category.

Condition factor range	Eels examined	Infected eels	Prevalence (%)	Number of *A. crassus*	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.04-0.12	33	7	21.2	20	0.6±1.3	2.9±0.9	2-5
0.13-0.14	37	9	24.3	39	1.1±2.3	4.3±2.8	1-9
0.15-0.16	27	7	25.9	32	1.2±2.4	4.6±2.6	2-10
0.17-0.29	43	12	27.9	44	1.0±2.3	3.7±3.1	1-7

5.4.2 Primary helminth infection data

All 14 catchment sites were positive for helminth infections and in total, 101 of the 140 (72.1%) European eels examined contained helminths (Table 5.6). As such, helminth parasites were not recovered from 39 (27.9%) eels and this subset was sampled from 4 English and 6 Welsh river systems (Table 5.7). As such, this uninfected group of eels only lacked specimens from South East England (rivers Crane and Crouch), one catchment site from North West England (river Leven) and 1 site from South Wales (river Rhymney). The mean body length and body weight of the uninfected eels was 23.6±7.9 cm and 25.5±30.2 g respectively. These morphometric parameters were significantly different to the mean eel length and weight measurements (33.6±16.0 cm and 120.8±244.2 g) for the 101 eels positive for helminth infections ($p = 10^{-4}$). In addition, there was a significant difference ($p = 0.009$) in the mean condition factor of the uninfected eels (0.14±0.02) compared to the helminth infected group (0.15±0.05).

At sites positive for helminth infections, the prevalence ranged from 20 to 100%. The rivers Crouch, Crane, Leven and Rhymney showed the highest prevalence of infection (100%), whereas the lowest prevalence (20%) was observed in the river Mawddach-eden. These sites with maximal helminth prevalence, and also the site with the lowest helminth prevalence, were statistically significantly different to the mean prevalence (72.9%) of the eel population ($p = 10^{-7}$ and $p = 0.002$ respectively).

Table 5.6 Primary infection data for helminth parasites in European eels sampled from 14 catchment sites across England and Wales. Ten eels were examined from each site and the full infection profiles are within the Appendix. Mean helminth intensity, or abundance, assessed as significantly different ($p < 0.05$) as follows: * relative to the river Crouch, # relative to the river Crane, ^ relative to the river Leven, $^{+}$ relative to the river Rhymney.

Eel catchment sites	Prevalence (%)	Total number of helminths	Mean helminth abundance (± sd)	Mean helminth intensity (± sd)
Crouch: C England	100	352	35.2±29.5	35.2±29.5
Crane: CN England	100	329	32.9±19.4	32.9±19.4
Leven: RL England	100	305	30.5±43.1	30.5±43.1
Petteril: RP England	90	85	8.6±7.5*#	10.8±6.8*#
Bela: B England	70	50	4.9±7.4*#^$^{+}$	7±8.0*#^
Gowy: RG England	60	26	2.6±3.3*#^$^{+}$	4.3±3.2*#$^{+}$
Hether Burn: HB England	50	51	5.1±11.1*#^$^{+}$	10.2±14.6
Rhymney: R Wales	100	124	12.4±8.5#	12.4±8.5#
Cadoxton: CD Wales	80	83	8.3±9.5*#	10.4±9.5*#
Clwyd-Meirchion: MC Wales	80	17	1.7±1.6*#^$^{+}$	2.1±1.5*#^$^{+}$
Taff: TB Wales	60	22	2.2±3.4*#^$^{+}$	3.1±3.8*#^$^{+}$
Clwyd-Elwy: CE Wales	60	25	2.5±3.5*#^$^{+}$	4.2±3.7*#^$^{+}$
Dee-Eitha: D Wales	40	29	2.9±7.2*#^$^{+}$	7.3±10.6*#
Mawddach-eden: M Wales	20	10	1±2.8*#^$^{+}$	5±5.7
	72.1	1504	10.7±19.2	14.9±21.3

Table 5.7 Eel morphometric data for non-helminth infected eels. These specimens were sampled from 10 of the 14 catchment sites in England and Wales.

Eel catchment sites	Eel codes	Body length (cm)	Body weight (g)	Condition factor
Mawddach-eden: M Wales	M1	45	154.6	0.17
Mawddach-eden: M Wales	M2	41	91.9	0.13
Mawddach-eden: M Wales	M3	25	21.8	0.14
Mawddach-eden: M Wales	M4	22	14.4	0.14
Mawddach-eden: M Wales	M5	26.5	25.4	0.14
Mawddach-eden: M Wales	M7	21	14.1	0.15
Mawddach-eden: M Wales	M8	18	7.5	0.13
Mawddach-eden: M Wales	M10	11	1.7	0.13
Dee-Eitha: D Wales	D2	32.5	60.2	0.17
Dee-Eitha: D Wales	D3	25.5	17.7	0.11
Dee-Eitha: D Wales	D4	32	55.6	0.17
Dee-Eitha: D Wales	D6	12	1.5	0.09
Dee-Eitha: D Wales	D7	15	3.1	0.09
Dee-Eitha: D Wales	D8	18	7.1	0.12
Clwyd-Elwy: CE Wales	CE2	24	17.5	0.13
Clwyd-Elwy: CE Wales	CE6	20.5	11.6	0.13
Clwyd-Elwy: CE Wales	CE7	10	1.2	0.12
Clwyd-Elwy: CE Wales	CE10	19.5	8	0.13
Clwyd-Meirchion: MC Wales	MC9	18	10.5	0.18
Clwyd-Meirchion: MC Wales	MC10	13.5	2.8	0.11
Taff: TB Wales	TB3	28	33	0.15
Taff: TB Wales	TB5	21.5	15.5	0.16
Taff: TB Wales	TB8	28	35.2	0.16
Taff: TB Wales	TB10	25	24.7	0.16
Cadoxton: CD Wales	CD7	25	17.1	0.19
Cadoxton: CD Wales	CD10	20	7.9	0.09
Hether Burn: HB England	HB1	21	12.2	0.13
Hether Burn: HB England	HB2	27	23.6	0.12
Hether Burn: HB England	HB6	16	4.8	0.12
Hether Burn: HB England	HB8	19	8.1	0.12
Hether Burn: HB England	HB9	22.5	13.3	0.12
Bela: B England	B5	21	13.2	0.14
Bela: B England	B6	23	13.3	0.11
Bela: B England	B9	24	18.3	0.13
Gowy: RG England	RG3	36	63.2	0.14
Gowy: RG England	RG4	36	58.9	0.13
Gowy: RG England	RG7	35	70.5	0.16
Gowy: RG England	RG9	26	27.1	0.15
Petteril: RP England	RP7	14.5	5.2	0.17
Mean		23.6±7.9	25.5±30.2	0.14±0.02

5.4.2.1 Primary helminth infection data: the environment

Overall, 1504 helminths were collected from the infected eels (mean abundance = 10.7 ± 19.3; mean intensity =14.9 ± 21.3) (Table 5.6). The number of helminths extracted from individual eel specimens ranged between 1 and 135; the latter being isolated from a fish (RL2) sampled from the river Leven (Table 5.9). The mean intensity of helminth infection at the different sites, ranged from 2.1±1.5 to 35.2±29.5 parasites; the former in eels from the river Clwyd-Meirchion in Wales and the latter from the river Crouch in England. Indeed, the rivers with the two highest (Crouch and Crane) and two lowest (Clwyd-Meirchion and Taff) mean intensity data were significantly different to the mean helminth intensity data for the eel population (*p*: 0.0018 – 0.018). The mean helminth abundance at the catchment sites ranged from 1±2.8 (river Mawddach-eden) to 35.2±29.5 (river Crouch). Overall, the mean helminth abundance for the eel population (10.7 ± 19.3) differed significantly to the mean helminth abundance at all catchment sites with 100% prevalence (*p*: 0.0002 - 0.027) and also, to the two rivers with the lowest prevalence data (*p*: 0.002 – 0.02).

With respect to mean helminth intensity, there were statistically significant differences between the data for eels sampled from the rivers Crouch, Crane, Leven and Rhymney and a number of the other catchment sites (*p*: 0.003 – 0.041) (Table 5.6).

With respect to mean helminth abundance, there were also statistically significant differences between the data for eels sampled from the rivers Crouch, Crane, Leven and Rhymney and a number of the other catchment sites (*p*: 0.0002 – 0.017) (Table 5.6).

A single helminth was extracted from 17.8% (18/101) of the infected eels. Moreover, the majority (61/101; 60.4%) of the infected hosts carried less than 10 helminths whereas 39.6% (40/101) of the infected eels harboured 10, or more helminths (Tables 5.8 and 5.9). With respect to the sampling sites, the river Crouch yielded most helminths (n = 352) whereas the

lowest number was recovered from the river Mawddach-eden (n = 10). Overall, the helminths were over-dispersed (dispersion index = 34.8) (Figure 5.3).

Table 5.8 Eel morphometric data for animals with helminth infections sampled from sites across Wales. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of helminths	Number of helminth taxa
Mawddach-eden	M6	28	36.9	0.17	1	1
	M9	23	15.5	0.13	9	1
Clwyd-Meirchion	MC1	42	104.4	0.14	1	1
	MC2	30	38.8	0.14	5	1
	MC3	35	54.6	0.13	2	2
	MC4	25	26.8	0.17	1	1
	MC5	19.5	21.8	0.29	3	1
	MC6	18.5	9.0	0.14	1	1
	MC7	22	12.0	0.11	3	1
	MC8	15.5	5.3	0.14	1	1
Dee-Eitha	D1	28	27.6	0.13	1	1
	D5	14	2.4	0.09	23	1
	D9	14.5	1.2	0.04	4	1
	D10	25.5	21.6	0.13	1	1
Cadoxton	CD1	33	59.9	0.17	18	2
	CD2	27	30.4	0.15	6	2
	CD3	24	21.4	0.06	12	3
	CD4	28	28.3	0.13	7	2
	CD5	28.5	32.6	0.05	6	2
	CD6	22	11.0	0.10	2	1
	CD8	20	9.2	012	2	1
	CD9	21	8.8	0.09	30	2
Rhymney	R1	32	48.9	0.15	10	3
	R2	31	48.6	0.16	10	1
	R3	30	42.3	0.16	31	3
	R4	31.5	67.2	0.22	21	3
	R5	27	29.3	0.15	13	2
	R6	30	39.7	0.15	5	2
	R7	29	53.5	0.22	1	1
	R8	30	43.9	0.16	11	2
	R9	23.5	12.7	0.10	15	2
	R10	23.5	18.5	0.14	7	2
Clwyd-Elwy	CE1	39	72.9	0.12	5	2
	CE3	31	46	0.15	1	1
	CE4	34	70.1	0.18	11	1
	CE5	29	39.3	0.16	3	2
	CE8	22	16.8	0.16	1	1
	CE9	20	10.9	0.14	4	2
Taff	TB1	20	10.2	0.13	11	2
	TB2	25	33.5	0.21	1	1
	TB4	22	15.6	0.15	2	2
	TB6	24	24.4	0.18	1	1
	TB7	25	21.6	0.16	5	1
	TB9	19	24.7	0.15	2	1

Table 5.9 Eel morphometric data for animals with helminth infections sampled from sites across England. Ten eels were examined from each site.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of helminths	Number of helminth taxa
Crouch	C157	33	65.8	0.18	79	4
	C107	32	47	0.14	28	3
	C117	28	27.5	0.13	37	3
	C59	32	50.1	0.15	27	3
	C74	50	209.2	0.17	55	2
	C116	29	36.4	0.15	4	2
	C71	47	198.2	0.19	21	3
	C131	39	112	0.19	83	2
	C40	32	43.9	0.13	7	2
	C48	31	44.7	0.15	7	3
Leven	RL1	21	13.9	0.16	17	3
	RL2	52	187.0	0.13	135	3
	RL3	27	35.8	0.18	75	4
	RL4	22.5	21.3	0.19	2	1
	RL5	34	55.9	0.14	13	2
	RL6	35	71.3	0.17	6	3
	RL7	27	26.3	0.13	7	1
	RL8	29.5	30.3	0.11	8	2
	RL9	30	38.1	0.14	3	3
	RL10	26	22.3	0.13	39	3
Crane	CN1	70	682.3	0.21	56	1
	CN2	74	702.5	0.17	24	1
	CN3	86	1380.3	0.22	32	3
	CN4	77	1058.8	0.23	39	1
	CN5	70	668.6	0.19	34	5
	CN6	74	839.7	0.21	33	4
	CN7	86	805.7	0.13	32	2
	CN8	77	661.9	0.14	6	4
	CN9	69	563.5	0.17	5	3
	CN10	74	743.4	0.18	68	4
Hether Burn	HB3	23	14.7	0.12	5	2
	HB4	24	16	0.12	2	1
	HB5	25	18.6	0.12	36	2
	HB7	20	7.8	0.09	1	1
	GB10	16.5	11.1	0.15	7	1
Petteril	RP1	36	110.7	0.24	22	1
	RP2	31.5	82.8	0.28	18	2
	RP4	28	35.3	0.13	4	1
	RP5	35.5	74.9	0.17	6	3
	RP6	27.5	38.2	0.18	5	2
	RP8	43	101.1	0.07	12	2
	RP9	33	98.3	0.27	14	1
	RP10	26	29.7	0.17	4	2
Bela	B1	30	42.0	0.16	5	1
	B2	32	49.1	0.15	9	2
	B3	27	33.0	0.17	1	1
	B4	19.5	11.9	0.16	1	1
	B7	24	22.2	0.16	6	1
	B8	48	204.5	0.18	24	3
	B10	49	209.0	0.18	4	1
Gowy	RG1	39	73.6	0.12	3	1
	RG2	37	61.7	0.12	3	1
	RG5	40	65.7	0.10	3	1
	RG6	32	41.8	0.13	6	2
	RG8	33	62.2	0.17	10	2
	RG10	27	24.4	0.12	1	1

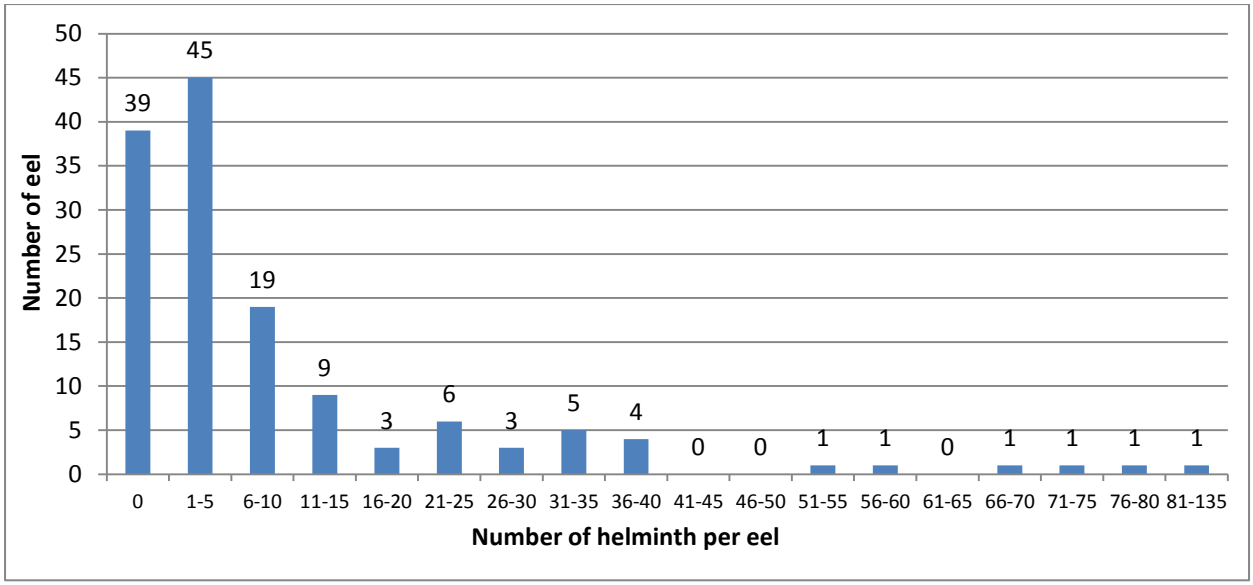

Figure 5.3 Dispersion of helminths within the sampled eel population.

Overall, the helminth prevalence data showed that infection was much more common in the eel population than non-infection (Table 5.10). Moreover, the pseudodactylids were the most prevalent helminth observed (35.7%) and also, the most dominant parasite within the eel population (n = 619; 41.2%). The pathogenic *A. crassus* also had relatively high prevalence and parasite numbers within the eel population (Table 5.10). The gastrointestinal nematodes and acanthocephalans were also highly prevalent and they were recovered from the eel population in large numbers. The least prevalent and least represented helminth class in terms of numbers isolated from the eel population was the tapeworms.

On examination of the helminth infection profile at the level of different helminth class, it was apparent that multiple different taxa were present in the majority of the river systems (Table 5.10). Indeed, the river Crane harboured eels with the most diverse array of helminth taxa (n = 5); pseudodactylids, *A. crassus*, GI tract nematodes, tapeworms and acanthocephalans. Four helminth taxa were commonly observed in the English catchment sites but in only one Welsh river, the Taff, in South Wales. The most common number of

helminth taxa observed at the Welsh catchment sites was 3; as exemplified by the river Clwyd-Elwy in North Wales and the rivers Rhymney and Cadoxton in South Wales. Only two different helminth taxa, the GI tract nematodes and tapeworms, were observed in eels sampled from the Clwyd-Meirchion in North Wales. Single taxa infections, both being of GI tract nematodes, were observed in one river in North Wales, and a further river in South Wales.

In total, 1504 helminths were recovered from the examined eels and pseudodactylids were the most common (n = 619). Indeed, pseudodactylids were the dominant class recovered from eels at 5 of the catchment sites; 3 Welsh rivers and 2 English rivers. Significant numbers of acanthocephalans (n = 469) and gastrointestinal nematodes (n = 263) were also extracted from the eels. The gastrointestinal nematodes were the dominant helminth class recovered from eels at 5 catchment sites; 3 Welsh rivers and 2 English rivers. Indeed at two of these Welsh sites, gastrointestinal nematodes were the only helminth recovered from eels. Acanthocephalans were the dominant helminth class recovered from eels at 3 catchment sites. Indeed, two of these sites, the rivers Crouch and Crane, provided 82.1% of all the acanthocephalans extracted from the eel population. The pathogenic *A. crassus* was the dominant helminth recovered from eels at one catchment site, the river Gowy, in North West England.

Table 5.10 Summary of the helminth infection data at the sampling sites across England and Wales. In total, 140 eels were analysed.

Eel Catchment Site	Number of eel without helminth infection	Number of eel infected with helminths	Number of pseudodactylids	Number of *A. crassus*	Number of GI nematodes	Number of tapeworms	Number of acanthocephalans	Number of helminths	Number of helminth taxa
Crouch: C England	0	10	141	29	0	1	177	348	4
Leven: RL England	0	10	232	34	35	0	4	305	4
Crane: CN England	0	10	74	15	26	6	208	329	5
Petteril: RP England	2	8	3	0	75	3	4	85	4
Bela: B England	3	7	5	0	40	1	4	50	4
Gowy: RG England	4	6	0	16	5	1	4	26	4
Hether Burn: HB England	5	5	33	0	16	2	0	51	3
Rhymney: R Wales	0	10	53	21	0	0	50	124	3
Cadoxton: CD Wales	2	8	60	18	5	0	0	83	3
Clwyd-Meirchion: MC Wales	2	8	0	0	14	3	0	17	2
Taff: TB Wales	3	7	1	2	1	0	18	22	4
Clwyd-Elwy: CE Wales	4	6	17	0	7	1	0	25	3
Dee-Eitha: D Wales	6	4	0	0	29	0	0	29	1
Mawddach-eden: M Wales	8	2	0	0	10	0	0	10	1
Prevalence	27.9%	72.1%	35.7%	25%	33.6%	9.3%	30%		
Total	39	101	619	135	263	18	469	1504	5

Upon analysis of the complete helminth infection data at the regional level, it was apparent that all examined eels in the two South East England catchment sites were positive for helminth infection (Table 5.11). As such, the greatest prevalence, and also the greatest mean abundance, and intensity of helminth infection, was observed in eels sampled from the rivers in South East England. At the remaining regions, prevalence of helminth infection varied between 50% and 83%. Overall, the regional helminth prevalence data was significantly different between North Wales and the other three geographic regions (p: 10^{-4}-0.048). There

was also a highly significant difference between prevalence data for South East England and both North West England and North Wales ($p = 10^{-4}$).

The region of North Wales had the lowest helminth abundance and intensity data; however, this data was only significantly different to that for South East England ($p = 10^{-4}$). Indeed, the abundance and intensity data observed for South East England was also significantly different compared to that for South Wales and North West England ($p = 10^{-4}$).

Table 5.11 Regional infection data for helminths isolated from European eel sampled across England and Wales.

Regions	Number of river sites examined	Number of helminth positive sites	Number of examined eel	Number of infected eel	Helminth prevalence (%)	Number of helminths	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
North West England	5	5	50	36	72%	517	10.3±22.2	14.4±25.1	1 - 135
South East England	2	2	20	20	100%	677	33.9±23.9	33.9±23.9	4 - 83
North Wales	4	4	40	20	50%	81	2.1±4.2	4.2±5.3	1 - 23
South Wales	3	3	30	25	83.3%	229	7.6±8.5	9.2±8.5	1 - 30
Total	14	14	140	101	72.1%	1504	10.7±19.3	14.9±21.3	1- 135

5.4.2.2 Primary helminth infection data: the host

For the helminth infected eels, the body length ranged from 14 to 86 cm (mean = 33.5 ±16.0 cm), body weight ranged from 1.2 to 1380.3 g (mean = 120±244.3 g) and the mean eel condition factor was 0.15±0.05. These length and weight parameters were significantly different to the 39 eels lacking a helminth infection (Table 5.7) ($p = 10^{-4}$). In addition, the mean condition factor for the uninfected eels (Table 5.7) was also significantly different to that of the helminth infected specimens ($p = 0.011$).

Upon analysis of different categories of eel length it was apparent that prevalence, as well as mean helminth abundance and intensity, generally increased as the eel body length category increased (Table 5.12). Indeed, the prevalence of helminth infection was significantly different between the two largest length category of eels and the two smallest length categories (*p*: 0.0364 – 0.0034). With respect to intensity of helminth infection, there were significant differences between the largest length category of eel and the two smallest length categories of eel (*p*: 0.0010 – 0.0039).

Table 5.12 Helminth infection data with respect to eel body length category.

Body length range (cm)	Eels examined	Infected eels	Prevalence (%)	Number of helminths	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
10-20	26	12	46.2	60	2.3±4.9	5±6.4	1 - 23
20.5-25	32	19	59.4	156	4.9±8.7	8.2±10.0	1 - 36
25.5-30	32	27	84.4	285	8.9±15.7	10.6±16.6	1 - 75
30.5-35	22	19	86.4	297	13.5±16.7	15.6±17.1	1 - 79
35.5-86	28	24	85.7	706	25.2±31.2	29.4±31.9	1 - 135

Upon analysis of different categories of eel condition factor it was apparent that prevalence, as well as mean helminth abundance and intensity, generally increased as the eel condition factor category increased (Table 5.13). There were significant differences (*p*: 0.01-0.03) in the prevalence of helminth infection between eels with the greatest condition factor category and the two smallest condition factor categories. There was no significant differences (*p*: 0.065 – 0.62) in the intensity of helminth infection between the different eel condition factor categories.

Table 5.13 Helminth infection data with respect to eel condition factor category.

Condition factor range	Eels examined	Infected eels	Prevalence (%)	Number of helminths	Mean abundance (±sd)	Mean intensity (±sd)	Intensity range
0.04-0.12	33	21	56.6	196	5.9±8.8	9.3±9.5	1 - 36
0.13-0.14	37	22	59.5	354	9.6±23.7	16.1±29.2	1 - 135
0.15-0.16	27	21	77.8	169	6.3±8.0	8.0±8.3	1 – 31
0.17-0.29	43	37	86.0	785	18.3±23.8	21.2±24.4	1 - 83

5.4.3 Helminth community structure analysis

Overall, the majority of eels examined were either infected with one class of helminth, or they were not infected (Table 5.14). Helminth co-infections occurred less frequently (n = 55; 39.5%) and not surprisingly, there was a negative correlation between the number of classes of helminth contributing to the co-infection and the proportion of eels harbouring these multiple classes of parasite.

Table 5.14 Summary of the helminth infection profile within the population of eels sampled across England and Wales. In total, 140 eels were analysed.

Number of helminth taxa	Number of eels	% of eel population
0	39	27.9%
1	46	32.9%
2	31	22.1%
3	18	12.9%
4	5	3.8%
5	1	0.7%

The known pathogens, *A. crassus*, and the monogenean pseudodactylids, were infrequently present as single taxon helminth infections (2.9% and 3.8 % respectively) (Table 5.15). The most commonly observed group of parasites responsible for single taxon helminth infection was the gastrointestinal nematodes (24/140, 17.1%) and this was followed by the acanthocephalans (7.9%). The least commonly encountered single helminth taxon infection was not surprisingly attributed to tapeworms (1.4%).

Table 5.15 Summary of the single taxon helminth infection profiles within the population of eels sampled across England and Wales. In total, 46 of the 140 analysed eels contained a single helminth taxon.

Helminth taxa	Number of infected eels	% of total eel population	Mean intensity (±sd)	Intensity range
Gastrointestinal nematodes	24	17.1%	5.7±6.6	1-23
Acanthocephalans	11	7.9%	13±18.8	1-56
Pseudodactylids	5	3.8%	3.4±4.3	1-11
A. crassus	4	2.9%	6±5.4	2-7
Tapeworms	2	1.4%	1	1
Total	46	32.9%		

With respect to parasite intensity for single helminth taxa, the range observed was highly variable and was greatest for the acanthocephalan infections (Table 5.15). Although the gastrointestinal nematode infections also exhibited a variable intensity range, a substantial number (9/24; 37.5%) were observed as single worm infections (mean intensity = 5.7±6.6).

5.4.3.1 Helminth community structure analysis: the host

For the 46 eels that were infected with one helminth taxon the body length ranged from 14 to 77 cm (mean = 29.3±14.0 cm), body weight ranged from 1.2g to 1058.8 g (mean = 87.1±202.7 g) and the mean condition factor was 0.16±0.05. The differences in mean body length and weight and also, mean condition factor, between the uninfected eels (Table 5.7) and eels infected eels with one helminth taxon were significant ($p = 0.0398$, $p = 0.0147$ and $p = 0.0172$ respectively).

On closer inspection of these single helminth taxon infections, it was apparent that in 17 eels, these infections comprised a single parasite (2 pseudodactylids, 9 gastrointestinal nematodes, 4 acanthocephalans and 2 tapeworms) and there were no significant differences ($p > 0.09$) in eel morphometric parameters relative to the uninfected group (Table 5.7). The remaining 29 eels with single taxon helminth infections harboured between 2 and 56 worms. The mean length and mean weight of these infected eels was 31.7±16.7 cm and 121.0±250.0 g respectively and these measurements were significantly different ($p = 0.038$ and $p = 0.018$) to the group of uninfected eels (Table 5.7).

For the 5 eels that were infected with just pseudodactylids the body length ranged from 20 to 34 cm (mean = 24.4±5.5 cm), body weight ranged from 7.8g to 70.1 g (mean = 24.3±25.8 g) and the mean condition factor was 0.16±0.07 (Table 5.16). The differences in mean body length and weight and also, mean condition factor, between the uninfected eels (Table 5.7)

and eels infected eels with just pseudodactylids were not significant (p = 0.78, p = 0.94 and p = 0.46 respectively).

Table 5.16 Summary of the eel morphometric data for fish infected with just pseudodactylids.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of pseudodactylids
Clwyd-Elwy: CE Wales	CE4	34	70.1	0.18	11
Clwyd-Elwy: CE Wales	CE8	22	16.8	0.16	1
Cadoxton: CD Wales	CD6	22	11	0.27	2
Hether Burn: HB England	HB4	24	16	0.12	2
Hether Burn: HB England	HB7	20	7.8	0.09	1
Mean		24.4±5.5	24.3±25.8	0.16±4.3	3.4±4.3

For the 4 eels that were infected with just *A. crassus* the body length ranged from 20 to 39 cm (mean = 30.8±8.9 cm), body weight ranged from 9.2 g to 73.6 g (mean = 42.7±30.0 g) and the mean condition factor was 0.12±0.005 (Table 5.17). The differences in mean body length and weight and also, mean condition factor, between the uninfected eels (Table 5.7) and eels infected just *A. crassus* were not significant (p = 0.12, p = 0.15 and p = 0.15 respectively).

Table 5.17 Summary of the eel morphometric data for fish infected with just *A. crassus*.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of *A. crassus*
Leven: RL England	RL7	27	26.3	0.13	7
Gowy: RG England	RG1	39	73.6	0.12	3
Gowy: RG England	RG2	37	61.7	0.12	3
Cadoxton: CD Wales	CD8	20	9.2	0.12	2
Mean		30.8±8.9	42.7±30.0	0.12±0.005	3.8±2.2

For the 2 eels infected with just tapeworms the body length ranged from 25 to 42 cm (mean = 33.5±12.0 cm), body weight ranged from 26.8 g to 104.4 g (mean = 65.6±54.9 g) and the mean condition factor was 0.16±0.02. The differences in mean body length and weight and also, mean condition factor, between the uninfected eels (Table 5.7) and eels infected with just tapeworms were not significant *(*p = 0.16, p = 0.10 and p = 0.25 respectively).

For the 24 eels that were infected with just gastrointestinal nematodes the body length ranged from 14.5 to 49 cm (mean = 24.8±7.9 cm), body weight ranged from 1.2 g to 209 g (mean = 35.7±45.6 g) and the mean condition factor was 0.16±0.06 (Table 5.18). The differences in mean body length and weight between the uninfected eels (Table 5.7) and eels infected with just gastrointestinal nematodes were not significant *(*p = 0.49 and p = 0.23 respectively). However, there was a significant difference in the mean condition factor between the uninfected eels and those with just gastrointestinal nematode infections (p = 0.027).

Table 5.18 Summary of the eel morphometric data for fish infected with just gastrointestinal nematodes.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of Gut nematodes
Mawddach-eden: M Wales	M6	28	36.9	0.17	1
Mawddach-eden: M Wales	M9	23	15.5	0.13	9
Clwyd-Meirchion: MC Wales	MC2	30	38.8	0.14	5
Clwyd-Meirchion: MC Wales	MC5	19.5	21.8	0.29	3
Clwyd-Meirchion: MC Wales	MC6	18.5	9	0.14	1
Clwyd-Meirchion: MC Wales	MC7	22	12	0.11	3
Clwyd-Meirchion: MC Wales	MC8	15.5	5.3	0.14	1
Clwyd-Elwy: CE Wales	CE3	31	46	0.15	1
Dee-Eitha: D Wales	D1	28	27.6	0.13	1
Dee-Eitha: D Wales	D5	14	2.4	0.09	23
Dee-Eitha: D Wales	D9	14.5	1.2	0.04	4
Dee-Eitha: D Wales	D10	25.5	21.6	0.13	1
Taff: TB Wales	TB4	22	15.6	0.15	1
Petteril: RP England	RP1	36	110.7	0.24	22
Petteril: RP England	RP3	18.5	8.2	0.13	16
Petteril: RP England	RP4	28	35.3	0.16	4
Petteril: RP England	RP9	33	98.3	0.27	14
Hether Burn: HB England	HB10	16.5	11.1	0.25	7
Leven: RL England	RL4	22.5	21.3	0.19	2
Bela: B England	B1	30	42	0.16	5
Bela: B England	B3	27	33	0.17	1
Bela: B England	B4	19.5	11.9	0.16	1
Bela: B England	B7	24	22.2	0.16	6
Bela: B England	B10	49	209	0.18	4
Mean		24.8±7.9	35.7±45.6	0.16±0.06	5.7±6.6

For the 11 eels that were infected with just acanthocephalans the body length ranged from 19 to 77 cm (mean = 40.1±22.2 cm), body weight ranged from 10.6 g to 1058.8 g (mean = 247.8±376.4 g) and the mean condition factor was 0.17±0.04 (Table 5.19). Interestingly, the differences in mean body length and weight and also, mean condition factor, between the uninfected eels (Table 5.7) and eels infected with just acanthocephalans were significant *(p* = 0.0075, *p* = 0.0034 and *p* = 0.0113 respectively).

Table 5.19 Summary of the eel morphometric data for fish infected with just acanthocephalans.

Catchment sites location	Infected eel codes	Eel body length (cm)	Eel body weight (g)	Condition factor	Numbers of acanthocephalans
Taff: TB Wales	TB2	25	33.5	0.21	1
Taff: TB Wales	TB6	24	24.4	0.18	1
Taff: TB Wales	TB7	25	21.6	0.14	5
Taff: TB Wales	TB9	19	10.6	0.15	2
Rhymney: R Wales	R2	31	48.6	0.16	10
Rhymney: R Wales	R7	29	53.5	0.22	1
Gowy: RG England	RG5	40	65.7	0.1	3
Gowy: RG England	RG10	27	24.4	0.12	1
Crane: CN England	CN1	70	682.3	0.21	56
Crane: CN England	CN2	74	702.5	0.17	24
Crane: CN England	CN4	77	1058.8	0.23	39
Mean		40.1±22.2	247.8±376	0.17±0.04	13±18.8

For the 31 eels that were co-infected with two helminth taxa, the dominant class observed was the pseudodactylids (n = 21) and these were most commonly present with acanthocephalans (n = 8) (Table 5.20). Indeed, this pairing was also the most commonly observed co-infection for the acanthocephalans. *A. crassus* was observed as a component of a double co-infection in 11 eels and it was most commonly present with the pseudodactylids (n = 6). Tapeworms were most commonly present as a double co-infection with the gastrointestinal nematodes (n = 3). For each of the aforementioned taxa, their occurrence as a

component of co-infection involving two helminth taxa was greater than observed for a single taxa infection. Contrary to that, the occurrence of the gastrointestinal nematodes as a component of double co-infection was reduced relative to their observed occurrence as a single taxa helminth infection. Indeed, the gastrointestinal nematodes were present as a double taxa co-infection in only 12 eels and they were most commonly present with the pseudodactylids (n = 6).

For these 31 eels that were co-infected with two helminth taxa, the body length ranged from 20 to 86 cm (mean = 32.1 ±12.0 cm), body weight ranged from 8.8 to 805.7 g (mean = 71.8±141.8 g) and the mean eel condition factor was 0.15±0.05. The length and weight parameters of these double taxa co-infections were significantly different (p = 0.0002) to the 39 eels lacking a helminth infection (Table 5.7). However, there was no significant difference in the mean condition factor for the uninfected (Table 5.7) and double helminth taxa infected eels (p = 0.20).

For the 6 eels that were co-infected with *A. crassus* and pseudodactylids, the body length ranged from 21 to 33 cm (mean = 27.9±3.9 cm), the body weight ranged from 8.8g to 59.9 g (mean = 33.3±30.2 g) and the mean condition factor was 0.14±0.05. There were no significant differences between these host parameters for the *A. crassus*/pseudodactylid co-infection group and the 39 uninfected eels (Table 5.7); however, the lack of significance for body length difference between the two groups of eels was marginal (p = 0.059).

For the 8 eels that were co-infected with acanthocephalans and pseudodactylids, the body length ranged from 25 to 39 cm (mean = 30.3±4.8 cm), body weight ranged from 18.6 g to 72.9 g (mean = 41.4±18.5.2 g) and the mean condition factor was 0.14±0.03. Interestingly, there was a significant difference (p = 0.019) in body length and also body weight between these acanthocephalan/pseudodactylid co-infected eels and the uninfected group (Table 5.7).

Table 5.20 Summary of the double helminth taxa co-infection profiles within the population of European eels sampled across England and Wales.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
MC3	35	54.6	0.13			**1**		**1**	6
RG6	32	41.8	0.13		**5**			**1**	
RG8	33	62.2	0.17		**5**	**5**			6
B2	32	49.1	0.15	**5**		**4**			9
TB1	20	10.2	0.13		**2**		**9**		11
RP2	31.5	82.8	0.28			**17**		**1**	18
RP6	27.5	38.2	0.18	**1**		**4**			5
RP8	43	101.1	0.07			**11**	**1**		12
RP10	26	29.7	0.17			**3**	**1**		4
CE1	39	72.9	0.12	**4**		**1**			5
CE5	29	39.3	0.16	**1**		**2**			3
CE9	20	10.9	0.14			**3**		**1**	4
HB3	23	14.7	0.12	**3**				**2**	4
HB5	25	18.6	0.12	**27**		**9**			36
R5	27	29.3	0.15	**11**			**2**		13
R6	30	39.7	0.15	**3**	**2**				5
R8	30	43.9	0.16		**4**		**7**		11
R9	23.5	12.7	0.1	**3**			**12**		15
R10	23.5	18.5	0.14		**2**		**5**		7
CD 1	33	59.9	0.17	**14**	**4**				18
CD2	27	30.4	0.17	**5**	**1**				6
CD4	28	28.3	0.1	**2**	**5**				7
CD5	28.5	32.6	0.05	**4**	**2**				6
CD9	21	8.8	0.18	**29**	**1**				30
CN7	86	805.7	0.13	**2**			**30**		32
C74	50	209.8	0.17	**2**			**53**		55
C116	29	36.4	0.15	**3**			**1**		4
C131	39	112	0.19	**2**			**81**		83
C40	32	43.9	0.32	**5**			**2**		7
RL5	34	55.9	0.14	**10**			**3**		13
RL8	29.5	30.5	0.11	**5**		**3**			8

For the 6 eels that were co-infected with gastrointestinal nematodes and pseudodactylids, the body length ranged from 23.5 to 86 cm (mean = 40.1±20.3 cm), body weight ranged from 12.7g to 805.7.9 g (mean = 163.2±267.3 g) and the mean condition factor was 0.16±0.07. Again, interestingly, there was a significant difference in body length (p = 0.002) and also body weight ($p < 10^{-4}$) between these gut nematode/pseudodactylid co-infected eels and the uninfected group (Table 5.7).

For the 3 eels that were co-infected with *A. crassus* and acanthocephalans, the body length ranged from 20 to 30 cm (mean = 24.5±4.1 cm), body weight ranged from 10.2g to 43.9 g (mean = 24.2±14.3 g) and the mean condition factor was 0.14±0.01. There were no significant differences in body length (p = 0.75), body weight (p = 0.63) and condition factor (p = 0.55) between these *A. crassus* /acanthocephalans co-infected eels and the uninfected group (Table 5.7).

For the 3 eels that were co-infected with gastrointestinal nematodes and tapeworms, the body length ranged from 20 to 35 cm (mean = 28.8±7.8 cm), body weight ranged from 10.9 g to 82.8 g (mean = 49.4±36.2 g) and the mean condition factor was 0.18±0.08. There was no significant differences in body length (p = 0.33), body weight (p = 0.26) and condition factor (p = 0.32) between these gastrointestinal nematodes/tapeworm co-infected eels and the uninfected group (Table 5.7).

For the 2 eels that were co-infected with gastrointestinal nematodes and acanthocephalans, the body length ranged from 26 to 43 cm (mean = 34.5±12.0 cm), body weight ranged from 29.7 g to 101.1 g (mean = 65.4±50.5 g) and the mean condition factor was 0.12±0.07. There was no significant differences in body length (p = 0.12), body weight (p = 0.08) and condition factor (p = 0.83) between these gastrointestinal nematodes/acanthocephalan co-infected eels and the uninfected group (Table 5.7).

For the 18 eels with triple helminth taxa co-infections, the dominant class observed was the pseudodactylids (n = 17) (Table 5.21). The next most common taxa represented as a component of triple co-infections was *A. crassus* (n = 15) and in all instances, the swim bladder nematode was also present with pseudodactylids. Acanthocephalans were present in 12 eels that harboured triple helminth taxa co-infections. Not surprisingly, the most commonly observed combination of taxa contributing to triple co-infections was the pseudodactylids, *A. crassus* and the acanthocephalans. Gastrointestinal nematodes were observed as a component of triple helminth taxa infections in 7 eels and in 6 instances, the other parasites observed were pseudodactylids and *A. crassus*. Only 1 eel harboured gastrointestinal nematodes with a tapeworm and acanthocephalan combination. Tapeworms were part of a triple taxa co-infection in 3 eels and 2 hosts harboured these parasites in combination with acanthocephalans and pseudodactylids.

For the 18 eels that were co-infected with three helminth taxa, the body length ranged from 21 to 86 cm (mean = 38.3±16.6 cm), body weight ranged from 13.9g to 1380.3 g (mean = 162.4±330.1 g) and the mean condition factor was 0.14±0.05. The length and weight parameters of these triple taxa co-infections were significantly different (p = 0.0001) to the 39 eels lacking a helminth infection (Table 5.7). However, there was no significant difference in the mean condition factor for the uninfected (Table 5.7) and triple helminth taxa infected eels (p = 0.21).

For the 9 eels that were co-infected with pseudodactylids, *A. crassus* and acanthocephalans the body length ranged from 28 to 69 cm (mean = 36.9±13.2 cm), body weight ranged from 27.5 to 563.5 g (mean = 121.0±173.6 g) and the mean condition factor was 0.14±0.05.

Interestingly, there was a significant difference (p = 0.0008) in body length and also body weight (p = 0.0007) between these pseudodactylid / *A. crassus* and acanthocephalans co-

infected eels compared to the uninfected group (Table 5.7). However, there was no significant difference in the condition factors between the two groups of eels ($p = 0.17$).

For the 6 eels that were co-infected with pseudodactylids, *A. crassus* and gut nematodes, the body length ranged from 21 to 52 cm (mean = 31.3±11.2 cm), body weight ranged from 21.4 g to 187 g (mean = 59±65.9 g) and the mean condition factor was 0.13±0.04. There was a significant difference ($p = 0.047$) in body weight between these pseudodactylid / *A. crassus* and gut nematode co-infected eels and the uninfected group (Table 5.7). However, there were no significant differences in body length ($p = 0.077$) and condition factor ($p = 0.87$) between these two groups of eels.

For the 2 eels that were co-infected with pseudodactylids, acanthocephalans and tapeworms, the body length ranged from 35.5 to 86 cm (mean = 60.8±35.7 cm), body weight ranged from 74.9 g to 1380.3 g (mean = 727.6±923.1 g) and the mean condition factor was 0.19±0.04. There was a significant difference in body length and body weight between ($p = 0.037$ and 0.027 respectively) between these pseudodactylid/acanthocephalan and tapeworm co-infected eels and the uninfected group (Table 5.7). Interestingly, there was also a significant difference in the mean condition factor ($p = 0.035$) between these two groups of eels.

Table 5.21 Summary of the triple helminth taxa co-infection profiles within the population of European eels sampled across England and Wales.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
B8	48	24.5	0.02			19	4	1	24
RP5	35.5	74.9	0.17	2			2	2	6
R1	32	48.9	0.15	5	4		1		10
R3	30	42.3	0.16	22	6		3		31
R4	31.5	67.2	0.22	9	3		9		21
CD3	24	21.4	0.06	4	3	5			12
CN3	86	1380.3	0.22	12			19	1	32
CN9	69	563.5	0.17	2	2		1		5
C107	32	47	0.14	19	7		2		28
C117	28	27.5	0.13	34	1		2		37
C59	32	50.1	0.15	12	10		5		27
C71	47	198.2	0.02	7	2		12		21
C48	31	44.7	0.15	1	2		4		7
RL1	21	13.9	0.16	5	4	8			17
RL2	52	187	0.13	125	9	1			139
RL6	35	71.3	0.17	2	1	3			6
RL9	30	38.1	0.14	1	1	1			3
RL10	26	22.3	0.13	33	5	1			39

Only 5 eels harboured quadruple taxa helminth infections and these were all sampled from three different English rivers (Table 5.22). Pseudodactylids and acanthocephalans were present in all 5 hosts, gastrointestinal nematodes and *A. crassus* were present in 4, and tapeworms were found in 2, of these eels. Not surprisingly, the most common quadruple taxa helminth infections comprised of pseudodactylids, acanthocephalans, gastrointestinal nematodes and *A. crassus* (n = 3).

For the 5 eels that were co-infected with four helminth taxa, the body length ranged from 27 to 77 cm (mean = 57±24.8 cm), body weight ranged from 35.8 g to 839.7 g (mean = 469.3±387.3 g) and the mean condition factor was 0.18±0.02. The length and weight parameters of these quadruple taxa co-infections were significantly different to the 39 eels lacking a helminth infection (Table 5.7) (p = 0.0023 and p = 0.0013 respectively).

Interestingly, there was also a significant difference in the mean condition factor for the uninfected (Table 5.7) and quadruple helminth taxa infected eels (p = 0.004).

For the 3 eels that were co-infected with *A. crassus*, pseudodactylids, acanthocephalans and gastrointestinal nematodes, the body length ranged from 27 to 74 cm (mean = 58.3±27.1 cm), body weight ranged from 35.8 g to 839.7 g (mean = 539.6±438.9 g) and the mean condition factor was 0.19±0.02. The length and weight parameters of these quadruple taxa co-infections were significantly different to the 39 eels lacking a helminth infection (Table 5.7) (p = 0.018 and p = 0.013 respectively). There was also a highly significant difference in the mean condition factor for the uninfected (Table 5.7) and quadruple helminth taxa infected eels (p = 0.007).

Table 5.22 Summary of the profile of quadruple taxa helminth co-infections within the population of eels sampled across England and Wales.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
CN6	74	839.7	0.21	1	11	1	20		33
CN8	77	661.9	0.14	3		1	1	1	6
CN10	74	743.4	0.18	52	1	3	12		68
C157	33	65.8	0.18	56	7		15	1	79
RL3	27	35.8	0.18	51	7	16	1		75

Only one eel (CN5) was found to be infected with all 5 helminth taxa and this specimen was sampled from the river Crane (Table 5.23). Since this was the only eel infected with 5 different helminth taxa, it was not possible to statistically analyse the data with respect to the uninfected eel group.

Table 5.23 Summary of the infection profile in the one eel (CN5) co-infected with five helminth taxa.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
CN5	70	668.6	0.19	2	1	21	6	4	34

5.4.3.2 Helminth community structure analysis: the environment

In order to analyse helminth community structure with respect to the environment, the individual catchment sites were grouped according to their geographic region, as was carried out in previous Chapters. This allowed for larger numbers of eels to be analysed with respect to their helminth community structure profile. The resulting data (Figure 5.4) shows that the English river systems analysed in this study have a richer combination of eel helminths compared to the Welsh river systems. As highlighted earlier (Table 5.10), eels from South East England were found to host between 1 helminth taxon and up to 5 co-infecting helminth taxa. In contrast, 50% of eels sampled from catchment sites in North Wales were not infected with helminths and only 10% of the surveyed fish from this region displayed a co-infection.

Figure 5.4 Summary of the different numbers of helminth taxa observed within the eels sampled from the four different geographic regions. No helminths: , One helminth taxon: , two helminth taxa: , three helminth taxa: , four helminth taxa: and five helminth taxa: .

With respect to eels sampled from the five rivers in North West England, there were 19 specimens with helminth co-infections (Table 5. 24). The most common class of helminth observed in these co-infections was the gastrointestinal nematodes (15/19; 78.9%). The most common combination of parasites responsible for a double taxa infection was the pseudodactylids and gut nematodes (n=4). Not surprisingly, triple taxa infections were dominated by pseudodactylids and gut nematodes in combination with *A. crassus* (n=5).

Table 5.24 Helminth co-infection profiles in the eels sampled from rivers in North West England.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
RG6	32	41.8	0.13		5			1	6
RG8	33	62.2	0.17		5	5			10
B2	32	49.1	0.15	5		4			9
RP2	31.5	82.8	0.28			17		1	18
RP6	27.5	38.2	0.18	1		4			5
RP8	43	101.1	0.07			11	1		12
RP10	26	29.7	0.17			3	1		4
HB3	23	14.7	0.12	3				2	5
HB5	25	18.6	0.12	27		9			36
RL5	34	55.9	0.14	10			3		13
RL8	29.5	30.5	0.11	5		3			8
B8	48	24.5	0.02			19	4	1	24
RP5	35.5	74.9	0.17	2			2	2	6
RL1	21	13.9	0.16	5	4	8			17
RL2	52	187	0.13	125	9	1			135
RL6	35	71.3	0.17	2	1	3			6
RL9	30	38.1	0.14	1	1	1			3
RL10	26	22.3	0.13	33	5	1			39
RL3	27	35.8	0.18	51	7	16	1		75

With respect to eels sampled from the two rivers in South East England, there were 17 specimens with helminth co-infections (Table 5. 25). Pseudodactylids and acanthocephalans were present in all of these co-infected eels (100% prevalence). For triple taxa co-infections, the most common combination of helminths observed was the aforementioned pseudodactylids and acanthocephalans with *A. crassus*. Not surprisingly, quadruple taxa infections were dominated by pseudodactylids, acanthocephalans and *A. crassus*, in combination with either gastrointestinal nematodes (n = 2) or tapeworms (n = 1).

Table 5.25 Helminth co-infection profiles in the eels sampled from rivers in South East England.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Tapeworms	Total number of helminths
CN7	86	805.7	0.13	2			30		32
C74	50	209.8	0.17	2			53		55
C116	29	36.4	0.15	3			1		4
C131	39	112	0.19	2			81		83
C40	32	43.9	0.32	5			2		7
CN3	86	1380.3	0.22	12			19	1	32
CN9	69	563.5	0.17	2	2		1		5
C107	32	47	0.14	19	7		2		28
C117	28	27.5	0.13	34	1		2		37
C59	32	50.1	0.15	12	10		5		27
C71	47	198.2	0.02	7	2		12		21
C48	31	44.7	0.15	1	2		4		7
CN6	74	839.7	0.21	1	11	1	20		33
CN8	77	661.9	0.14	3		1	1	1	6
CN10	74	743.4	0.18	52	1	3	12		68
C157	33	65.8	0.18	56	7		15	1	79
CN5	70	668.6	0.19	2	1	21	6	4	34

With respect to eels sampled from the four rivers in North Wales, there were only 4 specimens with helminth co-infections (Table 5.26). Gastrointestinal nematodes were present in all of these co-infected eels (100% prevalence). Only double taxa infections were observed and the other component of the co-infection in these specimens was either pseudodactylids (50% prevalence) or tapeworms (50% prevalence).

Table 5.26 Helminth co-infection profiles in the eels sampled from rivers in North Wales.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	Gut nematodes	Tapeworms	Total number of helminths
MC3	35	54.6	0.13		1	1	2
CE1	39	72.9	0.12	4	1		5
CE5	29	39.3	0.16	1	2		3
CE9	20	10.9	0.14		3	1	4

With respect to eels sampled from the three rivers in South Wales, there were 15 specimens with helminth co-infections (Table 5.27). Either the pathogenic *A. crassus* or the pseudodactylids were observed as a component of all helminth co-infections. Not surprisingly, these two pathogenic parasites were the dominant helminths in double taxa co-infections (6/11; 54.5%). *A. crassus* and pseudodactylids were also present in all triple taxa co-infections; most commonly in combination with acanthocephalans.

Table 5.27 Helminth co-infection profiles in the eels sampled from rivers in South Wales.

Eel code	Body length (cm)	Body weight (g)	Condition factor	Pseudodactylids	*A. crassus*	Gut nematodes	Acanthocephalans	Total number of helminths
TB1	20	10.2	0.13		2		9	11
R5	27	29.3	0.15	11			2	13
R6	30	39.7	0.15	3	2			5
R8	30	43.9	0.16		4		7	11
R9	23.5	12.7	0.1	3			12	15
R10	23.5	18.5	0.14		2		5	7
CD 1	33	59.9	0.17	14	4			18
CD2	27	30.4	0.17	5	1			6
CD4	28	28.3	0.1	2	5			7
CD5	28.5	32.6	0.05	4	2			6
CD9	21	8.8	0.18	29	1			30
R1	32	48.9	0.15	5	4		1	10
R3	30	42.3	0.16	22	6		3	31
R4	31.5	67.2	0.22	9	3		9	21
CD3	24	21.4	0.06	4	3	5		12

5.5 Discussion

Work in this chapter is focussed upon describing the helminth community structure and co-infection status of European eels sampled from sites across England and Wales. Prior to this analysis, a dataset of *A. crassus* infection was also analysed; the majority of this swim bladder nematode data was extracted from an earlier Ph.D study at The University of Salford (Ab Aziz, 2012), though eels from three additional catchment sites were surveyed for *A. crassus* infection during the course of this thesis work. To this end, the data described in this chapter contributes new knowledge on the helminth infection status of *A. anguilla* stocks in UK rivers. Indeed, although helminth infections were commonly found, the data also confirms that a sizeable minority (28%) of the eels lacked a helminth infection, as reported in other studies (Kennedy, 1993a; Kennedy & Guégan, 1996; Kennedy *et al.*, 1998). These uninfected eels were sampled from rivers across England and Wales, though not from South East England. The 100% helminth infection status of eels from the two catchment sites in South East England may be in part influenced by the large size and hence age/time of exposure to helminths in one of the rivers, the Crane; however, the eels from the other river in this region, the Crouch, were of average size. As such, it is also likely that the river environment in South East England is particularly favourable to helminth transmission to eels, as noted in a previous survey (Ab Aziz, 2012).

5.5.1 A. crassus

The pathogenic swim bladder nematode *A. crassus* was isolated from the majority of the eels sampled from the river Leven in North West England. Indeed, when combined with the earlier dataset (Ab Aziz, 2012), 25% of all the *A. crassus* were derived from just 7 eels sampled from the river Leven. A further 11% of the entire *A. crassus* individuals within the dataset were attributable to 4 infected eels sampled from the river Crane in South East England. The swim bladder of a number of these eels showed the distinct appearance of *A.*

crassus infection since they appeared discoloured due to haemorrhaging (Kirk, 2003). No *A. crassus* parasites were recovered from eels sampled from the river Petteril, the most northerly located sampling site in this study.

On extrapolating the analysis to the regional level, all 4 river sites in North Wales contained eels that were free of *A. crassus* infection. Moreover, a statistical analysis of the regional infection data confirmed significant differences in *A. crassus* prevalence between most of the regions. The data confirms that eels sampled from catchment sites in South East England had the highest prevalence of *A. crassus*; one contributory factor to this is likely to be that the parasite has been present in this area of the country for the longest period of time given the suspected invasion route (Kennedy & Fitch, 1990). Another contributory factor however is most probably host dependent since one of the rivers in South East England, the Crane, provided specimens that were significantly larger than the rest of the study population. As discussed earlier (Chapter 1), these fish were likely to be at a more mature developmental state and older than the rest of the eels in the study and hence exposed to the risk of *A. crassus* infection for a much longer period of time. Of potential importance was the finding that *A. crassus* infection was linked to a significant increase in the eel body length and weight (20% and 48% respectively). This corroborates a recent study on *A. crassus* infections in eels at sampling sites along the river Rhone in Southern France in which swim bladder nematode infection was linked to an 11% increase in eel body length and a 41% increase in body weight (Lefebvre *et al.*, 2013). Lefebvre and colleagues propose that this counter-intuitive finding is likely to be a consequence of eel panmixia contributing to variability in growth potential and infection risk as the most active foragers would not only be larger but that they would also have the highest probability of becoming infected.

The *A. crassus* dataset shows that this pathogenic parasite is present in 50% of the catchment sites analysed within this study. As there appears to be no published literature on *A. crassus*

infections in European eel from these catchment sites, this dataset contributes new information on the spread of *A. crassus* in the UK. Indeed, the finding that 70% of eels from the river Leven were infected with *A. crassus* may have implications for the spread of the parasite to Scotland; a region of the UK for which there exists very limited data on *A. crassus* (Barry *et al.*, 2014). Overall, *A. crassus* was found to be present in 35 eel specimens and in only 4 of this subset was the swim bladder nematode found to be the only helminth infection. As such, *A. crassus* was most commonly found as a component of a helminth co-infection.

5.5.2 Helminth community structure

In total, 1504 individual helminths were isolated from the eel population and analysis of the infection profiles at the 14 catchment sites showed that multiple different classes of helminth were present within the majority of the rivers. Indeed, 4 different helminth taxa were commonly found in eels sampled from the English rivers and 3 different helminth taxa were routinely isolated from eels recovered from the Welsh rivers. Moreover, the river containing eels with the most numerous helminth taxa (n = 5) was the Crane in South East England; in contrast, only gastrointestinal nematodes were recovered from eels sampled from two catchment sites in North Wales. Indeed, not only did the catchment sites in North Wales appear to support eels with a limited number of helminth taxon, acanthocephalans were absent from this subset of eels, in addition to *A. crassus*, as discussed above (5.2.1).

With respect to the numbers of helminths recovered from the eels, approximately 2/3 were isolated from 3 rivers; the Crouch, Crane and Leven in England. Not surprisingly therefore, the primary infection data at these sites was significantly different to the data obtained from eels sampled from the majority of the remaining catchment sites. Moreover, 1198 helminths (79.7%) were recovered from eels from the English rivers, including 135 from just one host (eel code: RL2), and hence these sites could be classed as being potentially richer in biodiversity than the Welsh rivers.

Indeed, one important factor contributing to eel helminth diversity is the habitat supporting the eels, the intermediate hosts and any paratenic hosts (Conneely & McCarthy, 1986; Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993b; Thielen, Muenderle, *et al.*, 2007). To this end, scrutiny of the Biodiversity Action Plan (Morgan *et al.*, 2011) may offer some insight with respect to the helminth community structure at the different catchment sites. The river Petteril appears to have the greatest number of qualifying criteria (n = 5) contributing

towards the BAP and eels sampled from this river harboured 4 different helminth taxa; as discussed above (5.2.1), *A. crassus* appeared to be absent from these fish. In contrast, the river Crouch in South East England is probably the least attractive catchment site in terms of qualifying criteria towards the BAP since it is categorised as only containing one category B species (*A. anguilla*). Despite this low categorisation, eels from the river Crouch were host to 4 different helminth taxa; no gastrointestinal nematodes were recovered. The two other rivers considered to have low qualifying criteria (n = 1) towards the BAP, the rivers Cadoxton and Gowy, supported eels with 3 and 4 different helminth taxa respectively. Furthermore, the eels sampled from the river Crane harboured the maximum number of helminth taxa observed in this study (n = 5) and yet according to the BAP, it has only 2 qualifying criteria. As such, it is not possible to draw any conclusions about how, taken in isolation, the overall environmental quality at the respective catchment sites may affect the observed helminth community structure within the European eel. Nonetheless, other environmental factors, such as differences in the physio-chemical properties of the water (Køie, 1988; Jakob, Hanel, *et al.*, 2009b; Mayo-Hernández *et al.*, 2015) and also, climate/seasonal variation across the UK, may contribute to the observed data, as has been reported in other studies (Kennedy, 1997a; Thielen, Muenderle, *et al.*, 2007; Filippi *et al.*, 2013).

Another important factor contributing towards eel helminth diversity is the characteristics of the host itself (Schneebauer *et al.*, 2016). Indeed, it is likely that the age and condition of an eel may influence the likelihood of it acquiring a helminth infection. To this end, it is interesting to note that the data produced in this study confirms that helminth infected eels are significantly larger and have a greater condition factor than the uninfected group of eels. Indeed, the helminth infected eels had mean length and weight measurements that were 42% and 371% greater respectively than the uninfected group of eels. Again, as reported above for *A. crassus* (5.5.1) (Lefebvre *et al.*, 2013), this may indicate that these larger eels are more

actively feeding and hence have an increased risk of exposure to helminth infected intermediate and paratenic hosts.

The river environment and host factors together influence whether, or not, the eel becomes infected with a helminth. Furthermore, these major influences are also crucial with regard to the type, if any, of co-infection that eels may harbour. To obtain a thorough understanding of any co-infection requires knowledge not only of the different parasitic taxa/species but also, the respective numbers of parasites. This may be further influenced by competition between the parasites themselves if they are localised to the same niche within the host (Holmes, 1973). The result may be different outcomes for both the parasites and the host (Mayo-Hernaez *et al.*, 2015). With regard to *A. anguilla*, there has been limited published information on helminth co-infections as most of the data is presented as a catalogue of parasites surveyed from specific locations (Kennedy, 1993a; Sures, Knopf, Würtz, *et al.*, 1999; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004; Aguilar, Álvarez, *et al.*, 2005; Mayo-Hernández *et al.*, 2015; Moravec & Scholz, 2015). However, there is some published co-infection data that describes helminth co-infections that can be found within a particular eel niche such as the gastrointestinal tract (Kennedy & Moriarty, 1987; Kennedy *et al.*, 1989), or, the gill (Buchmann, 1988b).

This study has already confirmed that the eel gill can be co-infected with *P. anguillae* and *P. bini* (Chapter 2) and that the gastrointestinal tract can be co-infected with *P. laevis* and non-bulb acanthocephalans (Chapter 4). Data within this chapter now extends knowledge on the occurrence of other co-infections with the European eel. In total, there were 55 eels with helminth co-infections and only 25% of these (n = 14) were localised to the gastrointestinal tract. This may indicate a resource limitation (Petney & Andrews, 1998; Rigaud *et al.*, 2010; Knowles *et al.*, 2013) within the eel gastrointestinal tract between the gut nematodes, acanthocephalans and tapeworms and hence competition between these parasites. Indeed,

such resource limitation has been proposed previously in the European eel following study of the distribution of different species of acanthocephalans within the gastrointestinal tract (Kennedy, 1985). Interestingly, tapeworms were encountered in combination with either gut nematodes and/or acanthocephalans, in 9 eels. There was no preference with regard to the other components of the gut helminth community in these 9 eels: in 3 eels tapeworms were present with acanthocephalans; in a further 3 eels tapeworms were present with gut nematodes and in the final 3 eels, tapeworms were in combination with both acanthocephalans and gut nematodes. To this end, it could be suggested that eel tapeworms have a neutral impact on the gut helminth community structure and this may be a consequence of spatial separation from other helminths within the gastrointestinal tract, as noted in a previous study (Schabuss *et al.*, 1997). The overall prevalence of tapeworm infection was 9.3% whereas the overall prevalence of acanthocephalans and gut nematodes was 30% and 33.6% respectively. As such, it is perhaps of note that 9 eel gut co-infections involved tapeworms, which is similar to the 11 eels that had gut co-infections involving acanthocephalans and the 11 eels that presented with gut co-infections involving nematodes; again, emphasizing that tapeworms have little, if any, influence on the gut helminth community of eels (Abdelmonem *et al.*, 2010).

The majority of the co-infections reported in this chapter (75%, n = 41) involved one, or both, of the pathogenic helminths *A. crassus* and the pseudodactylids. This is perhaps not surprising given that these helminths were highly prevalent and abundant; indeed together, they comprised 50% (n = 754) of all the helminth specimens analysed in this thesis. Moreover, as the swim bladder nematode and the gill monogeneans occupy distinct and separate organs within the host they are not in direct competition for resources either with each other, or with the gastrointestinal helminths. This presumably aids both *A. crassus* and the pseudodactylids establishing an infection that is then able to persist despite an immune

response from the host (Knopf, 2006; Knopf *et al.*, 2008). Indeed, infection with *A. crassus* has recently been reported to influence outcomes of the eel innate immune system to the extent that the host is likely to become more susceptible to infection with other parasites (Terech-Majewska *et al.*, 2015). Together, *A. crassus* and the gill monogeneans were both present as components of the eel helminth community in 26 (47.3%) of the co-infected hosts. Given the known pathogenicity of these helminths, this duel combination, either alone, or with one, or more, gut helminths, is potentially of concern with respect to eel health and capacity to successfully complete a migration to the spawning grounds (Kirk, 2003; Lefebvre *et al.*, 2013; Barry *et al.*, 2014; Schneebauer *et al.*, 2016).

Analysis of the host parameters; length, weight and condition factor, with respect to the infection profiles offered some interesting insights. It was apparent that helminth infection was generally associated with larger eels; indeed, the longer and heavier eels were more likely to be infected with helminths and have a greater intensity of infection. Moreover, this pattern was observed regardless of whether the eel harboured a single helminth taxon infection, or, co-infections of multiple taxa. This observation is once again, most likely to be a consequence of the larger fish being not only exposed to infection for greater periods of time as they are older but also, that they have a more varied and substantial diet (Nie & Kennedy, 1991d; Schabuss *et al.*, 2005; Kennedy, 2007a; Jakob, Hanel, *et al.*, 2009b).

With respect to the infection profile at the taxa level, infections involving acanthocephalans, as already noted above, were perhaps of most interest in terms of any association with the host length and weight parameters. Acanthocephalan infections, whether alone (11 eels), or in combination with pseudodactylids (8 eels), pseudodactylids/*A. crassus* (9 eels), pseudodactylids/tapeworms (2 eels), or pseudodactylids/*A. crassus*/gut nematodes (3 eels), appeared to be associated with the larger hosts. The only acanthocephalan co-infections that did not show any significant difference in host parameters relative to the uninfected group of

eels was the combination with *A. crassus* (3 eels) and with gastrointestinal nematodes (2 eels). It may seem counter-intuitive that these acanthocephalan infected eels are significantly larger than the uninfected specimens, particularly when the gut perforation often associated with these helminths is considered (Chapter 4). However, as discussed above, these hosts are larger/older and hence have not only been exposed to potential infections for a longer period of time but they are also likely to have a more varied and substantial diet (Jakob, Hanel, *et al.*, 2009b; Filippi *et al.*, 2013; Gerard *et al.*, 2013). The only other co-infections that appeared to have a significant association with the measured host parameters was that of the pseudodactylids/gastrointestinal nematodes (6 eels) and also, this pairing in combination with *A. crassus* (6 eels). From analysis of these acanthocephalan-free helminth co-infected hosts, it does appear that co-infections are of crucial importance since single infections with the gill monogeneans (5 eels), gastrointestinal nematodes (24 eels), or *A. crassus* (4 eels) are not significantly associated with eel body length and weight measurements.

On extrapolation of this analysis to the level of the condition factor, it was also of note that helminth infection (n = 101) was associated with a significantly greater eel condition factor (0.15±0.05) than the uninfected eels (0.14±0.02). Interestingly, this observed difference was noticed with single taxon infections caused by gastrointestinal nematodes ($p = 0.03$) and also, acanthocephalans ($p = 0.01$), though not with infections caused by either *A. crassus*, tapeworms, or the pseudodactylids. This in part may be a consequence of low numbers of eels harbouring a single taxon infection with the latter three, though also, the gill monogeneans route of infection is not linked to dietary intake. Furthermore, the mean intensity of the single taxon infections caused by gastrointestinal nematodes and also, acanthocephalans, was higher than the data observed for single taxon infections with either *A. crassus*, tapeworms or the pseudodacytlids. Not surprisingly, there is variation in the helminth intensity data for co-infections and this probably is reflected in the condition factor

not always being significantly different in the eels with helminth co-infections compared to the uninfected eels. For example, in the eels co-infected with gastrointestinal nematodes and acanthocephalans, the condition factor was not significantly different to the uninfected eels. However, only two eels had this specific co-infection and also, in both cases, only a single acanthocephalan was recovered from these eels and in one of them, only 3 nematodes were present. Examining the triple helminth co-infections, there was only 1 eel with a combination involving gastrointestinal nematodes and acanthocephalans. However, there were two eels that were co-infected with acanthocephalans/pseudodactylids/tapeworms and the mean condition factor was significantly higher (0.19 ±0.04) than the group of uninfected eels. This may again reflect feeding habits since one of these two eels was from the river Crane, which provided the largest/oldest group of fish; indeed, the triple co-infection in this specimen included 19 acanthocephalans.

Although the above helminth taxa data is of interest, a more detailed analysis of the helminth community structure in each co-infection, as indicated above, would require the intensity data to also be thoroughly scrutinised. Unfortunately, the relatively small numbers of eels with the different combinations of helminth taxa does not allow a more rigorous statistical analysis of the data with respect to the host parameters.

At the regional level, distinct differences in the helminth community structures were observed. The two rivers in South East England contained helminth co-infected eels (n = 17) with up to 5 helminth taxa and the number of different combinations of these taxa was 7. The 5 rivers analysed in North West England contained similar numbers of helminth co-infected eel specimens (n = 19) and these were observed to have a total of 11 different taxa combinations. Moreover, pseudodactylids and acanthocephalans were present in all the co-infected eels from South East England, whereas gastrointestinal nematodes were the dominant helminth observed in the co-infected eels sampled from North West England. The

Welsh river systems contained less helminth co-infected eels and the combinations of taxa observed were also reduced relative to the eels sampled from English rivers. Interestingly, there were also differences between the data for North and South Wales. Gastrointestinal nematodes were present in all the co-infected eels from North Wales (n = 4) and there were no co-infections involving either acanthocephalans (Chapter 4) or *A. crassus* (5.4.1) as these were not found in eels sampled from this region. As such, only 2 combinations of helminth taxa were observed in the co-infected eels sampled from North Wales. In contrast, the co-infected eels from South Wales (n = 15) had 5 combinations of different helminth taxa; the dominant taxa were *A. crassus* (n = 13) and the pseudodactylids (n = 12), whilst there was only 1 host with a co-infection involving gastrointestinal nematodes and no tapeworm co-infections as the latter were not present in the eels sampled from this region.

5.6 Concluding remarks

This study was conducted in order to contribute novel data on helminth parasites in *A. anguilla*, a species classified as threatened, from sites across England and Wales. In order to achieve this overall aim, 140 European eel specimens were acquired in an ad hoc fashion following routine surveys conducted by the Environment Agency at 14 rivers across South East England, North West England, South and North Wales. Most (n = 130) of these eels were close to the mean specimen size for the surveyed population; however, 10 eels, all from the river Crane, were substantially larger and most probably at a late developmental stage. These eels were not obtained via the routine sampling route as they were acquired following death due to a major pollution event in the river Crane.

The eel specimens were dissected and helminth analysis was conducted on the gills, the swim bladder and the gastrointestinal tract in order to generate data on pseudodactylids, *A. crassus*, acanthocephalans, gastrointestinal nematodes and tapeworms respectively. The resulting data set confirms the presence of all the aforementioned parasites and also, that no digenean trematodes were present in the eels. The absence of digenean infections was not unexpected given that previous reports indicate these helminths are generally associated with eels sampled from brackish waters (Køie, 1988). Overall, the catalogue of helminths produced in this study included 11 out of the 27 helminth species previously reported to infect *A. anguilla* in the UK (Table 5.28). Seven of these helminths; *P. anguillae*, *P. bini*, *A. crassus*, *S. inermis*, *P. tenerrima*, *P. macrocephalus* and *B. claviceps* are known eel specialist parasites. The remaining 4; *A. clavula*, *A. lucii*, *P. laevis* and *R. acus*, are non-specialist helminths of eels.

Table 5.28 Helminth species reported in *A. anguilla* from the UK (* reported in this study)

Helminth class	Helminth parasite	Reference
Monogenean	*Gyrodactylus anauillae*	(Kennedy, Nie, Kaspers, *et al.*, 1992)
	Pseudodactylogyrus anguillae *	(Nie & Kennedy, 1991d; Kennedy, Nie, Kaspers, *et al.*, 1992)
	P. bini *	**(Kirk, 2006)**
Digenean	*Deropristis inflate (marine)*	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Nicolla gallica	(Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Crepidostomum farionis	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a)
	Podocotyle atomon (marine)	(Kennedy, Nie, Kaspers, *et al.*, 1992)
	Sphaerostoma bramae	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a)
	Phyllodistomum folium	(Kennedy, Nie, Kaspers, *et al.*, 1992)
Acanthocephalan	*Acanthocephalus anguillae*	(Kennedy & Moriarty, 1987; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Acanthocephalus clavula *	(Kennedy & Lord, 1982; Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a)
	Acanthocephalus lucii *	(Kennedy & Moriarty, 1987; Kennedy, 1992; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Echinorhyncus truttae	(Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Neoechinorhynchus rutili	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Pomphorhynchus laevis *	(Kennedy *et al.*, 1989; Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
Nematode	*Anguillicoloides crassus* *	(Kennedy & Fitch, 1990; Kennedy, Nie, Kaspers, *et al.*, 1992; Kirk, 2003)
	Camallanus lacustris	(Nie & Kennedy, 1991a; Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Cucullanus truttae	(Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Capillaria sp.	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a)
	Daniconema anguillae	(Kennedy, Nie, Kaspers, *et al.*, 1992)
	Goezia inermis	(Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004)
	Raphidascaris acus *	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004)
	Paraquimperia tenerrima *	(Nie & Kennedy, 1991c; Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004)
	Spinitectus inermis *	(Kennedy, Nie, Kaspers, *et al.*, 1992; Norton *et al.*, 2003; Norton, Lewis, *et al.*, 2004; Norton, Rollinson, *et al.*, 2004; Kennedy, 2012)
	Streptocara sp.	(Kennedy, 1993a)
Tapeworm	*Bothriocephalus claviceps* *	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004)
	*Proteocephalus microcephalus**	(Kennedy, Nie, Kaspers, *et al.*, 1992; Kennedy, Nie, & Rostron, 1992; Kennedy, 1993a; Norton *et al.*, 2003; Norton, Rollinson, *et al.*, 2004)

The gill monogeneans were the dominant helminth isolated from the eel specimens, both in terms of numbers recovered and prevalence of infection at the study sites. Indeed, this known pathogen was present in eels at all sites examined from South East England and South Wales and at all but 1 of the sites in North West England. However, pseudodactylids were only recovered from eels at one river, the Clwyd-Elwy, in North Wales. Morphological examination of the monogeneans was inconclusive with regard to species identification. This was somewhat predictable given the mode of acquisition of the eel specimens; however, even if fresh eel specimens had been obtained, it remains challenging to discriminate between *P. bini* and *P. anguillae* (Zolovs *et al.*, 2016). Consequently, a species identification approach was developed that was based upon the small number of nucleotide differences that exist in the 18S rRNA gene of these pseudodactylids. This PCR/restriction digestion-based diagnostic approach allowed species characterisation of approximately 30% of the collected gill monogeneans and the data confirmed that *P. bini* was slightly more prevalent than *P. anguillae* (ratio: 55:45). Some of the catchment sites appeared to harbour just *P. anguillae* and other sites just *P. bini.* Additionally, there were further sites at which both of these parasites were detected. Interestingly, at the river site with the largest fish, the Crane, only *P. anguillae* was detected despite analysis of 25 PCR products and so this may suggest that both gill monogenean species are not necessarily present at all catchment sites positive for pseudodactylids. There may be unknown ecological reasons for this observation given that eels from the other river in South East England, the Crouch, produced both species of gill monogenean; moreover, *P. bini* was present at levels approximately 10-fold greater than *P. anguillae*. Given the lack of published literature on *P. bini* infections in European eels from UK rivers, this prevalence data is perhaps surprising.

The swim bladder nematode *A. crassus* has presented much concern with regard to eel health. Data produced in this thesis, along with data from another Ph.D. student at Salford University

(Ab Aziz, 2012), confirms the extent of *A. crassus* spread amongst the UK eel population. As observed for pseudodactylid infections, all sites examined from South East England and South Wales were also positive for *A. crassus* infection. Interestingly, this potentially devastating parasite was not recovered from any eels sampled from rivers in North Wales. Although *A. crasuss* was the dominant helminth recovered from eels sampled from the river Gowy, most of the catchment sites in North West England appeared not to harbour eels with swim bladder nematode infections. This may continue to reflect the dispersal of *A. crassus* across the UK eel population from its geographic origin of introduction (Kennedy & Fitch, 1990).

The gastrointestinal tracts of the 140 eel specimens yielded a total of 750 helminths (49.9% of all the parasites recovered). Very few tapeworms were recovered; 12 *B. claviceps* and 6 *P. macrocephalus* and they were present in all regions except South Wales. Moreover, half of the *B. claviceps* specimens were isolated from the catchment site containing the largest eels, the river Crane. As such, this suggests that tapeworm infection in eels is relatively uncommon compared to the other helminth infections (Nie & Kennedy, 1991b; Nie & Kennedy, 1992b).

Gastrointestinal nematodes were the dominant helminth taxa at 5 of the catchment sites; 2 in North West England and 3 in North Wales. Indeed, given the relative prevalence of gastrointestinal nematodes, it was somewhat surprising to find that none were recovered from eels sampled from the rivers Rhymney in Wales and Crouch in England. The latter is of most intrigue given that eels from the river Crouch generated the highest number of total helminths recovered from any site, including a substantial number of other gut infections (eg. acanthocephalans). This may reflect the array and abundance of intermediate hosts specific for acanthocephalans to complete their life cycle relative to those required for the nematodes. Indeed, overall, it did appear that the eels from the more northerly sampling sites were more likely to be infected with gastrointestinal nematodes and again, perhaps emphasizes that the

ecology of these sites is more suited to these helminths completing their life cycles. The gastrointestinal nematodes confirmed in this study were *S. inermis*, *P. tenerrima* and *R. acus*. In confirming the presence of *S. inermis* and also, *P. tenerrima*, novel sequence data for the 18S rRNA gene of these helminths was generated. These sequences should assist phylogenetic analysis of the genera *Spinitectus* and *Paraquimperia*.

Significant numbers of acanthocephalans were recovered from the eels; indeed, these helminths were dominant at the 2 rivers in South East England and the river Taff in South Wales. In contrast, acanthocephalans were not recovered from eels at any of the rivers sampled in North Wales. In total, 215 *P. laevis* individuals, a category 2 parasite (Environment Agency UK, 2014), were isolated from eels at 2 sites in South Wales and 4 sites in England. Of particular concern may be the high prevalence and also, high numbers of *P. laevis* isolated from eels sampled from the rivers Crouch and Rhymney. A large number, 254, non-bulb acanthocephalans were also isolated from the eels. A number of these acanthocephalans were analysed by PCR amplification of the 18S rRNA gene and sequence data confirmed the presence of *A. lucii*. In addition, novel sequence data was generated and a phylogenetic analysis offered strong support for some of the acanthocephalans being *A. clavula*. All of these non-bulb acanthocephalan infections were localised to rivers in England and the reasons for this can only be speculated given that *P. laevis*, which uses similar intermediate hosts (Dezfuli *et al.*, 2000), was recovered from sites in England and Wales. For example, other than the 2 rivers in South East England, prevalence of the non-bulb acanthocephalans is low. Indeed, 97% of all the non-bulb acanthocephalans were derived from eels sampled from the rivers Crane and Crouch and hence there may be specific environmental factors; and possibly also host factors, as the river Crane eel (n = 208 non-bulb acanthocephalans) were significantly larger than the rest, that contribute to the observed infection profile.

As a consequence of studying the individual taxa, it was possible to subsequently analyse the helminth community structure within the eel population. The interesting findings have been emphasized above (5.5) and the resulting co-infections provide new insights for helminth infections in *A. anguilla*. The data also stress how important the contribution of the river Crane eels was to the study. They were significantly larger than the rest of the eels and they not only provided almost 22% of the total helminths but also, the highest number of taxa. Furthermore, the Crane eels had the greatest mean condition factor which may indicate that prior to the pollution event; these fish were healthy and reasonably well nourished. The latter supports a notion that the Crane eels had access to a relatively good array of food sources and given their parasite infection status; this would include many of the intermediate and paratenic hosts (Thielen, Muenderle, *et al.*, 2007). Interestingly, the Crane eels also displayed a gut immune response to helminth infection as cysts were present in the majority of these eels and these contained encapsulated larvae of *A. crassus* and also *R. acus*, a phenomena noted in other studies (Køie, 1988; Knopf & Mahnke, 2004; Heitlinger *et al.*, 2009). Indeed, such a response may contribute to these eels being noted as able to tolerate multi-parasite infections (Mayo-Hernaez *et al.*, 2015). As pseudodactylids were also present in the Crane eels at relatively high levels, this supports age and hence longevity of exposure to parasitic threats, as being an important factor, along with diet, in contributing to the overall helminth community structure (Conneely & McCarthy, 1986; Aguilar, Alvarez, *et al.*, 2005a; Schabuss *et al.*, 2005; Filippi *et al.*, 2013).

Finally, some of the studied helminths are known to cause pathology and disease in the European eel (Buchmann, 1993a; Sures *et al.*, 2001; Kirk, 2003; Kennedy, 2007b; Sjöberg *et al.*, 2009; Abdelmonem *et al.*, 2010; Barry *et al.*, 2014). This study also corroborated some of the pathological findings associated with *A. crassus*, pseudodactylids and acanthocephalans. Indeed, there were a number of examined eels that exhibited combinations of swim bladder

haemorrhaging, gut perforations and external disruption to the gill surface. The precise health outcomes for these fish can only be speculated upon; however, the literature suggests that such eels are likely to be compromised in their ability to migrate to the spawning grounds (Schneebauer *et al.*, 2016). However, precise information concerning the health outcomes of the acanthocephalan infected eels are less well known and therefore worthy of further study.

5.7 Future directions

Due to *A. anguilla* being a threatened species, it proved difficult to access specimens for this study and hence there was a dependence upon ad hoc acquisition through routine Environment Agency sampling and also, a single pollution event that killed fish in the river Crane. This limited the numbers of eels available from any specific site and there were also limitations with respect to detailed data concerning the actual dates of sampling and the environmental conditions at the sites. To this end, subsequent data interpretations with respect to these parameters have proven impossible and any future study would address these concerns.

During the course of this study, a number of novel DNA sequences were generated. These were argued as being derived from the gastrointestinal nematodes *S. inermis* and *P. tenerrima* and also from the acanthocephalan *A. clavula*. However, given the mode of acquisition and subsequent processing of the eels, a rigorous approach of confirming species through detailed morphological recordings was not always possible. Consequently, it would be best to dissect fresh eels and work with fresh parasites in order to robustly link the novel 18S rRNA sequences to the above helminths.

In addition to a DNA based approach for species identification, it might prove worthwhile attempting to support morphological recordings with a parasite chemical signature. This approach, using Fourier Transform Infrared Microspectroscopy (FTIR), allows complex biological materials, including single cells and multicellular organisms such as the nematode *Caenorhabditis elegans*, to be rapidly distinguished based upon a chemical footprint (Davis, 2012). One of the advantages is the speed of the analysis and if the approach could be developed for some of the parasites isolated in this study it would circumvent the time consuming need to isolate genomic DNA, amplify the 18S rRNA gene by PCR and then

subject the resulting product to DNA sequencing. This might be particularly useful for parasites with a relatively similar morphology.

One of the important questions about eel parasites remains whether, or not, they impact upon eel migration. Moreover, if it is accepted that they do impact upon eel migration, the question then becomes to what extent and how that ultimately affects the European eel population. This study has confirmed that eels from many regions in the UK are infected with helminths; however, it offers no hypothesis with regard to these important questions. To this end, a concerted effort is needed to develop non-invasive approaches that allow parasite infections to be determined in the European eel and then sophisticated tracking mechanisms to be utilised to monitor fish migration. With regard to non-invasive parasite diagnostics, scanning techniques have been used to determine eel swim bladder infection with *A. crassus* (Székely *et al.*, 2004). However, other methodologies are needed for the gastrointestinal helminths. Options that could be developed for use on eels sampled at the silvering stage include helminth egg diagnosis within faecal samples (Cringoli *et al.*, 2010), or, antibody based approaches (Baszler *et al.*, 1995; Andersen *et al.*, 2000). If these techniques could be used on silvering eels, in conjunction with sophisticated tracking, it might be possible to begin to address the important questions above.

Finally, this thesis and the additional data produced at Salford University on eel parasites (Ab Aziz, 2012), has allowed a comprehensive survey of the extent of parasite infection in UK eels. This should support further development of Eel Management Plans in order to assist future management of the stocks of *A. anguilla* in UK waters.

References

Ab Aziz, R. (2012). Helminth, Protozoan and Viral infection of the European eel. Anguilla anguilla. *Thesis*.

Abdelmonem, A. A., Metwally, M. M., Hussein, H. S., & Elsheikha, H. M. (2010). Gross and microscopic pathological changes associated with parasitic infection in European eel (*Anguilla anguilla*, Linnaeus 1758). *Parasitology Research, 106*(2), 463-469. doi: 10.1007/s00436-009-1688-2

Abollo, E., Gestal, C., & Pascual, S. (2001). Anisakis infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitology Research, 87*(6), 492-499.

Acou, A., Laffaille, P., Legault, A., & Feunteun, E. (2008). Migration pattern of silver eel (*Anguilla anguilla*, L.) in an obstructed river system. *Ecology of Freshwater Fish, 17*(3), 432-442.

Aguilar, A., Alvarez, M., Leiro, J., & Sanmartin, M. (2005a). Parasite populations of the European eel (Anguilla anguilla L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture, 249*(1), 85-94.

Aguilar, A., Alvarez, M. F., Leiro, J. M., & Sanmartin, M. L. (2005b). Parasite populations of the European eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture, 249*(1-4), 85-94. doi: 10.1016/j.aquaculture.2005.04.052

Aguilar, A., Álvarez, M. F., Leiro, J. M., & Sanmartín, M. L. (2005). Parasite populations of the European eel (Anguilla anguilla L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture, 249*(1–4), 85-94. doi: http://dx.doi.org/10.1016/j.aquaculture.2005.04.052

Alcaide, E., Herraiz, S., & Esteve, C. (2006). Occurrence of Edwardsiella tarda in wild European eels Anguilla anguilla from Mediterranean Spain. *Diseases of Aquatic Organisms, 73*(1), 77-81.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology, 215*(3), 403-410.

Alvarez-Pellitero, P. (2008). Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. *Veterinary immunology and immunopathology, 126*(3), 171-198.

Alves, D. R., Luque, J. L., & Paraguassú, A. R. (2002). Community ecology of the metazoan parasites of pink cusk-eel, *Genypterus brasiliensis* (Osteichthyes: Ophidiidae), from the coastal zone of the State of Rio de Janeiro, Brazil. *Memorias Do Instituto Oswaldo Cruz, 97*(5), 683-689.

Amaro, C., & Biosca, E. G. (1996). Vibrio vulnificus biotype 2, pathogenic for eels, is also an opportunistic pathogen for humans. *Applied and Environmental Microbiology, 62*(4), 1454-1457.

Amaro, C., Biosca, E. G., Fouz, B., Alcaide, E., & Esteve, C. (1995). Evidence that water transmits Vibrio vulnificus biotype 2 infections to eels. *Applied and Environmental Microbiology, 61*(3), 1133-1137.

Amin, O. M. (1987). Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *The Journal of parasitology*, 1216-1219.

Amin, O. M. (2013). Classification of the Acanthocephala. *Folia Parasitologica, 60*(4), 273-305.

Amin, O. M., Abdullah, S. M., & Mhaisen, F. T. (2003). Description of Pomphorhynchus spindletruncatus n. sp.(Acanthocephala: Pomphorhynchidae) from freshwater fishes in northern Iraq, with the erection of a new pomphorhynchid genus, Pyriproboscis ng, and keys to genera of the Pomphorhynchidae and the species of Pomphorhynchus Monticelli, 1905. *Systematic Parasitology, 54*(3), 229-235.

Amin, O. M., & Heckmann, R. A. (1992). Description and pathology of Neoechinorhynchus idahoensis n. sp.(Acanthocephala: Neoechinorhynchidae) in Catostomus columbianus from Idaho. *The Journal of parasitology*, 34-39.

Amin, O. M., Heckmann, R. A., & Baldanova, D. R. (2015). Revisiting Echinorhynchid Acanthocephalans in Lake Baikal with the Use of Scanning Electron Microscopy, with some

Taxonomic Reconsiderations. *Comparative Parasitology, 82*(1), 29-39.
Amin, O. M., Heckmann, R. A., & El-Naggar, A. M. (2011). Revisiting the morphology of Acanthocephalus lucii (Acanthocephala: Echinorhynchidae) in Europe, using SEM. *Scientia Parasitologica, 12*, 185-189.
Amin, O. M., Thielen, F., Münderle, M., Taraschewski, H., & Sures, B. (2008). Description of a new echinorhynchid species (Acanthocephala) from the European eel, Anguilla anguilla, in Germany, with a key to species of Acanthocephalus in Europe. *Journal of Parasitology, 94*(6), 1299-1304.
Andersen, P., Munk, M., Pollock, J., & Doherty, T. (2000). Specific immune-based diagnosis of tuberculosis. *The Lancet, 356*(9235), 1099-1104.
Aoyama, J. (2009). *Life history and evolution of migration in catadromous eels (Genus Anguilla)*: Terrapub.
Aoyama, J., Wouthuyzen, S., Miller, M. J., Inagaki, T., & Tsukamoto, K. (2003). Short-distance spawning migration of tropical freshwater eels. *The Biological Bulletin, 204*(1), 104-108.
Aprahamian, M., Walker, A., Williams, B., Bark, A., & Knights, B. (2007). On the application of models of European eel (*Anguilla anguilla*) production and escapement to the development of Eel Management Plans: the River Severn. *ICES Journal of Marine Science: Journal du Conseil, 64*(7), 1472-1482.
Arafa, S. Z., & Reda, E. S. (2012). Surface features of the monogenean gill parasites *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini* from the European eel *Anguilla anguilla* in Egypt. *Parasitology Research, 110*(1), 127-133. doi: 10.1007/s00436-011-2459-4
Arai. (2014). Evidence of local short-distance spawning migration of tropical freshwater eels, and implications for the evolution of freshwater eel migration. *Ecology and Evolution, 4*(19), 3812-3819. doi: 10.1002/ece3.1245
Arai, T., Otake, T., & Tsukamoto, K. (2000). Timing of metamorphosis and larval segregation of the Atlantic eels *Anguilla rostrata* and *A. anguilla*, as revealed by otolith microstructure and microchemistry. *Marine Biology, 137*(1), 39-45.
Armitage, J., Hewlett, N. R., Twigg, M., Lewin, N. C., Reading, A. J., Williams, C. F., . . . Peeler, E. J. (2014). Detection of *Herpesvirus anguillae* during two mortality investigations of wild European eel in England: implications for fishery management. *Fisheries Management and Ecology, 21*(1), 1-12. doi: 10.1111/fme.12039
Ashworth, S. (1993). *Density dependence within the Anguillicola crassus eel and copepod system.* Paper presented at the International Workshop" Anguillicola and Anguillicolosis of eels.
Ashworth, S., & Kennedy, C. (1999). Density-dependent effects on Anguillicola crassus (Nematoda) within its European eel definitive host. *Parasitology, 118*(03), 289-296.
Awachie, J. (1965). The ecology of Echinorhynchus truttae Schrank, 1788 (Acanthocephala) in a trout stream in North Wales. *Parasitology, 55*(04), 747-762.
Awachie, J. (1966). The development and life history of Echinorhynchus truttae Schrank, 1788 (Acanthocephala). *Journal of Helminthology, 40*(1-2), 11-32.
Bakker, T. C., Mazzi, D., & Zala, S. (1997). Parasite-induced changes in behavior and color make Gammarus pulex more prone to fish predation. *Ecology, 78*(4), 1098-1104.
Baldauf, S. A., Thünken, T., Frommen, J. G., Bakker, T. C., Heupel, O., & Kullmann, H. (2007). Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology, 37*(1), 61-65.
Bandin, I., Souto, S., Cutrin, J. M., Lopez-Vazquez, C., Olveira, J. G., Esteve, C., . . . Dopazo, C. P. (2014). Presence of viruses in wild eels Anguilla anguilla L, from the Albufera Lake (Spain). *Journal of Fish Diseases, 37*(7), 597-607. doi: 10.1111/jfd.1392
Bark, A., Williams, B., & Knights, B. (2007). Current status and temporal trends in stocks of European eel in England and Wales. *ICES Journal of Marine Science: Journal du Conseil, 64*(7), 1368-1378.
Barry, J., McLeish, J., Dodd, J., Turnbull, J., Boylan, P., & Adams, C. (2014). Introduced parasite

Anguillicola crassus infection significantly impedes swim bladder function in the European eel Anguilla anguilla (L.). *Journal of Fish Diseases, 37*(10), 921-924.
Baszler, T., Evermann, J., Kaylor, P., Byington, T., & Dilbeck, P. (1995). Diagnosis of naturally occurring bovine viral diarrhea virus infections in ruminants using monoclonal antibody-based immunohistochemistry. *Veterinary Pathology Online, 32*(6), 609-618.
Bates, R., & Kennedy, C. (1991). Potential interactions between Acanthocephalus anguillae and Pomphorhynchus laevis in their natural hosts chub, Leuciscus cephalus and the European eel, Anguilla anguilla. *Parasitology, 102*(02), 289-297.
Bauer, A., & Rigaud, T. (2015). Identifying a key host in an acanthocephalan-amphipod system. *Parasitology, 142*(13), 1588-1594.
Bauer, A., Trouvé, S., Grégoire, A., Bollache, L. c., & Cézilly, F. (2000). Differential influence of Pomphorhynchus laevis (Acanthocephala) on the behaviour of native and invader gammarid species. *International Journal for Parasitology, 30*(14), 1453-1457.
Belpaire, C., Reyns, T., Geeraerts, C., & Van Loco, J. (2015). Toxic textile dyes accumulate in wild European eel Anguilla anguilla. *Chemosphere, 138*, 784-791.
Benesh, D. P., Hasu, T., Suomalainen, L.-R., Valtonen, E. T., & Tiirola, M. (2006). Reliability of mitochondrial DNA in an acanthocephalan: the problem of pseudogenes. *International Journal for Parasitology, 36*(2), 247-254.
Beullens, K., Eding, E., Gilson, P., Ollevier, F., Komen, J., & Richter, C. (1997). Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguill* L.) maintained in captivity. *Aquaculture, 153*(1), 135-150.
Bibby, M. (1972). Population biology of the helminth parasites of Phoxinus phoxinus (L.), the minnow, in a Cardiganshire lake. *Journal of Fish Biology, 4*(2), 289-300.
Biró, P. (1977). Effects of exploitation, introductions, and eutrophication on percids in Lake Balaton. *Journal of the Fisheries Board of Canada, 34*(10), 1678-1683.
Boeger, W. A., & Kritsky, D. C. (1993). Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology, 26*(1), 1-32.
Boeger, W. A., & Kritsky, D. C. (1997). Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology, 27*(12), 1495-1511.
Bogitsh, B. J., Carter, C. E., & Oeltmann, T. N. (2013). *Human parasitology*: Academic Press.
Bollache, L., Rigaud, T., & Cézilly, F. (2002). Effects of two acanthocephalan parasites on the fecundity and pairing status of female Gammarus pulex (Crustacea: Amphipoda). *Journal of invertebrate pathology, 79*(2), 102-110.
Bonhommeau, S., Chassot, E., & Rivot, E. (2008). Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. *Fisheries Oceanography, 17*(1), 32-44.
Boon, J., Cannaerts, V., Augustijn, H., Machiels, M., De Charleroy, D., & Ollevier, F. (1990). The effect of different infection levels with infective larvae of Anguillicola crassus on haematological parameters of European eel (Anguilla anguilla). *Aquaculture, 87*(3-4), 243-253.
Borgsteede, F. (1996). The effect of parasites on wildlife. *Veterinary quarterly, 18*(sup3), 138-140.
Borgsteede, F., Haenen, O., Bree, J. d., & Lisitsina, O. (1999). Parasitic infections of European eel (*Anguilla anguilla* L.) in the Netherlands. *Helminthologia, 36*(4), 251-260.
Borgsteede, F. H. M., Haenen, O. L. M., De Bree, J., & Lisitsina, O. I. (1999). Parasitic infections of European eel (*Anguilla anguilla* L.) in the Netherlands. *Helminthologia, 36*(4), 251-260.
Bosi, G., & Dezfuli, B. S. (2015). Responses of Squalius cephalus intestinal mucous cells to Pomphorhynchus laevis. *Parasitology International, 64*(2), 167-172.
Bracamonte, S. E., Baltazar-Soares, M., & Eizaguirre, C. (2015). Characterization of MHC class II genes in the critically endangered European eel (Anguilla anguilla). *Conservation Genetics Resources, 7*(4), 859-870.
Brattey, J. (1986). Life history and population biology of Acanthocephalus lucii on the pigmentation,

reproduction and susceptibility to predation of the isopod Asellus aquaticus. *J. Parasitol, 72*, 633-645.
Brattey, J. (1988). Life history and population biology of adult Acanthocephalus lucii (Acanthocephala: Echinorhynchidae). *The Journal of parasitology*, 72-80.
Brázová, T., Poddubnaya, L. G., Miss, N. R., & Hanzelová, V. (2014). Ultrastructure and chemical composition of the proboscis hooks of Acanthocephalus lucii (Müller, 1776)(Acanthocephala: Palaeacanthocephala) using X-ray elemental analysis. *Folia Parasitologica, 61*(6), 549-557.
Bremermann, H. J., & Pickering, J. (1983). A game-theoretical model of parasite virulence. *Journal of Theoretical Biology, 100*(3), 411-426.
Brown, A. (1989). Seasonal dynamics of the acanthocephalan Pomphorhynchm laevis (Müller, 1776) in its intermediate and preferred definitive hosts. *Journal of Fish Biology, 34*(2), 183-194.
Brown, A., Chubb, J., & Veltkamp, C. (1986). A key to the species of Acanthocephala parasitic in British freshwater fishes. *Journal of Fish Biology, 28*(3), 327-334.
Brown, A. F. (1987). Anatomical variability and secondary sexual characteristics in Pomphorhynchus laevis (Müller, 1776)(Acanthocephala). *Systematic Parasitology, 9*(3), 213-219.
Brown, A. S., Butterfield, D. M., Brown, R. J., Hughey, P., Whiteside, K. J., Goddard, S. L., & Williams, M. (2011). Annual report for 2010 on the UK PAH monitoring and analysis network. *NPL Report AS, 62*.
Buchmann, K. (1987). The effects of praziquantel on the monogenean gill parasite *pseudodactylogyrus bini*. *Acta Veterinaria Scandinavica, 28*(3-4), 447-450.
Buchmann, K. (1988a). Epidemiology of pseudodactylogyrosis in an intensive eel-culture system. *Diseases of Aquatic Organisms, 5*(2), 81-85. doi: 10.3354/dao005081
Buchmann, K. (1988b). Interactions between the gill-parasitic monogeneans *Pseudodactylogyrus anguillae* and P. bini and the fish host *Anguilla anguilla*. *Bull. Eur. Ass. Fish Pathol, 8*(5), 98.
Buchmann, K. (1988c). Spatial-distribution of *Pseudodactylogyrus-anguillae* and *Pseudodactylogyrus-bini* (monogenea) on the gills of the european eel, *Anguilla-anguilla*. *Journal of Fish Biology, 32*(5), 801-802. doi: 10.1111/j.1095-8649.1988.tb05422.x
Buchmann, K. (1988d). Temperature-dependent reproduction and survival of *Pseudodactylogyrus-bini* (monogenea) on the european eel (*Anguilla-anguilla*). *Parasitology Research, 75*(2), 162-164. doi: 10.1007/bf00932717
Buchmann, K. (1989a). Microhabitats of monogenean gill parasites on european eel (*Anguilla-anguilla*). *Folia Parasitologica, 36*(4), 321-329.
Buchmann, K. (1989b). Relationship between host size of *Anguilla-anguilla* and the infection level of the monogeneans pseudodactylogyrus spp. *Journal of Fish Biology, 35*(4), 599-601. doi: 10.1111/j.1095-8649.1989.tb03011.x
Buchmann, K. (1990). INFLUENCE OF TEMPERATURE ON REPRODUCTION AND SURVIVAL OF PSEUDODACTYLOGYRUS-ANGUILLAE (MONOGENEA) FROM THE EUROPEAN EEL. *Folia Parasitologica, 37*(1), 59-62.
Buchmann, K. (1993a). Epidemiology and control of pseudodactylogyrus infections in intensive eel culture systems - recent trends. *Bulletin Francais De La Peche Et De La Pisciculture*(328), 66-73. doi: 10.1051/kmae:1993012
Buchmann, K. (1993b). Epidemiology and control of Pseudodactylogyrus infections in intensive eel culture systems: Recent trends. *Bulletin Francais De La Peche Et De La Pisciculture, 0*(328), 66-73.
Buchmann, K. (2012). *Pseudodactylogyrus anguillae and Pseudodactylogyrus bini*.
Buchmann, K., Køie, M., & Prentø, P. (1987). The nutrition of the gill parasitic monogenean Pseudodactylogyrus anguillae. *Parasitology Research, 73*(6), 532-537.
Buchmann, K., & Lindenstrøm, T. (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology, 32*(3), 309-319.
Buchmann, K., Mellergaard, S., & Koie, M. (1987). Pseudodactylogyrus infections in eel - a review. *Diseases of Aquatic Organisms, 3*(1), 51-57. doi: 10.3354/dao003051

Budke, C. M., White Jr, A. C., & Garcia, H. H. (2009). Zoonotic larval cestode infections: neglected, neglected tropical diseases? *PLoS Negl Trop Dis, 3*(2), e319.
Bullock, W. L., & Schmidt, G. (1969). Morphological features as tools and as pitfalls in acanthocephalan systematics". *Problems in systematics of parasites.*, 9-43.
Bylund, B. G. (2003). 17 Diphyllobothrium latum. *Parasites of the colder climates*, 169.
Byrne, C., Holland, C., Walsh, E., Mulligan, C., Kennedy, C., & Poole, W. (2004). Utilization of brown trout Salmo trutta by Acanthocephalus clavula in an Irish lake: is this evidence of a host shift? *Journal of Helminthology, 78*(03), 201-206.
Carder, N., Reitz, E. J., & Crock, J. G. (2007). Fish communities and populations during the post-Saladoid period (AD 600/800–1500), Anguilla, Lesser Antilles. *Journal of Archaeological Science, 34*(4), 588-599.
Casselman, J. M. (2003). Dynamics of resources of the American eel, Anguilla rostrata: declining abundance in the 1990s *Eel biology* (pp. 255-274): Springer.
Castonguay, M., Hodson, P., Couillard, C., Eckersley, M., Dutil, J.-D., & Verreault, G. (1994). Why is recruitment of the American eel, Anguilla rostrata, declining in the St. Lawrence River and Gulf? *Canadian Journal of Fisheries and Aquatic Sciences, 51*(2), 479-488.
CDC. (2012). parasites/diphyllobothrium/biology, http://www.cdc.gov/parasites/diphyllobothrium/biology.html.
Chan, B. Z., & Wu, B. W. (1984). Studies on the pathogenicity, biology and treatment of pseudodactylogyrus for the eels in fishfarms. *Acta Zoologica Sinica, 30*(2), 173-&.
Chen, S., Ai, L., Zhang, Y., Chen, J., Zhang, W., Li, Y., . . . Xu, X. (2014). Molecular detection of Diphyllobothrium nihonkaiense in humans, China. *Emerging infectious diseases, 20*(2), 315-318.
Christison, K. W., & Baker, G. C. (2007). First record of Pseudodactylogyrus anguillae (Yin & Sproston, 1948) (Monogenea) from South Africa. *African Zoology, 42*(2), 279-285. doi: 10.3377/1562-7020(2007)42[279:fropay]2.0.co;2
Chubb, J., Pool, D., & Veltkamp, C. (1987). A key to the species of cestodes (tapeworms) parasitic in British and Irish freshwater fishes. *Journal of Fish Biology, 31*(4), 517-543.
Chung, H.-Y., Lin, I.-H., & Kou, G.-H. (1984). Study of the parasites on the gill of cultured eel in Taiwan. *COA Fisheries Series*(10), 24-33.
Collado, R., Fouz Rodríguez, B., Sanjuán Caro, E., & Amaro González, C. (2000). Effectiveness of different vaccine formulations against vibriosis caused by Vibrio vulnificus serovar E (biotype 2) in European eels Anguilla anguilla.
Combes, C. (1997). Fitness of parasites: pathology and selection. *International Journal for Parasitology, 27*(1), 1-10.
Cone, D. K., & Marcogliese, D. J. (1995). *Pseudodactylogyrus anguilla*e on *Anguilla-rostrata i*n nova-scotia - an endemic or an introduction. *Journal of Fish Biology, 47*(1), 177-178. doi: 10.1006/jfbi.1995.0124
Conneely, J., & McCarthy, T. (1986). Ecological factors influencing the composition of the parasite fauna of the European eel, Anguilla anguilla (L.), in Ireland. *Journal of Fish Biology, 28*(2), 207-219.
Copley, L., & McCarthy, T. (2001). The first record of the monogenean gill fluke Pseudodactylogyrus bini (Kikuchi 1929) in Ireland, with observations on other ectoparasites of River Erne eels. *The Irish Naturalists' Journal*, 405-413.
Cringoli, G., Rinaldi, L., Maurelli, M. P., & Utzinger, J. (2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature Protocols, 5*(3), 503-515.
Crompton, D. W. T., & Nickol, B. B. (1985). *Biology of the Acanthocephala*: Cambridge University Press.
Davey, A. J., & Jellyman, D. J. (2005). Sex determination in freshwater eels and management options for manipulation of sex. *Reviews in Fish Biology and Fisheries, 15*(1-2), 37-52.

Davidson, N. C. (2014). How much wetland has the world lost? Long-term and recent trends in global wetland area. *Marine and Freshwater Research, 65*(10), 934-941.
Davis, L. (2012). *Basic methods in molecular biology* (Vol. Chapter 7): Elsevier.
Dekker, W. (2000). The fractal geometry of the European eel stock. *ICES Journal of Marine Science: Journal du Conseil, 57*(1), 109-121.
Dekker, W. (2003a). Did lack of spawners cause the collapse of the European eel, Anguilla anguilla? *Fisheries Management and Ecology, 10*(6), 365-376.
Dekker, W. (2003b). On the distribution of the European eel (Anguilla anguilla) and its fisheries. *Canadian Journal of Fisheries and Aquatic Sciences, 60*(7), 787-799.
Dekker, W., Pawson, M., & Wickström, H. (2007). Is there more to eels than slime? An introduction to papers presented at the ICES Theme Session in September 2006. *ICES Journal of Marine Science: Journal du Conseil, 64*(7), 1366-1367.
Denmark, F. C. (1987). Pseudodactylogyrus infections in eel: a review. *Diseases of Aquatic Organisms, 3*, 51-57.
Dezfuli, B., Bo, T., Lorenzoni, M., Shinn, A., & Giari, L. (2015). Fine structure and cellular responses at the host–parasite interface in a range of fish–helminth systems. *Veterinary Parasitology, 208*(3), 272-279.
Dezfuli, B., Castaldelli, G., Bo, T., Lorenzoni, M., & Giari, L. (2011). Intestinal immune response of Silurus glanis and Barbus barbus naturally infected with Pomphorhynchus laevis (Acanthocephala). *Parasite immunology, 33*(2), 116-123.
Dezfuli, B., Giari, L., Simoni, E., Bosi, G., & Manera, M. (2002). Histopathology, immunohistochemistry and ultrastructure of the intestine of Leuciscus cephalus (L.) naturally infected with Pomphorhynchus laevis (Acanthocephala). *Journal of Fish Diseases, 25*(1), 7-14.
Dezfuli, B. S., Giari, L., & Poulin, R. (2000). Species associations among larval helminths in an amphipod intermediate host. *International Journal for Parasitology, 30*(11), 1143-1146.
Dezfuli, B. S., Rossetti, E., Rossi, R., & Fano, E. A. (1994). Occurrence of larval Acanthocephalus anguillae (Acanthocephala) in the Asellus aquaticus (Crustacea, Isopoda) from the River Brenta. *Italian Journal of Zoology, 61*(1), 77-81.
Di Cave, D., Berrilli, F., De Liberato, C., Orecchia, P., & Kennedy, C. (2001). Helminth communities in eels Anguilla anguilla from Adriatic coastal lagoons in Italy. *Journal of Helminthology, 75*(1), 7-14.
Dörner, H., & Benndorf, J. (2003). Piscivory by large eels on young-of-the-year fishes: its potential as a biomanipulation tool. *Journal of Fish Biology, 62*(2), 491-494.
Dorucu, M., & Huntingfordl, F. (1995). The ecology of endoparasitic helminth infections of brown trout. *Folia Parasitologica, 42*, 29-35.
Durif, C. M., Dufour, S., & Elie, P. (2006). Impact of silvering stage, age, body size and condition on reproductive potential of the European eel. *Marine Ecology-Progress Series, 327*, 171.
Durif, C. M., Gjøsæter, J., & Vøllestad, L. A. (2010). Influence of oceanic factors on Anguilla anguilla (L.) over the twentieth century in coastal habitats of the Skagerrak, southern Norway. *Proceedings of the Royal Society B: Biological Sciences*, rspb20101547.
Emde, S., Rueckert, S., Kochmann, J., Knopf, K., Sures, B., & Klimpel, S. (2014). Nematode eel parasite found inside acanthocephalan cysts-a" Trojan horse" strategy? *Parasites & Vectors, 7*(1), 1.
Emde, S., Rueckert, S., Palm, H. W., & Klimpel, S. (2012). Invasive Ponto-Caspian amphipods and fish increase the distribution range of the acanthocephalan Pomphorhynchus tereticollis in the river Rhine. *Plos One, 7*(12), e53218.
Environment Agency UK. (2014). Aquatic animal health and movements guides. *www.environment.agency.gov.uk*(1-5).
Esch, G., Kennedy, C., Bush, A., & Aho, J. (1988). Patterns in helminth communities in freshwater fish in Great Britain: alternative strategies for colonization. *Parasitology, 96*(03), 519-532.
Evans, D., Matthews, M., & McClintock, C. (2001). First record of Pomphorhynchus laevis

(Acanthocephala) in fishes from Northern Ireland. *Journal of Fish Biology, 59*(1), 166-168.
Fang, J., Shirakashi, S., & Ogawa, K. (2008a). Comparative Susceptibility of Japanese and European Eels to Infections with Pseudodactylogyrus spp. (Monogenea). *Fish Pathology, 43*(4), 144-151.
Fang, J., Shirakashi, S., & Ogawa, K. (2008b). Comparative susceptibility of Japanese and European eels to infections with Pseudodactylogyrus spp.(Monogenea). *Fish Pathology, 43*(4), 144-151.
Feunteun, E. (2002). Management and restoration of European eel population (Anguilla anguilla): an impossible bargain. *Ecological Engineering, 18*(5), 575-591.
Filippi, J.-J., Quilichini, Y., Foata, J., & Marchand, B. (2013). Influence of site, season, silvering stage, and length on the parasites of the European eel Anguilla anguilla in two Mediterranean coastal lagoons of the island of Corsica, France using indicator species method. *Parasitology Research, 112*(8), 2959-2969. doi: 10.1007/s00436-013-3468-2
Fitzsimons, J. D., Brown, S. B., Brown, L. R., Verreault, G., Tardif, R., Drouillard, K. G., . . . Lantry, J. R. (2013). Impacts of Diet on Thiamine Status of Lake Ontario American Eels. *Transactions of the American Fisheries Society, 142*(5), 1358-1369. doi: 10.1080/00028487.2013.811100
Fontenelle, G., Knoff, M., Felizardo, N. N., Lopes, L. M. S., & Sao Clemente, S. C. d. (2013). Nematodes of zoonotic importance in Cynoscion guatucupa (Pisces) in the state of Rio de Janeiro. *Revista Brasileira de Parasitologia Veterinária, 22*(2), 281-284.
Freyhof, J., & Kottelat, M. (2008). Anguilla anguilla. *IUCN 2010. IUCN Red List of Threatened Species*.
Friedland, K. D., Miller, M. J., & Knights, B. (2007). Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. *ICES Journal of Marine Science: Journal du Conseil, 64*(3), 519-530.
Galli, P., Crosa, G., Mariniello, L., Ortis, M., & D'amelio, S. (2001). Water quality as a determinant of the composition of fish parasite communities. *Hydrobiologia, 452*(1-3), 173-179.
García-Varela, M., de León, G. P.-P., De La Torre, P., Cummings, M. P., Sarma, S., & Laclette, J. P. (2000). Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution, 50*(6), 532-540.
García-Varela, M., & Nadler, S. A. (2005). Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology, 91*(6), 1401-1409.
Garcia, H. H., Moro, P. L., & Schantz, P. M. (2007). Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fascioliasis. *Current opinion in infectious diseases, 20*(5), 489-494.
Geeraerts, C., & Belpaire, C. (2010). The effects of contaminants in European eel: a review. *Ecotoxicology, 19*(2), 239-266.
Gelnar, M., Scholz, T., Matejusova, I., & Konecny, R. (1996). Occurrence of Pseudodactylogyrus anguillae (YIN and SPROSTON, 1948) and P. bini (KIKUCHI, 1929), parasites of eel, Anguilla anguilla L., in Austria (Monogenea: Dactylogyridae). *Annalen des Naturhistorischen Museums in Wien Serie B Botanik und Zoologie, 98*(0), 1-4.
Gerard, C., Trancart, T., Amilhat, E., Faliex, E., Virag, L., Feunteun, E., & Acou, A. (2013). Influence of introduced vs. native parasites on the body condition of migrant silver eels. *Parasite, 20*. doi: 10.1051/parasite/2013040
Gérard, C., Trancart, T., Amilhat, E., Faliex, E., Virag, L., Feunteun, E., & Acou, A. (2013). Influence of introduced vs. native parasites on the body condition of migrant silver eels. *Parasite, 20*.
Gibson, D. (1993). Monobothrium wageneri: another imported tapeworm established in wild British freshwater fishes? *Journal of Fish Biology, 43*(2), 281-285.
Gibson, D., Bray, R., & Harris, E. (2005). Host-parasite database of the natural history museum, London. *URL http://www. nhm. ac. uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/database/index. jsp*.
Gollock, M., Kennedy, C., & Brown, J. (2005). European eels, Anguilla anguilla (L.), infected with

Anguillicola crassus exhibit a more pronounced stress response to severe hypoxia than uninfected eels. *Journal of Fish Diseases, 28*(7), 429-436.
Golovin, P., & Shukhgalter, O. (1979). The biology of the monogenean parasites of eels from the genus Pseudodactylogyrus. *Sb. Nauchn. Tr., Vses. Nauchno-Issled. Inst. Prudnogo Rybn. Khoz.(Bolezni rybi bor'ba s nimi), 23*, 107-116.
Guimaraes, L., Gravato, C., Santos, J., Monteiro, L. S., & Guilhermino, L. (2009). Yellow eel (*Anguilla anguilla*) development in NW Portuguese estuaries with different contamination levels. *Ecotoxicology, 18*(4), 385-402. doi: 10.1007/s10646-008-0294-x
Gussev, A. (1965). A new genus of monogenetic trematodes from the eel, genus Anguilla. *Trudy Zoologicheskogo Instituta Leningrad, 35*, 119-125.
Haenen, O., Mladineo, I., Konecny, R., Yoshimizu, M., Groman, D., Munoz, P., . . . Van Beurden, S. (2012). Diseases of eels in an international perspective: Workshop on Eel Diseases at the 15th. *Bull. Eur. Ass. Fish Pathol, 32*(3), 109.
Haenen, O., van Ginneken, V., Engelsma, M., & van den Thillart, G. (2009). Impact of eel viruses on recruitment of European eel *Spawning migration of the European Eel* (pp. 387-400): Springer.
Han, Y.-S., Chang, Y.-T., Taraschewski, H., Chang, S.-L., Chen, C.-C., & Tzeng, W.-N. (2008). The swimbladder parasite Anguillicola crassus in native Japanese eels and exotic American eels in Taiwan. *Zoological Studies, 47*(6), 667-675.
Han, Y.-S., Tzeng, W.-N., Huang, Y.-S., & Liao, I. (2001). Silvering in the eel: changes in morphology, body fat content, and gonadal development. *Journal of Taiwan Fisheries Research, 9*(1&2), 119-127.
Hanel, R., Stepputtis, D., Bonhommeau, S., Castonguay, M., Schaber, M., Wysujack, K., . . . Miller, M. J. (2014). Low larval abundance in the Sargasso Sea: new evidence about reduced recruitment of the Atlantic eels. *Naturwissenschaften, 101*(12), 1041-1054. doi: 10.1007/s00114-014-1243-6
Hasu, T. (2013). Host-related factors affectingisopod (Asellus aquaticus) susceptibility to and interaction with an acanthocephalan (Acanthocephalus lucii) parasite.
Hayward, C. J., Iwashita, M., Crane, J. S., & Ogawa, K. (2001). First report of the invasive eel pest *Pseudodactylogyrus bini* in North America and in wild American eels. *Diseases of Aquatic Organisms, 44*(1), 53-60. doi: 10.3354/dao044053
Hedger, R. D., Atkinson, P. M., Thibault, I., & Dodson, J. J. (2008). A quantitative approach for classifying fish otolith strontium: calcium sequences into environmental histories. *Ecological Informatics, 3*(3), 207-217.
Heinen, J., & Mangino, K. (2007). Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).
Heitlinger, E. G., Laetsch, D. R., Weclawski, U., Han, Y.-S., & Taraschewski, H. (2009). Massive encapsulation of larval Anguillicoloides crassus in the intestinal wall of Japanese eels. *Parasites and Vectors, 2*(48), 15.
Hine, P. (1978). Distribution of some parasites of freshwater eels in New Zealand. *New Zealand journal of marine and freshwater research, 12*(2), 179-187.
Hine, P., & Kennedy, C. (1974a). Observations on the distribution, specificity and pathogenicity of the acanthocephalan *Pomphorhynchus laevis* (Müller). *Journal of Fish Biology, 6*(4), 521-535.
Hine, P., & Kennedy, C. (1974b). The population biology of the acanthocephalan Pomphorhynchus laevis (Müller) in the River Avon. *Journal of Fish Biology, 6*(5), 665-679.
Holmes, J. C. (1973). Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology, 51*(3), 333-347.
Holmgren, K., Wickström, H., & Clevestam, P. (1997). Sex-related growth of European eel, *Anguilla anguilla*, with focus on median silver eel age. *Canadian Journal of Fisheries and Aquatic Sciences, 54*(12), 2775-2781.

ICES. (2006). Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Rome, 23–27 January 2006. *EIFAC Occasional Paper*(38), 352.

Imada, R., & Muroga, K. (1978). *Pseudodactylogyrus-microrchis* (monogenea) on gills of cultured eels .2. oviposition, hatching and development on host. *Bulletin of the Japanese Society of Scientific Fisheries, 44*(6), 571-576.

Jackson, Y., Pastore, R., Sudre, P., Loutan, L., & Chappuis, F. (2007). Diphyllobothrium latum outbreak from marinated raw perch, Lake Geneva, Switzerland. *Emerg Infect Dis, 13*(12), 1957-1958.

Jacobsen, M. W., Pujolar, J. M., Bernatchez, L., Munch, K., Jian, J. B., Niu, Y. C., & Hansen, M. M. (2014). Genomic footprints of speciation in Atlantic eels (*Anguilla anguilla* and *A. rostrata*). *Molecular Ecology, 23*(19), 4785-4798. doi: 10.1111/mec.12896

Jacoby, D. M., Casselman, J. M., Crook, V., DeLucia, M.-B., Ahn, H., Kaifu, K., . . . Smith, K. G. (2015). Synergistic patterns of threat and the challenges facing global anguillid eel conservation. *Global Ecology and Conservation, 4*, 321-333.

Jahantab, M., Haseli, M., & Salehi, Z. (2014). Morphological and genetic characteristics of the anisakid nematode Raphidascaris acus from the southwest Caspian Sea: evidence for the existence of sibling species within a species complex. *Parasitology Research, 113*(9), 3419-3425. doi: 10.1007/s00436-014-4007-5

Jakob, E., Hanel, R., Klimpel, S., & Zumholz, K. (2009a). Salinity dependence of parasite infestation in the European eel Anguilla anguilla in northern Germany. *Ices Journal of Marine Science, 66*(2), 358-366. doi: 10.1093/icesjms/fsn160

Jakob, E., Hanel, R., Klimpel, S., & Zumholz, K. (2009b). Salinity dependence of parasite infestation in the European eel Anguilla anguilla in northern Germany. *ICES Journal of Marine Science: Journal du Conseil, 66*(2), 358-366.

Jakob, E., Walter, T., & Hanel, R. (2009). A checklist of the protozoan and metazoan parasites of European eel (Anguilla anguilla): checklist of Anguilla anguilla parasites. *Journal of Applied Ichthyology*.

Jellyman, D. J. (1989). Diet of two species of freshwater eel (Anguilla spp.) in Lake Pounui, New Zealand. *New Zealand journal of marine and freshwater research, 23*(1), 1-10.

Johnson, S., Fries, L., Williams, J., & Huffman, D. (1995). Presence of the parasitic swim bladder nematode, Anguillicola crassus. *Texas aquaculture. World Aquaculture, 26*(3), 35-26.

Kangur, A., & Kangur, K. (1998). Relationship between the Population Dynamics of Chironomidae and the Condition Factor of European Eel, Anguilla anguilla(L.) in Lake Vortsjaerv. *Limnologica, 28*(1), 103-107.

Kania, P. W., Taraschewski, H., Han, Y. S., Cone, D. K., & Buchmann, K. (2010). Divergence between Asian, European and Canadian populations of the monogenean Pseudodactylogyrus bini indicated by ribosomal DNA patterns. *Journal of Helminthology, 84*(4), 404-409. doi: 10.1017/s0022149x10000088

Kelly, C., Kennedy, C., & Brown, J. (2000). Physiological status of wild European eels (Anguilla anguilla) infected with the parasitic nematode, Anguillicola crassus. *Parasitology, 120*(02), 195-202.

Kennedy, C. (1974). A checklist of British and Irish freshwater fish parasites with notes on their distribution. *Journal of Fish Biology, 6*(5), 613-644.

Kennedy, C. (1984a). The dynamics of a declining population of the acanthocephalan Acanthocephalus clavula in eels Anguilla anguilla in a small river. *Journal of Fish Biology, 25*(6), 665-677.

Kennedy, C. (1984b). The status of flounders, Platichthys flesus L., as hosts of the acanthocephalan Pomphorhynchus laevis (Müller) and its survival in marine conditions. *Journal of Fish Biology, 24*(2), 135-149.

Kennedy, C. (1985). Site segregation by species of Acanthocephala in fish, with special reference to eels, Anguilla anguilla. *Parasitology, 90*(02), 375-390.

Kennedy, C. (1992). Field evidence for interactions between the acanthocephalans Acanthocephalus anguillae and A. lucii in eels, Anguilla anguilla. *Ecological Parasitology, 1*(2), 122-134.
Kennedy, C. (1993a). The dynamics of intestinal helminth communities in eels Anguilla anguilla in a small stream: long-term changes in richness and structure. *Parasitology, 107*(01), 71-78.
Kennedy, C. (1993b). Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. *Journal of Fish Biology, 43*(2), 287-301.
Kennedy, C. (1996). Colonization and establishment of Pomphorhynchus laevis (Acanthocephala) in an isolated English river. *Journal of Helminthology, 70*(01), 27-31.
Kennedy, C. (2001a). Metapopulation and community dynamics of helminth parasites of eels Anguilla anguilla in the River Exe system. *Parasitology, 122*(06), 689-698.
Kennedy, C. (2007a). The pathogenic helminth parasites of eels. *Journal of Fish Diseases, 30*(6), 319-334.
Kennedy, C., Bates, R., & Brown, A. (1989). Discontinuous distributions of the fish acanthocephalans *Pomphorhynchm laevis* and *Acanthocephalus anguillae* in Britain and Ireland: an hypothesis. *Journal of Fish Biology, 34*(4), 607-619.
Kennedy, C., Berrilli, F., Di Cave, D., De Liberato, C., & Orecchia, P. (1998). Composition and diversity of helminth communities in eels Anguilla anguilla in the River Tiber: long-term changes and comparison with insular Europe. *Journal of Helminthology, 72*(04), 301-306.
Kennedy, C., Broughton, P., & Hine, P. (1978). The status of brown and rainbow trout, Salmo trutta and S. gairdneri as hosts of the acanthocephalan, Pomphorhynchus laevis. *Journal of Fish Biology, 13*(2), 265-275.
Kennedy, C., & Di Cave, D. (1998). Gyrodactylus anguillae (Monogenea): the story of an appearance and a disappearance. *Folia Parasitologica, 45*(1), 77-78.
Kennedy, C., Di Cave, D., Berrilli, F., & Orecchia, P. (1997). Composition and structure of helminth communities in eels Anguilla anguilla from Italian coastal lagoons. *Journal of Helminthology, 71*(01), 35-40.
Kennedy, C., & Fitch, D. (1990). Colonization, larval survival and epidemiology of the nematode Anguillicola crassus, parasitic in the eel, Anguilla anguilla, in Britain. *Journal of Fish Biology, 36*(2), 117-131.
Kennedy, C., Galaktionov, K., Pugachev, O., MacKenzie, K., & Skírnisson, K. (2000). *Freshwater fish parasites and habitat change.* Paper presented at the Bulletin of the Scandinavian Society for Parasitology.
Kennedy, C., & Guégan, J.-F. (1996). The number of niches in intestinal helminth communities of Anguilla anguilla: are there enough spaces for parasites? *Parasitology, 113*(03), 293-302.
Kennedy, C., & Lord, D. (1982). Habitat specificity of the acanthocephalan Acanthocephalus clavula (Dujardin, 1845) in eels Anguilla anguilla (L). *Journal of Helminthology, 56*(02), 121-130.
Kennedy, C., & Moriarty, C. (1987). Co-existence of congeneric species of Acanthocephala: Acanthocephalus lucii and A. anguillae in eels Anguilla anguilla in Ireland. *Parasitology, 95*(02), 301-310.
Kennedy, C., Nie, P., Kaspers, J., & Paulisse, J. (1992). Are eels (Anguilla anguilla L.) planktonic feeders? Evidence from parasite communities. *Journal of Fish Biology, 41*(4), 567-580.
Kennedy, C., Nie, P., & Rostron, J. (1992). An insect, Sialis lutaria, as a host for larval Proteocephalus sp. *Journal of Helminthology, 66*(01), 7-16.
Kennedy, C. R. (1997a). Long-term and seasonal changes in composition and richness of intestinal helminth communities in eels Anguilla anguilla of an. *Folia Parasitologica, 44*, 267-273.
Kennedy, C. R. (1997b). Long-term and seasonal changes in composition and richness of intestinal helminth communities in eels Anguilla anguilla of an isolated English river. *Folia Parasitologica, 44*(4), 267-273.
Kennedy, C. R. (1998). Aquatic birds as agents of parasite dispersal: a field test of the effectiveness of helminth colonisation strategies. *Bulletin of the Scandinavian Society for Parasitology, 8*, 23-

28.
Kennedy, C. R. (1999). Post-cyclic transmission in Pomphorhynchus laevis (Acanthocephala). *Folia Parasitologica, 46*(2), 111-116.
Kennedy, C. R. (2001b). Metapopulation and community dynamics of helminth parasites of eels Anguilla anguilla in the River Exe system. *Parasitology, 122*, 689-698.
Kennedy, C. R. (2007b). The pathogenic helminth parasites of eels. *Journal of Fish Diseases, 30*(6), 319-334. doi: 10.1111/j.1365-2761.2007.00821.x
Kennedy, C. R. (2012). Can a specialist parasite species of a widespread and common host species be rare? The case of Spinitectus inermis (Nematoda: Cystidicolidae) in eels Anguilla anguilla. *Folia Parasitologica, 59*(2), 131-138.
Kennedy, C. R., & Moriarty, C. (2002). Long-term stability in the richness and structure of helminth communities in eels, Anguilla anguilla, in Lough Derg, River Shannon, Ireland. *Journal of Helminthology, 76*(04), 315-322.
Keppel, M., Dangel, K., & Sures, B. (2014). Comparison of infection success, development and swim bladder pathogenicity of two congeneric Anguillicola species in experimentally infected Anguilla anguilla and A. japonica. *Parasitology Research, 113*(10), 3727-3735.
Khan, R. (2012). Host-parasite interactions in some fish species. *Journal of parasitology research, 2012*.
Kikuchi, H. (1929). Two new species of Japanese trematodes belonging to Gyrodactylidae. *Annotations Zoologicae Japonensis, 12*, 175-186.
Kimura, S., Tsukamoto, K., & Sugimoto, T. (1994). A model for the larval migration of the Japanese eel: roles of the trade winds and salinity front. *Marine Biology, 119*(2), 185-190.
Kirk, R. (2003). The impact of Anguillicola crassus on European eels. *Fisheries Management and Ecology, 10*(6), 385-394.
Kirk, R., Kennedy, C., & Lewis, J. (2000). Effect of salinity on hatching, survival and infectivity of Anguillicola crassus (Nematoda: Dracunculoidea) larvae. *Diseases of Aquatic Organisms, 40*(3), 211-218.
Kirk, R., Lewis, J., & Kennedy, C. (2000). Survival and transmission of Anguillicola crassus Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology, 120*(03), 289-295.
Kirk, R., Morritt, D., Lewis, J., & Kennedy, C. (2002). The osmotic relationship of the swimbladder nematode Anguillicola crassus with seawater eels. *Parasitology, 124*(03), 339-347.
Kirk, R. S. (2006). Pseudodactylogyrus species on European eels (Anguilla anguilla L.) in the River Test and the River Thames, England. *ICOPA XI, Glasgow.*
Kleckner, R., & McCleave, J. (1982). Entry of migrating American eel leptocephali into the Gulf Stream system. *Helgoländer Meeresuntersuchungen, 35*(3), 329-339.
Knights, B. (2003). A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *Science of the total Environment, 310*(1), 237-244.
Knights, B., Bark, A., Ball, M., Williams, F., Winter, E., & Dunn, S. (2001). *Eel and elver stocks in England and Wales: status and management options*: Environment Agency United Kingdom.
Knoff, M., Felizardo, N. N., Iñiguez, A. M., Maldonado Jr, A., Torres, E. J. L., Pinto, R. M., & Gomes, D. C. (2012). Genetic and morphological characterisation of a new species of the genus Hysterothylacium (Nematoda) from Paralichthys isosceles Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, state of Rio de Janeiro, Brazil. *Memorias Do Instituto Oswaldo Cruz, 107*(2), 186-193.
Knopf, K. (2006). The swimbladder nematode Anguillicola crassus in the European eel Anguilla anguilla and the Japanese eel Anguilla japonica: differences in susceptibility and immunity between a recently colonized host and the original host. *Journal of Helminthology, 80*(02), 129-136.
Knopf, K., Helm, A. M., Lucius, R., Bleiss, W., & Taraschewski, H. (2008). Migratory response of European eel (Anguilla anguilla) phagocytes to the eel swimbladder nematode Anguillicola

crassus. *Parasitology Research, 102*(6), 1311-1316.
Knopf, K., & Mahnke, M. (2004). Differences in susceptibility of the European eel (*Anguilla anguilla*) and the Japanese eel (*Anguilla japonica*) to the swim-bladder nematode *Anguillicola crassus*. *Parasitology, 129*, 491-496. doi: 10.1017/s0031182004005864
Knopf, K., Naser, K., van der Heijden, M., & Taraschewski, H. (2000). Humoral immune response of European eel Anguilla anguilla experimentally infected with Anguillicola crassus. *Diseases of Aquatic Organisms, 42*(1), 61-69.
Knowles, S. C., Fenton, A., Petchey, O. L., Jones, T. R., Barber, R., & Pedersen, A. B. (2013). *Stability of within-host–parasite communities in a wild mammal system.* Paper presented at the Proc. R. Soc. B.
Kodedová, I., Doležel, D., Broučková, M., Jirků, M., Hypša, V., Lukeš, J., & Scholz, T. (2000). On the phylogenetic positions of the Caryophyllidea, Pseudophyllidea and Proteocephalidea (Eucestoda) inferred from 18S rRNA. *International Journal for Parasitology, 30*(10), 1109-1113.
Koie, M. (1991). Swimbladder nematodes (anguillicola-spp) and gill monogeneans (pseudodactylogyrus-spp) parasitic on the european eel (*anguilla-anguilla*). *Journal Du Conseil, 47*(3), 391-398.
Køie, M. (1988). Parasites in European eel Anguilla Anguilla (L.) from Danish Freshwater, Brackishand Marine Localities. *Ophelia, 29*(2), 93-118.
Køie, M. (1991). Swimbladder nematodes (Anguillicola spp.) and gill monogeneans (Pseudodactylogyrus spp.) parasitic on the European eel (Anguilla anguilla). *Journal du Conseil: ICES Journal of Marine Science, 47*(3), 391-398.
Køie, M. (1993). Aspects of the life cycle and morphology of Hysterothylacium aduncum (Rudolphi, 1802)(Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Zoology, 71*(7), 1289-1296.
Koops, H., & Hartmann, F. (1989). Anguillicola-infestations in Germany and in German eel imports. *Journal of Applied Ichthyology, 5*(1), 41-45.
Kopp, D. A., Elke, D. A., Caddigan, S. C., Raj, A., Rodriguez, L., Young, M. K., & Sparkes, T. C. (2011). Dispersal in the acanthocephalan Acanthocephalus dirus. *Journal of Parasitology, 97*(6), 1101-1105.
Kristmundsson, Á., & Helgason, S. (2007). Parasite communities of ecls Anguilla anguilla in freshwater and marine habitats in Iceland in comparison with other parasite communities of eels in Europe. *Folia Parasitologica, 54*(2), 141.
Kuchta, R., Brabec, J., Kubáčková, P., & Scholz, T. (2013). Tapeworm Diphyllobothrium dendriticum (Cestoda)—neglected or emerging human parasite? *PLoS Negl Trop Dis, 7*(12), e2535.
Kuchta, R., Burianova, A., Jirku, M., de Chambrier, A., Oros, M., Brabec, J., & Scholz, T. (2012). Bothriocephalidean tapeworms (Cestoda) of freshwater fish in Africa, including erection of Kirstenella n. gen. and description of Tetracampos martinae n. sp. (vol 3309, pg 1, 2012). *Zootaxa*(3325), 68-68.
Kuchta, R., Scholz, T., & Bray, R. A. (2008). Revision of the order Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008 (Eucestoda) with amended generic diagnoses and keys to families and genera. *Systematic Parasitology, 71*(2), 81-136.
Laetsch, D. R., Heitlinger, E. G., Taraschewski, H., Nadler, S. A., & Blaxter, M. L. (2012). The phylogenetics of Anguillicolidae (Nematoda: Anguillicoloidea), swimbladder parasites of eels. *Bmc Evolutionary Biology, 12*(1), 60.
Lafferty, K. D., & Kuris, A. M. (1999). How environmental stress affects the impacts of parasites. *Limnology and Oceanography, 44*(3part2), 925-931.
Larkin, M. A., Blackshields, G., Brown, N., Chenna, R., McGettigan, P. A., McWilliam, H., . . . Lopez, R. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*(21), 2947-2948.
Lasne, É., & Laffaille, P. (2008). Assessing the freshwater distribution of yellow eel. *Knowledge and Management of Aquatic Ecosystems*(390-391), 04.
Lassiere, O. L. (1988). Host-parasite relationships between larval Sialis lutaria (Megaloptera) and

Neoechinorhynchus rutili (Acanthocephala). *Parasitology, 97*(02), 331-338.
Lassiere, O. L., & Crompton, D. (1988). Evidence for post-cyclic transmission in the life-history of Neoechinorhynchus rutili (Acanthocephala). *Parasitology, 97*(02), 339-343.
Laycock, H., Moran, D., Smart, J., Raffaelli, D., & White, P. (2009). Evaluating the cost-effectiveness of conservation: The UK Biodiversity Action Plan. *Biological Conservation, 142*(12), 3120-3127.
Leander, N. J., Shen, K. N., Chen, R. T., & Tzeng, W. N. (2012). Species Composition and Seasonal Occurrence of Recruiting Glass Eels (Anguilla spp.) in the Hsiukuluan River, Eastern Taiwan. *Zoological Studies, 51*(1), 59-71.
Lecomte-Finiger, R. (2003). The genus Anguilla Schrank, 1798: current state of knowledge and questions. *Reviews in Fish Biology and Fisheries, 13*(3), 265-279. doi: 10.1023/B:RFBF.0000033072.03829.6d
Lee, E. B., Song, J. H., Park, N. S., Kang, B. K., Lee, H. S., Han, Y. J., . . . Chai, J.-Y. (2007). A case of Diphyllobothrium latum infection with a brief review of diphyllobothriasis in the Republic of Korea. *The Korean Journal of Parasitology, 45*(3), 219-223.
Lee, R. (1981). Ecology of *Acanthocephalus lucii* (Müller, 1776) in perch, Perca fluviatilis, in the Serpentine, London, UK. *Journal of Helminthology, 55*(02), 149-154.
Lefebvre, F., Contournet, P., & Crivelli, A. (2002). The health state of the eel swimbladder as a measure of parasite pressure by Anguillicola crassus. *Parasitology, 124*(04), 457-463.
Lefebvre, F., Fazio, G., Mounaix, B., & Crivelli, A. J. (2013). Is the continental life of the European eel Anguilla anguilla affected by the parasitic invader Anguillicoloides crassus? *Proceedings of the Royal Society of London B: Biological Sciences, 280*(1754), 20122916.
Lenz, T. L., Wells, K., Pfeiffer, M., & Sommer, S. (2009). Diverse MHC IIB allele repertoire increases parasite resistance and body condition in the Long-tailed giant rat (Leopoldamys sabanus). *Bmc Evolutionary Biology, 9*(1), 269.
Liermann, C. R., Nilsson, C., Robertson, J., & Ng, R. Y. (2012). Implications of Dam Obstruction for Global Freshwater Fish Diversity. *Bioscience, 62*(6), 539-548. doi: 10.1525/bio.2012.62.6.5
Lintas, C., Hirano, J., & Archer, S. (1998). Genetic variation of the European eel (*Anguilla anguilla*). *Molecular Marine Biology and Biotechnology, 7*, 263-269.
Lobon-Cervil, J., & Iglesias, T. (2008). Long-term numerical changes and regulation in a river stock of European eel Anguilla anguilla. *Freshwater Biology, 53*(9), 1832-1844.
Lowe, R. H. (1952). The influence of light and other factors on the seaward migration of the silver eel (Anguilla anguilla L.). *The Journal of Animal Ecology*, 275-309.
Lyndon, A. R., & Kennedy, C. R. (2001). Colonisation and extinction in relation to competition and resource partitioning in acanthocephalans of freshwater fishes of the British Isles. *Folia Parasitologica, 48*(1), 37-46.
Magnadóttir, B. (2006). Innate immunity of fish (overview). *Fish & Shellfish Immunology, 20*(2), 137-151.
Maitland, P. S., & Lyle, A. A. (1991). Conservation of freshwater fish in the British Isles: the current status and biology of threatened species. *Aquatic Conservation: Marine and Freshwater Ecosystems, 1*(1), 25-54.
Marco-Noales, E., Biosca, E. G., & Amaro, C. (1999). Effects of salinity and temperature on long-term survival of the eel pathogen Vibrio vulnificus biotype 2 (serovar E). *Applied and Environmental Microbiology, 65*(3), 1117-1126.
Marco-Noales, E., Milán, M., Fouz, B., Sanjuán, E., & Amaro, C. (2001). Transmission to eels, portals of entry, and putative reservoirs of Vibrio vulnificus serovar E (biotype 2). *Applied and Environmental Microbiology, 67*(10), 4717-4725.
Marcogliese, D. J., & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology, 27*(3), 123-130.
Matejusova, I., Simkova, A., Sasal, P., & Gelnar, M. (2003). Microhabitat distribution of Pseudodactylogyrus anguillae and Pseadodactylogyrus bini among and within gill arches of the European eel (Anguilla anguilla L.). *Parasitology Research, 89*(4), 290-296. doi:

10.1007/s00436-002-0682-8
Mayo-Hernaez, E., Serrano, E., PEÑALVER, J., GARCÍA-AYALA, A., RUIZ DE YBÁÑEZ, R., & MUÑOZ, P. (2015). The European eel may tolerate multiple infections at a low biological cost. *Parasitology, 142*(07), 968-977.
Mayo-Hernandez, E.-H., Elvira, Serrano, E., Penaver, J., GARCÍA-AYALA, A., RUIZ DE YBÁÑEZ, R., & MUÑOZ, P. (2015). The European eel may tolerate multiple infections at a low biological cost. *Parasitology*, 1-10.
Mayo-Hernández, E., Peñalver, J., García-Ayala, A., Serrano, E., Muñoz, P., & de Ybáñez, R. R. (2015). Richness and diversity of helminth species in eels from a hypersaline coastal lagoon, Mar Menor, south-east Spain. *Journal of Helminthology, 89*(03), 345-351.
Mayo-Hernandez, E., Serrano, E., Penalver, J., Garcia-Ayala, A., DE YBÁÑEZ, R. R., & Munoz, P. (2015). The European eel may tolerate multiple infections at a low biological cost. *Parasitology, 142*(07), 968-977.
Médoc, V., Rigaud, T., Motreuil, S., Perrot-Minnot, M.-J., & Bollache, L. (2011). Paratenic hosts as regular transmission route in the acanthocephalan Pomphorhynchus laevis: potential implications for food webs. *Naturwissenschaften, 98*(10), 825-835.
Melià, P., Bevacqua, D., Crivelli, A., De Leo, G., Panfili, J., & Gatto, M. (2006). Age and growth of Anguilla anguilla in the Camargue lagoons. *Journal of Fish Biology, 68*(3), 876-890.
Mellergaard, S., & Dalsgaard, I. (1987). Disease problems in Danish eel farms. *Aquaculture, 67*(1–2), 139-146. doi: http://dx.doi.org/10.1016/0044-8486(87)90019-6
Mello Sampaio, J. L., Piana de Andrade, V., Lucas, M. d. C., Fung, L., Gagliardi, S. M. B., Santos, S. R. P., . . . Dick, T. (2005). Diphyllobothriasis, Brazil. *Emerging infectious diseases*, 1598-1600.
Mercado, R., Yamasaki, H., Kato, M., Muñoz, V., Sagua, H., Torres, P., & Castillo, D. (2010). Molecular identification of the Diphyllobothrium species causing diphyllobothriasis in Chilean patients. *Parasitology Research, 106*(4), 995-1000.
Miller, M. J., Bonhommeau, S., Munk, P., Castonguay, M., Hanel, R., & McCleave, J. D. (2014). A century of research on the larval distributions of the Atlantic eels: a re-examination of the data. *Biological Reviews*.
Molnár, K., Baska, F., Csaba, G., Glávits, R., & Székely, C. (1993). Pathological and histopatnotogical studies of the swimbladder of eels Anguilla anguitla infected by Angutlticpta crassus (Nemafoda: Dracuncuioidea). *Diseases of Aquatic Organisms, 15*(1), 41-50.
Moravec, F. (1970). On life history of nematode *raphidascaris-acus* (bloch, 1779) in natural environment of river-bystrice, czechoslovakia. *Journal of Fish Biology, 2*(4), 313-&. doi: 10.1111/j.1095-8649.1970.tb03290.x
Moravec, F. (1974). Some remarks on the development of *paraquimperia-tenerrima* nematoda quimperiidae. *Scripta Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis, 4*(5), 135-141.
Moravec, F. (1977). First record of the nematode Spinitectus inermis (Zeder, 1800) from eels of Czechoslovakia. *Folia Parasitologica, 24*(1), 85.
Moravec, F. (2004a). *Metazoan parasites of salmonid fishes of Europe*: Academia.
Moravec, F. (2004b). Observations on the transmission and the seasonality of infection of the nematode Raphidascaris acus in Salmo trutta fario in a small trout stream in North Bohemia, Czech Republic. *Helminthologia, 41*(2), 91-97.
Moravec, F. (2006). *Dracunculoid and anguillicoloid nematodes parasitic in vertebrates*: Academia.
Moravec, F. (2007). Some aspects of the taxonomy and biology of adult spirurine nematodes parasitic in fishes: a review. *Folia Parasitologica, 54*(4), 239.
Moravec, F., Boomker, J., & Taraschewski, H. (2000). Paraquimperia africana n. sp (Nematoda : Quimperiidae), a new intestinal parasite of the eel Anguilla mossambica Peters, in South Africa. *Journal of Parasitology, 86*(1), 113-117. doi: 10.1645/0022-3395(2000)086[0113:pansnq]2.0.co;2
Moravec, F., & Konecny, R. (1994). Some new data on the intermediate and paratenic hosts of the

nematode Anguillicola crassus Kuwahara, Niimi et Itagaki, 1974. *Folia Parasitologica, 41*, 65-70.
Moravec, F., & Scholz, T. (1991). Observations on the biology of Pomphorhynchus laevis (Zoega in Müller, 1776)(Acanthocephala) in the Rokytná River, Czech and Slovak Federative Republic. *Helminthologia, 28*(1), 23-29.
Moravec, F., & Scholz, T. (1994). Seasonal occurrence and maturation of Neoechinorhynchus rutili (Acanthocephala) in barbel, Barbus barbus (Pisces), of the Jihlava River, Czech Republic. *Parasite, 1*(3), 271-278.
Moravec, F., & Scholz, T. (2015). Macroparasites and their communities of the European eel Anguilla anguilla (Linnaeus) in the Czech Republic. *Folia Parasitologica, 62*, 033.
Morgan, V., Brewer, A., & Durham, E. (2011). The identification of UK BAP priority rivers: generating a spreadsheet of potential priority river water bodies.
Muñoz, P., Peñalver, J., de Ybañez, R. R., & Garcia, J. (2015). Influence of adult Anguillicoloides crassus load in European eels swimbladder on macrophage response. *Fish & Shellfish Immunology, 42*(2), 221-224.
Musumeci, V. L., Able, K. W., Sullivan, M. C., & Smith, J. M. (2014). Estuarine predator-prey interactions in the early life history of two eels (Anguilla rostrata and Conger oceanicus). *Environmental Biology of Fishes, 97*(8), 929-938. doi: 10.1007/s10641-013-0194-7
Myers, B. J. (1976). Research then and now on the Anisakidae nematodes. *Transactions of the American Microscopical Society*, 137-142.
Nagasawa, K., Kim, Y., & Hirose, H. (1994). Anguillicola crassus and A. globiceps (Nematoda: Dracunculoidea) parasitic in the swimbladder of eels (Anguilla japonica and A. anguilla) in East Asia: a review. *Folia Parasitologica, 41*(2), 127-137.
Naismith, I., & Knights, B. (1993). The distribution, density and growth of the European eel, *Anguilla anguilla*, in the freshwater catchment of the River Thames. *Journal of Fish Biology, 42*(2), 217-226.
Newbold, L., Hockley, F., Williams, C., Cable, J., Reading, A., Auchterlonie, N., & Kemp, P. (2015). Relationship between European eel Anguilla anguilla infection with non-native parasites and swimming behaviour on encountering accelerating flow. *Journal of Fish Biology, 86*(5), 1519-1533.
Nickol, B. B., & Crompton, D. W. T. (1985). *Biology of the Acanthocephala*: Cambridge University Press.
Nie, P., & Kennedy, C. (1991a). The population biology of Camallanus lacustris (Zoega) in eels, Anguilla anguilla (Linnaeus), and their status as its host. *Journal of Fish Biology, 38*(5), 653-661.
Nie, P., & Kennedy, C. (1991b). Population biology of *Proteocephalus macrocephalus* (Creplin) in the European eel, *Anguilla anguilla* (Linnaeus), in two small rivers. *Journal of Fish Biology, 38*(6), 921-927.
Nie, P., & Kennedy, C. (1991c). Seasonal population dynamics of Paraquimpevia tenevvima (Linstow)(Nematoda) in the European eel Anguilla anguilla (Linnaeus) in three localities of southwest England. *Journal of Fish Biology, 39*(3), 441-449.
Nie, P., & Kennedy, C. (1992a). Occurrence of Phyllodistomum folium (Olfers)(Digenea) in the eel, Anguilla anguilla (Linnaeus), in a small river. *Journal of Fish Biology, 40*(3), 469-470.
Nie, P., & Kennedy, C. R. (1991d). Occurrence and seasonal dynamics of *Pseudodactylogyrus-anguillae* (yin and sproston) (monogenea) in eel, *Anguilla-anguilla* (l), in england. *Journal of Fish Biology, 39*(6), 897-900. doi: 10.1111/j.1095-8649.1991.tb04419.x
Nie, P., & Kennedy, C. R. (1991e). Seasonal population-dynamics of *Paraquimperia-tenerrima* (linstow) (nematoda) in the european eel *Anguilla-anguilla* (linnaeus) in 3 localities of southwest england. *Journal of Fish Biology, 39*(3), 441-449. doi: 10.1111/j.1095-8649.1991.tb04375.x
Nie, P., & Kennedy, C. R. (1992b). Populations of *Bothriocephalus-claviceps* (goeze) (cestoda) in the

european eel, *Anguilla-anguilla* (l), in 3 localities in Southwest England. *Journal of Fish Biology, 41*(4), 521-531. doi: 10.1111/j.1095-8649.1992.tb02680.x
Nie, P., & Kennedy, C. R. (1993). Infection dynamics of larval *Bothriocephalus-claviceps* in *Cyclops-vicinus*. *Parasitology, 106*, 503-509.
Nielsen, M. (1999). An enhanced humoral immune response against the swimbladder nematode, Anguillicola crassus, in the Japanese eel, Anguilla japonica, compared with the European eel, A. anguilla. *Journal of Helminthology, 73*(03), 227-232.
Norton, J., Lewis, J., & Rollinson, D. (2003). Parasite infracommunity diversity in eels: a reflection of local component community diversity. *Parasitology, 127*(05), 475-482.
Norton, J., Lewis, J., & Rollinson, D. (2004). Temporal and spatial patterns of nestedness in eel macroparasite communities. *Parasitology, 129*(02), 203-211.
Norton, J., Rollinson, D., & Lewis, J. (2004). Patterns of infracommunity species richness in eels, Anguilla anguilla. *Journal of Helminthology, 78*(02), 141-146.
O'mahony, E., Bradley, D., Kennedy, C., & Holland, C. (2004). Evidence for the hypothesis of strain formation in Pomphorhynchus laevis (Acanthocephala): an investigation using mitochondrial DNA sequences. *Parasitology, 129*(03), 341-347.
O'Mahony, E., Kennedy, C., & Holland, C. (2004). Comparison of morphological characters in Irish and English populations of the acanthocephalan *Pomphorhynchus laevis* (Müller, 1776). *Systematic Parasitology, 59*(2), 147-157.
Ogawa, K., Chung, H. Y., Kou, G. H., & Imada, R. (1985). The validity of an eel monogenean *Pseudodactylogyrus-microrchis*. *Nippon Suisan Gakkaishi, 51*(3), 381-386.
Ogawa, K., & Egusa, S. (1976). Studies on eel pseudodactylogyrosis .1. morphology and classification of 3 eel dactylogyrids with a proposal of a new species, *pseudodactylogyrus-microrchis*. *Bulletin of the Japanese Society of Scientific Fisheries, 42*(4), 395-404.
Okada, H., Kuhn, C., Feillet, H., & Bach, J. F. (2010). The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clinical & Experimental Immunology, 160*(1), 1-9.
Orr, H., Des Clers, S., Simpson, G., Hughes, M., Battarbee, R., Cooper, L., . . . Hannah, D. (2010). Changing water temperatures: a surface water archive for England and Wales.
Palstra, A., Heppener, D., Van Ginneken, V., Székely, C., & Van den Thillart, G. (2007). Swimming performance of silver eels is severely impaired by the swim-bladder parasite Anguillicola crassus. *Journal of Experimental Marine Biology and Ecology, 352*(1), 244-256.
Palstra, A., Van Ginneken, V., Murk, A., & Van Den Thillart, G. (2006). Are dioxin-like contaminants responsible for the eel (Anguilla anguilla) drama? *Naturwissenschaften, 93*(3), 145-148.
Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in ecology & evolution, 22*(3), 133-139.
Pedersen, B. H. (2003). Induced sexual maturation of the European eel *Anguilla anguilla* and fertilisation of the eggs. *Aquaculture, 224*(1), 323-338.
Pedersen, B. H. (2004). Fertilisation of eggs, rate of embryonic development and hatching following induced maturation of the European eel *Anguilla anguilla*. *Aquaculture, 237*(1), 461-473.
Peirson, G., Tingley, D., Spurgeon, J., & Radford, A. (2001). Economic evaluation of inland fisheries in England and Wales. *Fisheries Management and Ecology, 8*(4-5), 415-424.
Pelster, B. (2014). Swimbladder function and the spawning migration of the European eel Anguilla anguilla. *Frontiers in Physiology, 5*.
Pelster, B. (2015). Swimbladder function and the spawning migration of the European eel *Anguilla anguilla*. *Frontiers in Physiology, 5*, 10. doi: 10.3389/fphys.2014.00486
Perrot-Minnot, M.-J. (2004). Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of Pomphorhynchus laevis (Acanthocephala). *International Journal for Parasitology, 34*(1), 45-54.
Petney, T. N., & Andrews, R. H. (1998). Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal for Parasitology, 28*(3), 377-393.

Pilcher, M., & Moore, J. (1993). Distribution and prevalence of Anguillicola crassus in eels from the tidal Thames catchmentb. *Journal of Fish Biology, 43*(3), 339-344.
Piper, A. T., Wright, R. M., Walker, A. M., & Kemp, P. S. (2013). Escapement, route choice, barrier passage and entrainment of seaward migrating European eel, *Anguilla anguilla*, within a highly regulated lowland river. *Ecological Engineering, 57*, 88-96. doi: 10.1016/j.ecoleng.2013.04.030
Plaistow, S. J., Troussard, J.-P., & Cézilly, F. (2001). The effect of the acanthocephalan parasite Pomphorhynchus laevis on the lipid and glycogen content of its intermediate host Gammarus pulex. *International Journal for Parasitology, 31*(4), 346-351.
Polzer, M., & Taraschewski, H. (1994). Proteolytic-enzymes of *Pomphorhynchus-laevis* and in 3 other acanthocephalan species. *Journal of Parasitology, 80*(1), 45-49. doi: 10.2307/3283343
Privitera, L., Aarestrup, K., & Moore, A. (2014). Impact of a short-term exposure to tributyl phosphate on morphology, physiology and migratory behaviour of European eels during the transition from freshwater to the marine environment. *Ecology of Freshwater Fish, 23*(2), 171-180. doi: 10.1111/eff.12043
Proctor, C., & Thresher, R. (1998). Effects of specimen handling and otolith preparation on concentration of elements in fish otoliths. *Marine Biology, 131*(4), 681-694.
Proman, J., & Reynolds, J. (2000). Differences in head shape of the European eel, *Anguilla anguilla* (L.). *Fisheries Management and Ecology, 7*(4), 349-354.
Rahman, H. (1964). On the morphology of the hitherto undescribed male of *Spinitectus oviflagellis* Fourment, 1884 (Nematoda: Rhabdochonidae). *Parasitology, 54*(04), 695-698.
Rego, A. A., de Chambrier, A., Hanzelová, V., Hoberg, E., Scholz, T., Weekes, P., & Zehnder, M. (1998). Preliminary phylogenetic analysis of subfamilies of the Proteocephalidea (Eucestoda). *Systematic Parasitology, 40*(1), 1-19.
Rigaud, T., Perrot-Minnot, M.-J., & Brown, M. J. (2010). Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proceedings of the Royal Society of London B: Biological Sciences, 277*(1701), 3693-3702.
Roberts, L. S., & Janovy, J. (2005). *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology*: McGraw-Hill Higher Education Boston.
Rohde, K., & Watson, N. (1985). Morphology, microhabitats and geographical variation of Kuhnia spp.(Monogenea: Polyopisthocotylea). *International Journal for Parasitology, 15*(5), 569-586.
Rosen, G. E., & Smith, K. F. (2010). Summarizing the evidence on the international trade in illegal wildlife. *EcoHealth, 7*(1), 24-32.
Sahay, U., & Prasad, D. (1965). On a new species of a nematode (*thelaziidae, spinitectinae, spinitectus*, fourment, 1883) with a key to the species of the genus *spinitectus*. *Japanese Journal of Medical Science and Biology, 18*(3), 143-150.
Saraiva, A., Moravec, F., Pereira, A., & Cruz, C. (2002). Development of Spinitectus inermis (Nematoda : Cystidicolidae), a parasite of eel, *Anguilla anguilla,* in Europe. *Folia Parasitologica, 49*(2), 118-126.
Saraiva, A., Pereira, A., & Cruz, C. (2002). Observations on the occurrence and maturation of Spinitectus inermis (Nematoda: Cystidicolidae) in the Sousa River, Portugal. *Folia Parasitologica, 49*(2), 167-168.
Sasal, P., Taraschewski, H., Valade, P., Grondin, H., Wielgoss, S., & Moravec, F. (2008). Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. *Parasitology Research, 102*(6), 1343-1350. doi: 10.1007/s00436-008-0916-5
Sasal, P., Trouvé, S., Müller-Graf, C., & Morand, S. (1999). Specificity and host predictability: a comparative analysis among monogenean parasites of fish. *Journal of Animal Ecology, 68*(3), 437-444.
Schabuss, M., Kennedy, C., Konecny, R., Grillitsch, B., Schiemer, F., & Herzig, A. (2005). Long-term investigation of the composition and richness of intestinal helminth communities in the stocked population of eel, Anguilla anguilla, in Neusiedler See, Austria. *Parasitology, 130*(02),

185-194.
Schabuss, M., Konecny, R., Belpaire, C., & Schiemer, F. (1997). Endoparasitic helminths of the European eel, Anguilla anguilla, from four disconnected meanders from the rivers Leie and Scheldt in western Flanders, Belgium. *Folia Parasitologica, 44*(1), 12-18.
Schjørring, S., & Koella, J. C. (2003). Sub-lethal effects of pathogens can lead to the evolution of lower virulence in multiple infections. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1511), 189-193.
Schneebauer, G., Hanel, R., & Pelster, B. (2016). Anguillicola crassus impairs the silvering-related enhancements of the ROS defense capacity in swimbladder tissue of the European eel (Anguilla anguilla). *Journal of Comparative Physiology B*, 1-11.
Scholz, T. (1997a). Life-cycle of *Bothriocephalus claviceps*, a specific parasite of eels. *Journal of Helminthology, 71*(3), 241-248.
Scholz, T. (1997b). A revision of the species of *Bothriocephalus Rudolphi,* 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. *Systematic Parasitology, 36*(2), 85-107.
Scholz, T., Drabek, R., & Hanzelová, V. (1998). Scolex morphology of Proteocephalus tapeworms (Cestoda: Proteocephalidae), parasites of freshwater fish in the Palaearctic Region. *Folia Parasitologica, 45*(1), 27-43.
Scholz, T., Garcia, H. H., Kuchta, R., & Wicht, B. (2009). Update on the human broad tapeworm (genus Diphyllobothrium), including clinical relevance. *Clinical Microbiology Reviews, 22*(1), 146-160.
Scholz, T., Škeříková, A., Shimazu, T., & Grygier, M. J. (2004). A taxonomic study of species of *Bothriocephalus Rudolphi*, 1808 (Cestoda: Pseudophyllidea) from eels in Japan: morphological and molecular evidence for the occurrence of B. claviceps (Goeze, 1782) and confirmation of the validity of B. japonicus Yamaguti, 1934. *Systematic Parasitology, 57*(2), 87-96.
Scholz, T., Špakulov, M., Šn, V., Kráíová, I., & Hanzelová, V. (1997). A multidisciplinary approach to the systematics of *Proteocephalus macrocephalus* (Creplin, 1825)(Cestoda: Proteocephalidae). *Systematic Parasitology, 37*(1), 1-12.
Secombes, C., & Chappell, L. (1996). Fish immune responses to experimental and natural infection with helminth parasites. *Annual Review of Fish Diseases, 6*, 167-177.
Shears, J. A., & Kennedy, C. R. (2005). The life cycle of Paraquimperia tenerrima: a parasite of the European eel *Anguilla anguilla*. *Journal of Helminthology, 79*(2), 169-176. doi: 10.1079/joh2004270
Simon, J. (2007). Age, growth, and condition of European eel (Anguilla anguilla) from six lakes in the River Havel system (Germany). *Ices Journal of Marine Science, 64*(7), 1414-1422. doi: 10.1093/icesjms/fsm093
Sjöberg, N. B., Petersson, E., Wickström, H., & Hansson, S. (2009). Effects of the swimbladder parasite Anguillicola crassus on the migration of European silver eels Anguilla anguilla in the Baltic Sea. *Journal of Fish Biology, 74*(9), 2158-2170.
Škoríková, B., Scholz, T., & Moravec, F. (1996). Spreading of introduced monogeneans Pseudodactylogyrus anguillae and P. bini among eel populations in the Czech Republic. *Folia Parasitologica, 43*(2), 155-156.
Smith, J. D. (1984). Taxonomy of raphidascaris spp (nematoda, anisakidae) of fishes, with a redescription of raphidascaris-acus (bloch-1772). *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 62*(4), 685-694.
Smrzlić, I. V., Valić, D., Kapetanović, D., Marijić, V. F., Gjurčević, E., & Teskeredžić, E. (2015). Pomphorhynchus laevis (Acanthocephala) from the Sava River basin: New insights into strain formation, mtDNA-like sequences and dynamics of infection. *Parasitology International, 64*(5), 243-250.
Starkie, A. (2003). Management issues relating to the European eel, *Anguilla anguilla*. *Fisheries*

Management and Ecology, 10(6), 361-364.
Sures, B., Knopf, K., & Kloas, W. (2001). Induction of stress by the swimbladder nematode Anguillicola crassus in European eels, Anguilla anguilla, after repeated experimental infection. *Parasitology, 123*(02), 179-184.
Sures, B., Knopf, K., Wurtz, J., & Hirt, J. (1999). Richness and diversity of parasite communities in European eels Anguilla anguilla Of the River Rhine, Germany, with special reference to helminth parasites. *Parasitology, 119*, 323-330. doi: 10.1017/s0031182099004655
Sures, B., Knopf, K., Würtz, J., & Hirt, J. (1999). Richness and diversity of parasite communities in European eels Anguilla anguilla of the River Rhine, Germany, with special reference to helminth parasites. *Parasitology, 119*(03), 323-330.
Sures, B., & Streit, B. (2001). Eel parasite diversity and intermediate host abundance in the River Rhine, Germany. *Parasitology, 123*(02), 185-191.
Székely, C., Molnár, K., Müller, T., Szabó, A., Romvári, R., Hancz, C., & Bercsényi, M. (2004). Comparative study of X-ray computerised tomography and conventional X-ray methods in diagnosis of swimbladder infection in eels caused by Anguillicola crassus. *Diseases of Aquatic Organisms, 58*(2-3), 157-164.
Tabeta, O., Tanimoto, T., Takai, T., Matsui, I., & Imamura, T. (1976). Seasonal occurrence of anguillid elvers in Cagayan River, Luzon Island, the Philippines. *Bulletin of the Japanese Society of Scientific Fisheries*.
Tain, L., Perrot-Minnot, M.-J., & Cézilly, F. (2007). Differential influence of Pomphorhynchus laevis (Acanthocephala) on brain serotonergic activity in two congeneric host species. *Biology letters, 3*(1), 69-72.
Tamam, O. A. (2014). The Aetiology, Histopathology, and Ultrastructural Features of Perianal Erythema (Red Anus Syndrome) in the European Eel (Anguilla anguilla). *Plos One, 9*(3), e90790.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution, 30*(12), 2725-2729.
Tarachewski, H. (1988). Host-parasite interface of fish acanthocephalans. I: Acanthocephalus anguillae (Palaeacanthocephala) in naturally infected fishes: LM and TEM investigations. *Diseases of Aquatic Organisms, 4*(2), 109-119.
Taraschewski, H. (1989). *Acanthocephalus anguillae* in intra-and extraintestinal positions in experimentally infected juveniles of goldfish and carp and in sticklebacks. *The Journal of parasitology*, 108-118.
Teng, H. Y., Lin, Y. S., & Tzeng, C. S. (2009). A New Anguilla Species and a Reanalysis of the Phylogeny of Freshwater Eels. *Zoological Studies, 48*(6), 808-822.
Terech-Majewska, E., Schulz, P., & Siwicki, A. K. (2015). Influence of nematode Anguillicoloides crassus infestation on the cellular and humoral innate immunity in European eel (Anguilla anguilla L.). *Central-European Journal of Immunology, 40*(2), 127.
Thielen, F., Muenderle, M., Taraschewski, H., & Sures, B. (2007). Do eel parasites reflect the local crustacean community? A case study from the Rhine river system. *Journal of Helminthology, 81*(2), 179-189. doi: 10.1017/s0022149x07753725
Thielen, F., Münderle, M., Taraschewski, H., & Sures, B. (2007). Do eel parasites reflect the local crustacean community? A case study from the Rhine river system. *Journal of Helminthology, 81*(02), 179-189.
Thoney, D., & Hargis, W. (1991). Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases, 1*, 133-153.
Torgerson, P. R., & Macpherson, C. N. (2011). The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary Parasitology, 182*(1), 79-95.
Tsai, I. J., Zarowiecki, M., Holroyd, N., Garciarrubio, A., Sanchez-Flores, A., Brooks, K. L., . . . Sciutto, E. (2013). The genomes of four tapeworm species reveal adaptations to parasitism. *Nature, 496*(7443), 57-63.

Tsukamoto, K. (2009). Oceanic migration and spawning of anguillid eels. *Journal of Fish Biology, 74*(9), 1833-1852.
Tsukamoto, K., Aoyama, J., & Miller, M. J. (2002). Migration, speciation, and the evolution of diadromy in anguillid eels. *Canadian Journal of Fisheries and Aquatic Sciences, 59*(12), 1989-1998.
Tzeng, W., Lin, H., Wang, C., & Xu, S. (2000). Differences in size and growth rates of male and female migrating Japanese eels in Pearl River, China. *Journal of Fish Biology, 57*(5), 1245-1253.
Tzeng, W., Wu, H., & Wlckström, H. (1994). Scanning electron microscopic analysis of annulus microstructure in otolith of European eel, *Anguilla anguilla*. *Journal of Fish Biology, 45*(3), 479-492.
Unsworth, K. (1944). Observations on the life-cycle of a species of Diphyllobothrium found parasitizing trout in Great Britain. *Annals of Tropical Medicine & Parasitology, 38*(3-4), 213-219.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic acids research, 40*(15), e115-e115.
Valtonen, E. T. (1979). Neoechinorhynchus rutili (Müller, 1780)(Acanthocephala) in the whitefish Coregonm nasus (Pallas) sensu Svärdson from the Bay of Bothnia. *Journal of Fish Diseases, 2*(2), 99-103.
Valtonen, E. T., Haaparanta, A., & Hoffmann, R. W. (1994). Occurrence and histological response of raphidascaris-acus (nematoda, ascaridoidea) in roach from 4 lakes differing in water-quality. *International Journal for Parasitology, 24*(2), 197-206. doi: 10.1016/0020-7519(94)90026-4
Van Banning, P., Haenen, O., Perkins, F., & Cheng, T. (1990). Effects of the swimbladder nematode Anguillicola crassus in wild and farmed eel, Anguilla anguilla. *Pathology in marine science*, 317-330.
van Ginneken, V., Antonissen, E., Müller, U. K., Booms, R., Eding, E., Verreth, J., & van den Thillart, G. (2005). Eel migration to the Sargasso: remarkably high swimming efficiency and low energy costs. *The Journal of experimental biology, 208*(7), 1329-1335.
von Bonsdorff, B., & Bylund, G. (1981). The ecology of Diphyllobothrium latum. *Ecology of disease, 1*(1), 21-26.
Walkey, M. (1967). The ecology of *Neoechinorhynchus rutili* (Müller). *The Journal of parasitology*, 795-804.
Wang, C., & Tzeng, W. (2000). The timing of metamorphosis and growth rates of American and European eel leptocephali:: A mechanism of larval segregative migration. *Fisheries Research, 46*(1), 191-205.
Watanabe, S., Aoyama, J., & Tsukamoto, K. (2009). A new species of freshwater eel *Anguilla luzonensis* (Teleostei: Anguillidae) from Luzon Island of the Philippines. *Fisheries Science, 75*(2), 387-392. doi: 10.1007/s12562-009-0087-z
Watanabe, S., Iida, M., Lord, C., Keith, P., & Tsukamoto, K. (2014). Tropical and temperate freshwater amphidromy: a comparison between life history characteristics of Sicydiinae, ayu, sculpins and galaxiids. *Reviews in Fish Biology and Fisheries, 24*(1), 1-14. doi: 10.1007/s11160-013-9316-8
Wayland, M. T. (2010). Proboscis profiler: a tool for detecting acanthocephalan morphotypes. *Systematic Parasitology, 76*(3), 159-167.
Wenner, C. A., & Musick, J. (1974). Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia. *Journal of the Fisheries Board of Canada, 31*(8), 1387-1391.
Wenner, C. A., & Musick, J. A. (1975). Food habits and seasonal abundance of the American eel, *Anguilla rostrata*, from the lower Chesapeake Bay. *Chesapeake Science, 16*(1), 62-66.
Wheeler, A. (1977). The origin and distribution of the freshwater fishes of the British Isles. *Journal of Biogeography*, 1-24.
Whittington, I. D. (2004). The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity,

classification and phylogeny with a note about species complexes. *Folia Parasitologica, 51*(2/3), 109-122.
Whyte, S., Chappell, L., & Secombes, C. (1989). Cytotoxic reactions of rainbow trout, Salmo gairdneri Richardson, macrophages for larvae of the eye fluke Diplostomum spathaceum (Digenea). *Journal of Fish Biology, 35*(3), 333-345.
Wicht, B., Scholz, T., Peduzzi, R., & Kuchta, R. (2008). First record of human infection with the tapeworm Diphyllobothrium nihonkaiense in North America. *The American journal of tropical medicine and hygiene, 78*(2), 235-238.
Wickstrom, H. (1986). Growth of cultured eels stocked in two Swedish lakes. *Vie et Milieu, 36*(4), 273-278.
Wills-Karp, M., Santeliz, J., & Karp, C. L. (2001). The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nature Reviews Immunology, 1*(1), 69-75.
Würtz, J., & Taraschewski, H. (2000). Histopathological changes in the swimbladder wall of the European eel Anguilla anguilla due to infections with Anguillicola crassus. *Diseases of Aquatic Organisms, 39*(2), 121-134.
Yera, H., Estran, C., Delaunay, P., Gari-Toussaint, M., Dupouy-Camet, J., & Marty, P. (2006). Putative Diphyllobothrium nihonkaiense acquired from a Pacific salmon (Oncorhynchus keta) eaten in France; genomic identification and case report. *Parasitology International, 55*(1), 45-49.
Yin, W.-Y., & Sproston, N. G. (1949). Studies on the monogenetic trematodes of China: parts 1-5. *Sinensia, 19*(1/6).
Žďárská, Z., & Nebesářová, J. (1999). Regional ultrastructural differences of the scolex and neck tegument of *Proteocephalus macrocephalus* (Eucestoda: Proteocephalidae). *Folia Parasitol, 46*, 279-283.
Zhang, W., Che, F., Tian, S., Shu, J., & Zhang, X. (2015). Molecular Identification of Diphyllobothrium nihonkaiense from 3 Human Cases in Heilongjiang Province with a Brief Literature Review in China. *The Korean Journal of Parasitology, 53*(6), 683-688.
Zhu, L.-y., Nie, L., Zhu, G., Xiang, L.-x., & Shao, J.-z. (2013). Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. *Developmental & Comparative Immunology, 39*(1), 39-62.
Zolovs, M., Deksne, G., DaukÅ¡ te, J. n., Aizups, J. n., & KirjuÅ¡ ina, M. (2016). Morphometric Analysis of the Hard Parts of Pseudodactylogyrus anguillae and Pseudodactylogyrus bini (Monogenea: Dactylogyridae) On the Gill Apparatus of the European Eels (Anguilla anguilla) from the Freshwaters of Latvia. *Journal of Parasitology*.

Appendix.1 Eel morphometric and associated helminth infection data for the **River Mawddach-eden**; Wales.

Eel code	Body length(cm)	Body weight(g)	Condition factor	Gill monogenean	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non Bulb	Total number	*B. claviceps*	*P. macrocephalus*		
M1	45	154.6	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0
M2	41	91.9	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
M3	25	21.8	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
M4	22	14.4	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
M5	26.5	25.4	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
M6	28	36.9	0.17	0	0	1	0	1	0	0	0	0	0	0	1	1
M7	21	14.1	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0
M8	18	7.5	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
M9	23	15.5	0.13	0	0	9	0	9	0	0	0	0	0	0	1	9
M10	11	1.7	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	26.1±10.2	38.4±48.1	0.14±0.01	0	0	10	0	10	0	0	0	0	0	0	1	10
%				0%	0%	20%			0%			0%				

= Ab Aziz (2012)

Gut nematodes were the only helminths found 100% (10/10), prevalence 20%.

Appendix.2 Eel morphometric and associated helminth infection data for the **River Clwyd-Meirchion**; Wales

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
MC1	42	104.4	0.14	0	0	0	0	0	0	0	0	1	1	0	1	1
MC2	30	38.8	0.14	0	0	5	0	5	0	0	0	0	0	0	1	5
MC3	35	54.6	0.13	0	0	1	0	1	0	0	0	1	1	0	2	2
MC4	25	26.8	0.17	0	0	0	0	0	0	0	0	1	1	0	1	1
MC5	19.5	21.8	0.29	0	0	3	0	3	0	0	0	0	0	0	1	3
MC6	18.5	9.0	0.14	0	0	1	0	1	0	0	0	0	0	0	1	1
MC7	22	12.0	0.11	0	0	3	0	3	0	0	0	0	0	0	1	3
MC8	15.5	5.3	0.14	0	0	1	0	1	0	0	0	0	0	0	1	1
MC9	18	10.5	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0
MC10	13.5	2.8	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	23.9± 8.7	28.6±31.22	0.16±0.05	0	0	14	0	14	0	0	0	3	3	0	2	17
%				0%	0%	60%			0%			30%				

= Ab Aziz (2012)

Gut nematodes were the dominant helminths 82.4% (14/17), prevalence 60%. And tapeworms 30%

Appendix.3 Eel morphometric and associated helminth infection data for the **River Dee-Eitha**; Wales

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
D1	28	27.6	0.13	0	0	1	0	1	0	0	0	0	0	0	1	1
D2	32.5	60.2	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0
D3	25.5	17.7	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0
D4	32	55.6	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0
D5	14	2.4	0.09	0	0	23	23	0	0	0	0	0	0	0	1	23
D6	12	1.5	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0
D7	15	3.1	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0
D8	18	7.1	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
D9	14.5	1.2	0.04	0	0	4	4	0	0	0	0	0	0	0	1	4
D10	25.5	21.6	0.13	0	0	1	0	1	0	0	0	0	0	0	1	1
T0tal	21.7±7.5	19.8±22.1	0.11±0.04	0	0	29	27	2	0	0	0	0	0	0	1	29
%				0%	0%	40%			0%			0%				

= Ab Aziz (2012)

Gut nematodes were the only helminths found 100% (29/29), prevalence 40%.

Appendix.4 Eel morphometric and associated helminth infection data for the **River Taff**; Wales

Eel code	Body length(cm)	Body weight(g)	Condition factor	Gill monogenean*	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
TB1	20	10.2	0.13	0	2	0	0	0	9	9	0	0	0	0	2	11
TB2	25	33.5	0.21	0	0	0	0	0	1	1	0	0	0	0	1	1
TB3	28	33	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0
TB4	22	15.6	0.15	1	0	1	1	0	0	0	0	0	0	0	2	2
TB5	21.5	15.5	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0
TB6	24	24.4	0.18	0	0	0	0	0	1	1	0	0	0	0	1	1
TB7	25	21.6	0.14	0	0	0	0	0	5	5	0	0	0	0	1	5
TB8	28	35.2	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0
TB9	19	10.6	0.15	0	0	0	0	0	2	2	0	0	0	0	1	2
TB10	25	24.7	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	23.8±2.9	22.4±9.4	0.16±0.02	1	2	1	1	0	18	18	0	0	0	0	4	22
				10%	10%	10%			50%			0%				

*= *P. bini* = 100% (1/1)

= Ab Aziz (2012)

Acanthocephalans were the dominant helminths 81.8% (18/22), prevalence 50%.

Appendix.5 Eel morphometric and associated helminth infection data for the **River Clwyd-Elwy**; Wales

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean *	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
			Total	Total		Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
CE1	39	72.9	0.12	4	0	1	0	1	0	0	0	0	0	0	2	5
CE2	24	17.5	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
CE3	31	46	0.15	0	0	1	0	1	0	0	0	0	0	0	1	1
CE4	34	70.1	0.18	11	0	0	0	0	0	0	0	0	0	0	1	11
CE5	29	39.3	0.16	1	0	2	2	0	0	0	0	0	0	0	2	3
CE6	20.5	11.6	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
CE7	10	1.2	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
CE8	22	16.8	0.16	1	0	0	0	0	0	0	0	0	0	0	1	1
CE9	20	10.9	0.14	0	0	3	3	0	0	0	0	1	0	1	2	4
CE10	19.5	8.0	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	24.9±8.4	29.4±26.1	0.14±0.02	17	0	7	5	2	0	0	0	1	0	1	3	25
%				40%	0%	40%			0%			10%				

***=** *P. bini* = 100% (10/10)
= Ab Aziz (2012)

Monogeneans were the dominant helminths 68% (17/25), prevalence 40%, and the gut nematodes 40%

Appendix.6 Eel morphometric and associated helminth infection data for the the **River Rhymney**; Wales

Eel code	Body length (cm)	Body weight (g)	Condition factor	Gill monogenean *	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number		Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
R1	32	48.9	0.15	5	4	0	0	0	1	1	0	0	0	0	3	10
R2	31	48.6	0.16	0	0	0	0	0	10	10	0	0	0	0	1	10
R3	30	42.3	0.16	22	6	0	0	0	3	3	0	0	0	0	3	31
R4	31.5	67.2	0.22	9	3	0	0	0	9	9	0	0	0	0	3	21
R5	27	29.3	0.15	11	0	0	0	0	2	2	0	0	0	0	2	13
R6	30	39.7	0.15	3	2	0	0	0	0	0	0	0	0	0	2	5
R7	29	53.5	0.22	0	0	0	0	0	1	1	0	0	0	0	1	1
R8	30	43.9	0.16	0	4	0	0	0	7	7	0	0	0	0	2	11
R9	23.5	12.7	0.10	3	0	0	0	0	12	12	0	0	0	0	2	15
R10	23.5	18.5	0.14	0	2	0	0	0	5	5	0	0	0	0	2	7
Total	28.8±3.1	40.5±16.4	0.16±0.04	53	21	0	0	0	50	50	0	0	0	0	3	124
%				60%	60%	0%			90%			0%				

*= *P. anguillae* = 100 (8/8)
= Ab Aziz (2012)

Monogeneans were the dominant helminths 42.7% (53/124), prevalence 60%, and the acanthocephalan 90%

Appendix.7 Eel morphometric and associated helminth infection data for the **River Cadoxton**; Wales

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean *	Swim bladder nematode#	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number		Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
CD 1	33	59.9	0.17	14	4	0	0	0	0	0	0	0	0	0	2	18
CD2	27	30.4	0.15	5	1	0	0	0	0	0	0	0	0	0	2	6
CD3	24	21.4	0.06	4	3	5	0	5	0	0	0	0	0	0	3	12
CD4	28	28.3	0.13	2	5	0	0	0	0	0	0	0	0	0	2	7
CD5	28.5	32.6	0.05	4	2	0	0	0	0	0	0	0	0	0	2	6
CD6	22	11.0	0.10	2	0	0	0	0	0	0	0	0	0	0	1	2
CD7	25	17.1	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0
CD8	20	9.2	0.12	0	2	0	0	0	0	0	0	0	0	0	1	2
CD9	21	8.8	0.09	29	1	0	0	0	0	0	0	0	0	0	2	30
CD10	20	7.9	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	24.9±4.3	22.7±16.1	0.11±0.03	60	18	5	0	5	0	0	0	0	0	0	3	83
%				70%	70%	10%			0%			0%				

***=** *P. anguillae* = (7/9) *P. bini* = (2/9)
= Ab Aziz (2012)
Monogeneans were the dominant helminths 72.3% (60/83), prevalence 70%, and the *A. crassus* 70%

Appendix.8 Eel morphometric and associated helminth infection data for the the **River Gowy**; England

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
RG1	39	73.6	0.12	0	3	0	0	0	0	0	0	0	0	0	1	3
EG2	37	61.7	0.12	0	3	0	0	0	0	0	0	0	0	0	1	3
RG3	36	63.2	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
RG4	36	58.9	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
RG5	40	65.7	0.10	0	0	0	0	0	3	0	3	0	0	0	1	3
RG6	32	41.8	0.13	0	5	0	0	0	0	0	0	1	0	1	2	6
RG7	35	70.5	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0
RG8	33	62.2	0.17	0	5	5	0	5	0	0	0	0	0	0	2	10
RG9	26	27.1	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0
RG10	27	24.4	0.12	0	0	0	0	0	1	0	1	0	0	0	1	1
Total	34.1±4.7	54.9±17.5	0.13±0.02	0	20	5	0	5	4	0	4	1	0	1	4	26
%				0	40%	10%			20%			10%				

= Ab Aziz (2012)

A. crassus was the dominant helminths 76.9% (20/26), prevalence 40%.

Appendix.9 Eel morphometric and associated helminth infection data for the **River Bela**; England,

Eel code	Body length(cm)	Body weight(g)	Condition factor	Gill monogenean	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
B1	30	42.0	0.16	0	0	5	0	5	0	0	0	0	0	0	1	5
B2	32	49.1	0.15	5 n.d.	0	4	0	4	0	0	0	0	0	0	2	9
B3	27	33.0	0.17	0	0	1	0	1	0	0	0	0	0	0	1	1
B4	19.5	11.9	0.16	0	0	1	0	1	0	0	0	0	0	0	1	1
B5	21	13.2	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
B6	23	13.3	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0
B7	24	22.2	0.16	0	0	6	0	6	0	0	0	0	0	0	1	6
B8	48	204.5	0.18	0	0	19	0	19	4	4	0	1	1	0	3	24
B9	24	18.3	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
B10	49	209.0	0.18	0	0	4	0	4	0	0	0	0	0	0	1	4
Total	29.8±10.6	43.6±59.5	0.15±0.02	5	0	40	0	40	4	4	0	1	1	0	4	50
				10%	0%	70%			10%			10%				

n.d. = not determined
# = Ab Aziz (2012)

Gut nematodes were the dominant helminths 88.4% (40/50), prevalence 70%.

Appendix.10 Eel morphometric and associated helminth infection data for the **River Petteril**; England

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean *	Swim bladder nematode	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
RP1	36	110.7	0.24	0	0	22	0	22	0	0	0	0	0	0	1	22
RP2	31.5	82.8	0.28	0	0	17	0	17	0	0	0	1	0	1	2	18
RP3	18.5	8.2	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
RP4	28	35.3	0.16	0	0	4	0	4	0	0	0	0	0	0	1	4
RP5	35.5	74.9	0.17	2	0	0	0	0	2	2	0	2	0	2	3	6
RP6	27.5	38.2	0.18	1	0	4	0	4	0	0	0	0	0	0	2	5
RP7	14.5	5.2	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0
RP8	43	101.1	0.07	0	0	11	0	11	1	1	0	0	0	0	2	12
RP9	33	98.3	0.27	0	0	14	0	14	0	0	0	0	0	0	1	14
RP10	26	29.7	0.17	0	0	4	0	4	1	1	0	0	0	0	2	5
Total	29.4±8.4	58.4±39.6	0.18±0.06	3	0	76	0	76	4	4	0	3	0	3	4	86
				20%		70%			30%			20%				

*= *P .bini* 100% (2/2)

Gut nematodes were the dominant helminths 88.4% (76/86), prevalence 70%, and the acanthocephalans 30%

Appendix.11 Eel morphometric and associated helminth infection data for the **River Hether Burn**; England

Eel code	Body length (cm)	Body weight(g)	Condition factor	Gill monogenean *	Swim bladder nematode #	Gut nematode				Acanthocephalan		Tape worm			Numbers of helminth classes	Number of helminth
				Total	Total	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	B. claviceps	*P. macrocephalus*		
HB1	21	12.2	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0
HB2	27	23.6	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
HB3	23	14.7	0.12	3	0	0	0	0	0	0	0	2	2	0	2	5
HB4	24	16	0.12	2	0	0	0	0	0	0	0	0	0	0	1	2
HB5	25	18.6	0.12	27	0	9	0	9	0	0	0	0	0	0	2	36
HB6	16	4.8	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
HB7	20	7.8	0.09	1	0	0	0	0	0	0	0	0	0	0	1	1
HB8	19	8.1	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
HB9	22.5	13.3	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
HB10	16.5	11.1	0.25	0	0	7	7	0	0	0	0	0	0	0	1	7
Total	21.4±3.6	13.0±5.6	0.13±0.04	33	0	16	7	9	0	0	0	2	2	0	3	51
%				40%	0%	20%			0%			10%				

*= *P. anguillae* = 100% (14/14)
= Ab Aziz (2012)

Monogeneans were the dominant helminths 64.7% (33/51), prevalence 40%, and the gut nematodes 20%

Appendix.12 Eel morphometric and associated helminth infection data for the **River Crane**; England

Eel code	Body length (cm)	Body weight (g)	Condition factor	Gill monogenean*	Swim bladder nematode	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total	Total number	Total number	*S. inermis*	Small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
CN1	70	682.3	0.21	0	0	0	0	0	56	0	56	0	0	0	1	56
CN2	74	702.5	0.17	0	0	0	0	0	24	0	24	0	0	0	1	24
CN3	86	1380.3	0.22	12	0	0	0	0	19	0	19	1	1	0	3	32
CN4	77	1058.8	0.23	0	0	0	0	0	39	0	39	0	0	0	1	39
CN5	70	668.6	0.19	2	1	21	0	21	6	0	6	4	4	0	5	34
CN6	74	839.7	0.21	1	11	1	0	1	20	0	20	0	0	0	4	33
CN7	86	805.7	0.13	2	0	0	0	0	30	0	30	0	0	0	2	32
CN8	77	661.9	0.14	3	0	1	0	1	1	0	1	1	1	0	4	6
CN9	69	563.5	0.17	2	2	0	0	0	1	0	1	0	0	0	3	5
CN10	74	743.4	0.18	52	1	3	0	3	12	0	12	0	0	0	4	68
Total	75.7±6.1	810.7± 241.1	0.19±0.03	74	15	26	0	26	208	0	208	6	6	0	5	329
%				70%	40%	40%			100%			30%				

***=** *P. anguillae* = 100% (25/25)

Acanthocephalans were the dominant helminths 63.2% (208/329), prevalence 100%, and the monogenean 70%

Appendix.13 Eel morphometric and associated helminth infection data for the **River Crouch**; England

Eel code	Body length (cm)	Body weight (g)	Condition factor	Gill monogenean *	Swim bladder nematode #	Gut nematode			Acanthocephalan			Tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
C157	33	65.8	0.18	56	7	0	0	0	15	0	15	1	0	1	4	83
C107	32	47.0	0.14	19	7	0	0	0	2	0	2	0	0	0	3	28
C117	28	27.5	0.13	34	1	0	0	0	2	0	2	0	0	0	3	37
C59	32	50.1	0.15	12	10	0	0	0	5	0	5	0	0	0	3	27
C74	50	209.8	0.17	2	0	0	0	0	53	0	53	0	0	0	2	55
C116	29	36.4	0.15	3	0	0	0	0	1	0	1	0	0	0	2	4
C71	47	198.2	0.19	7	2	0	0	0	12	0	12	0	0	0	3	21
C131	39	112	0.19	2	0	0	0	0	81	0	81	0	0	0	2	83
C40	32	43.9	0.13	5	0	0	0	0	2	0	2	0	0	0	2	7
C48	31	44.7	0.15	1	2	0	0	0	4	0	4	0	0	0	3	7
Total	35.3±7.6	83.5±67.6	0.16±0.02	141	29	0	0	0	177	0	177	1	0	1	4	352
%				100%	60%	0%			100%			10%				

***=** *P. anguillae* = (5/60) *P.bini* = (55/60)
= Ab Aziz (2012)

Acanthocephalans were the dominant helminths 48.6% (171/352), prevalence 100%, and the monogenean 100%

Appendix 14 Eel morphometric and associated helminth infection data for the **River Leven**; England

Eel code	Body length (cm)	Body weight (g)	Condition factor	Gill monogenean*	Swim bladder nematode	Gut nematode			Acanthocephalan			tape worm			Numbers of helminth classes	Number of helminth
				Total number	Total number	Total number	*S. inermis*	small nematode	Total number	*P. laevis*	Non bulb	Total number	*B. claviceps*	*P. macrocephalus*		
RL1	21	13.9	0.16	5	4	8	0	8	0	0	0	0	0	0	3	17
RL2	52	187.0	0.13	125	9	1	1	0	0	0	0	0	0	0	3	135
RL3	27	35.8	0.18	51	7	16	16	0	1	1	0	0	0	0	4	75
RL4	22.5	21.3	0.19	0	0	2	2	0	0	0	0	0	0	0	1	2
RL5	34	55.9	0.14	10	0	0	0	0	3	0	3	0	0	0	2	13
RL6	35	71.3	0.17	2	1	3	0	3	0	0	0	0	0	0	3	6
RL7	27	26.3	0.13	0	7	0	0	0	0	0	0	0	0	0	1	7
RL8	29.5	30.5	0.11	5	0	3	3	0	0	0	0	0	0	0	2	8
RL9	30	38.1	0.14	1	1	1	0	1	0	0	0	0	0	0	3	3
RL10	26	22.3	0.13	33	5	1	0	1	0	0	0	0	0	0	3	39
Total	35.4±8.8	50.2±51.0	0.15±03	232	34	35	22	13	4	1	3	0	0	0	5	305
%				80%	70%	80%			30%			0%				

* = *P. anguillae* = 44.2% (19/43) *P.bini* 55.8% (24/43)

Monogeneans were the dominant helminths 76.1% (232/305), prevalence 80%, and the gut nematodes 80%

Table 5.1 Summary of the primary helminth infection data and mean host morphometric parameters. Ten eels were examined from each site from England and Wales.

Eel Catchment Site Code	ML(cm) (±sd)	MW(g) (±sd)	MK (±sd)	Number of eel without helminth infection	Number of eel infected with helminths	Number of pseudodactylids	Number of *A. crassus*	Number of GI nematodes	Number of tapeworms	Number of acanthocephalans
Crouch: England; C	35.4±8.8	50.2±51.0	0.15±03	0	10	141	29	0	1	177
Leven: England; RL	30.4±8.3	50.3±48.4	0.15±0.02	0	10	232	34	35	0	4
Crane: England; CN	75.7±6.1	810.7±241.1	0.19±0.03	0	10	74	15	26	6	208
Hether Burn: England; HB	21.4±3.6	13.0±5.6	0.13±0.04	5	5	33	0	16	2	0
Petteril: England; RP	29.4±8.4	58.4±39.6	0.18±0.06	1	9	3	0	75	3	4
Bela: England; B	29.8±10.6	43.6±59.5	0.14±0.05	3	7	5	0	40	1	4
Gowy: England; RG	34.1±4.7	54.9±17.5	0.13±0.02	4	6	0	16	5	1	4
Mawddach-eden: Wales; M	26.1±10.2	38.4±48.1	0.14±0.01	8	2	0	0	10	0	0
Clwyd-Meirchion: Wales; MC	23.9± 8.7	28.6±31.2265	0.16±0.05	2	8	0	0	14	3	0
Dee-Eitha: Wales; D	21.7±7.5	19.8±22.1	0.11±0.04	6	4	0	0	29	0	0
Cadoxton: Wales; CD	24.9±4.3	22.7±16.1	0.14±0.07	2	8	60	18	5	0	0
Rhymney: Wales; R	28.8±3.1	40.5±16.4	0.16±0.04	0	10	53	21	0	0	50
Clwyd-Elwy: Wales; CE	24.9±8.4	29.4±26.1	0.14±0.02	4	6	17	0	7	1	0
Taff: Wales; TB	23.8±2.9	22.4±9.4	0.16±0.02	4	6	1	2	1	0	18
Prevalence				27.9% (39/140)	72.1% (101/140)	35.7% (50/140)	25% (35/140)	33.6% (47/140)	9.3% (13/140)	30% (42/140)
Total helminth				39	101	619	135	263	18	469