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The Use of Non-Invasive Sampling Methods for the Detection of *Anguillicola crassus*, a Swimbladder Parasite of the European Eel (*Anguilla anguilla*), and an Investigation into the Morphological Changes Associated With Swimbladder Infection

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Non-technical Summary

THE USE OF NON-INVASIVE SAMPLING METHODS FOR THE DETECTION OF *ANGUILLICOLA CRASSUS*, A SWIMBLADDER PARASITE OF THE EUROPEAN EEL (*ANGUILLA ANGUILLA*), AND AN INVESTIGATION INTO THE MORPHOLOGICAL CHANGES ASSOCIATED WITH SWIMBLADDER INFECTION

Describing the physiological and morphological implications of parasitic nematode infection in the eel and investigating non-destructive methods for its detection

BACKGROUND

The persistent large-scale decline in European eel stocks since the 1980's requires a closer investigation of the factors known to increase eel mortality. Of these, the introduction of the parasitic swimbladder nematode *Anguillicola crassus* to European waters has been cited as a likely contributor to the recorded loss in juvenile recruitment. Through induced cellular and structural modifications, the consequences of hosting the *A. crassus* parasite can affect the integrity of the swimbladder, the buoyancy control organ vital to the successful completion of the migration out to the European eel's Sargasso Sea spawning grounds. Individuals incapable of making this lengthy migration are therefore lost from the viable reproductive stock.

Closer examination of the morphological and physiological effects incurred by infected individuals is therefore necessary to further examine the long-term implication of *A. crassus* infection. The critically endangered status of wild European eels requires that alternative methods of sampling are established in order to minimise unnecessary loss to standing stock. Several such methods were tested in order to provide both an efficient and non-destructive method of *A. crassus* detection; by examining alternative parasite hosts within the surrounding water body and utilising ultrasonography diagnostics. Individuals used in sampling provided both infected and uninfected tissues for histological examination of the swimbladder tissue and physiological testing of swimbladder extensibility. A more detailed understanding of the consequences of *A. crassus* infection from a species life history perspective was obtained, and discussed alongside managerial tactics for the improved monitoring, diagnosis and potential treatment of European eel populations in Northern Irish and Scottish waters.

Physiological and Morphological Changes

Histopathological examination showed a catalogue of morphological changes induced by long-term exposure to the *A. crassus* parasite. Overall thickening of the swimbladder wall, fibrosis, inflammation and folding of internal epithelial layers coupled with changes to cellular structure were documented in infected individuals. Comparison across a spectrum of infection levels was possible and showed an overall decrease in swimbladder condition, both on a microscopic scale and in terms of generic condition score of macroscopic factors. Swimbladder length to body length ratios showed that noticeable shortening and fattening of the swimbladder structure occurred with prolonged infection. Infected swimbladders therefore carry a decreased volume of air. Further experimental analysis was conducted to assess whether the internal structural changes documented compromised swimbladder functionality. Although statistical analysis was inconclusive, isolated swimbladders subjected to increased internal pressure sustained a range of internal pressures, suggesting that all swimbladders sampled would tolerate the pressure change that occurs with depth when completing their deep dives in the open ocean, thus remaining reproductively viable.

Non-destructive methods of sampling for *A. crassus*

The use of copepod intermediate hosts and perch paratenic host sampling does not provide a viable method of *A. crassus* detection. Infection rates in these hosts vary by location and food-web dynamics, and proved too low to allow for the efficient diagnosis of *A. crassus* infection within a given water body. Results from ultrasound diagnostics were far more favourable and provided immediate and accurate analysis of an individual's infection status. This method required little training and allowed for the determination of precise infection rates within a population, although quantification of parasite density is not possible. Alternative methods of non-invasive testing have been proposed, such as specific antigen detection in blood plasma and radio-diagnostic tools, but ultrasonography provides a cost-effective and relatively simple presence/absence diagnostic tool.

Techniques for the treatment and management of *A. crassus*

Several techniques, such as the use of the anti-helminth drugs levamisole HCl and metrifonate, antibacterial drugs flumequine and oxytetracycline, and the movement of infected stocks to more saline waters have been proposed for the treatment of *A. crassus*. The use of drug treatments requires repeated application and none of the methods described have provided a viable long-term solution to parasitic infection. Continued monitoring of freshwater populations through non-invasive diagnostic methods is therefore necessary to ensure early identification of infected populations and allow for the monitoring of parasitic spread throughout a catchment.

ABSTRACT

Mass declines in European eel stocks throughout its distributional range require a closer examination of the factors known to increase eel mortality. Several compounding factors have been suggested as likely causes. Of these, the arrival of *Anguillicola crassus*, a non-native parasitic swimbladder nematode, coincides with the mass mortalities witnessed, and the morphological changes incurred by infected individuals have been suggested to decrease the viability of infected eels. Due to this decrease in eel numbers, non-destructive and non-invasive methods of testing for *A. crassus* are highly beneficial. The use of intermediate and paratenic hosts as indicators for *A. crassus* habitat presence were examined, alongside a non-invasive diagnostic method using ultrasonography. Morphological and physiological changes to swimbladder tissue resulting from *A. crassus* infection were also determined using samples from two Scottish and two Northern Irish freshwater lochs. A suite of swimbladder health parameters were assessed using visual assessment, microscopy, histology, and a laboratory experiment to assess swimbladder response to increasing internal pressure in infected and parasite-free samples. Sample sites containing *A. crassus* showed a decrease in overall swimbladder condition, as determined by an index of swimbladder health. Histological results showed thickening of the swimbladder wall in areas of long-term parasite exposure and structural change at a cellular level. A noticeable reduction in swimbladder length in relation to body length was also recorded in infected individuals. Results of the pressure test were inconclusive in showing a physiological consequence to the changes in swimbladder morphology described. These results were analysed in a life history context and management options for continued species survival in the face of long-term *A. crassus* exposure were discussed.

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1 INTRODUCTION

Recent declines in the population of European eel (*Anguilla anguilla*) have been reported throughout much of its distributional range. Present-day stocks are suggested to be ten times lower than those of thirty years ago and juvenile recruitment has shown a 95% reduction over the same period (Feunteun, 2002; Freyhof and Kottelat, 2010). Stocks are currently critically low, and intervention is necessary wherever possible to ensure that the species is able to recover to a sustainable level. Such rapid, large-scale, population depletion requires an investigation into the factors known to increase eel mortality and more detailed study of those highlighted to be of critical importance. These factors need to be considered from a life history perspective and action taken wherever possible to ensure the species continues to thrive.

The short time interval over which this rapid population decline has occurred can represent the life span of only one or two generations in the European eel. This suggests the overall population decline to be a result of several compounding factors, each playing a role at different stages during the eel's life cycle. The species is catadromous, spending the majority of its life growing and feeding in continental waters, freshwater rivers and streams, before migrating out to sea to spawn in the outer reaches of the Sargasso Sea. Relatively little is known of the marine and reproductive phase of the European eel's life cycle. Reproduction is generally accepted to occur at depth of up to 500m, although this has never been witnessed, and no trace of the millions of spawned female eggs has ever been recorded (Lucas and Baras, 2001). In the last few decades, the marine phase is thought to have become a significant source of eel mortality; a result of changes in ocean climate brought about by global climate change. The decrease in flow of the Gulf Stream is thought to impede the ability of leptocephalus larvae to migrate east across the Atlantic and settle around the European continent (Knights, 2003). Seasonal and long-term fluctuations in plankton production affect leptocephalus growth during this stage, and play a role in determining subsequent survival rates (Desaunay and Guerault, 1997; Bonhommeau *et al.* 2008). Larvae then cease feeding and growth, developing into glass eels that settle around the continental shelf. Local climatic factors such as unfavourable wind-driven currents, also exacerbated by global climate change, may limit their ability to become established in these areas (Knights, 2003). Environmental factors are extremely important but are essentially outwith the reaches of human intervention and, as such, not a viable outlet for stock management.

Continental glass eels have historically been an important resource for the fishing industry. Moriarty and Dekker (1997) considered glass eel fisheries an important source of stock removal, with markets in Asia and Europe importing large quantities in the 1990's. In the face of the recent crash in standing

stock this practice has now been banned. In preparation for residency in inland waters, glass eels undergo pigmentation and develop into elvers, which will maintain an estuarine existence in brackish water or proceed to migrate upstream to freshwater rivers and lakes. Elvers mature into yellow eels, and will remain in inland water bodies or estuaries for a number of years before silvering prompts their subsequent return to the Sargasso Sea to spawn. The average age of silver eels varies greatly between individuals and locations, but is approximately 7-12 years for males and 9-19 years for females in Scotland (Maitland, 2007). Sources of stock removal during the resident yellow eel phase are plentiful, but fisheries throughout most of Europe have historically been responsible for removing vast quantities of both silver and yellow eels. Dekker (2003) analysed historic landing data for European eel fisheries and reported that fisheries' landings have been in steady decline for more than forty years, peaking at approximately 50, 000 tonnes in the 1930's and 40's, and dropping to 15,000 to 20,000 tonnes in the 1990's. It was concluded that the decline in recruitment witnessed in the 1980's was preceded by a decrease in landing some twenty years earlier, and therefore a reduction in migratory spawning stock may have been responsible for the decrease in recruitment that followed. Fishing of the European eel is now limited to stocked populations to allow recovery of the natural standing stock, and fishing pressure is therefore not considered to be of present concern.

Further freshwater threats have been linked to habitat-related alteration and degradation. The effects of habitat loss, localized pollution events and the implementation of hydroelectric schemes have been discussed in the literature (Castonguay *et al.* 1994; Moriarty and Dekker, 1997). The fallout from these sources is felt locally and, although incidences of each have increased throughout the European eel's distribution, habitat alteration is not considered significant enough to be independently responsible for large-scale mortality. The successful colonization of the invasive swimbladder nematode *Anguillicola crassus* throughout much of the European eel's distribution has, however, been identified as a potential large-scale source of eel mortality, and it's occurrence in European waters coincides with the rapid decline in eel stocks witnessed in the 1980's.

A.crassus originated from the Eastern Asian continent and is now widespread throughout Asia, Europe, Africa and Eastern North America. The origins of European eel infection and the parasite's subsequent trans-continental spread are summarized by Kirk (2003), and have been traced to the mass import/export of European eels to Japan, in order to support the substantial Asian eel trade. European elvers, immunologically naive to the swimbladder nematode, became infected by mixing with its native host, the Japanese eel *Anguilla japonica*, and were then exported carrying the infection to other regions of Asia. *A.crassus* is thought to have been introduced to European waters by the importation of infected Japanese eels to Germany in 1980 (Koops and Hartmann, 1989). Incidences of *A.crassus* infection were first reported in Britain in 1987, with infected individuals found in the Rivers Thames, Welland and Trent (Kennedy and Fitch, 1990). The parasite it thought to have been introduced

through escaped imports from the eel trade and accidental contamination caused by transportation lorries using local freshwater sources to refresh water containers during the long-haul transport of infected eel stocks. The colonization of *A.crassus* in both natural and farmed stocks throughout England and Wales then ensued.

A.crassus is a highly efficient parasite that has been able to rapidly exploit an empty niche (Kirk, 2003). Its capacity to spread throughout a catchment is a direct consequence of the organism's adaptability, high reproductive output and short life cycle. This success is ultimately contingent upon predator/prey interactions, with larval stages transmitted trophically through freshwater food-web interactions (figure one).

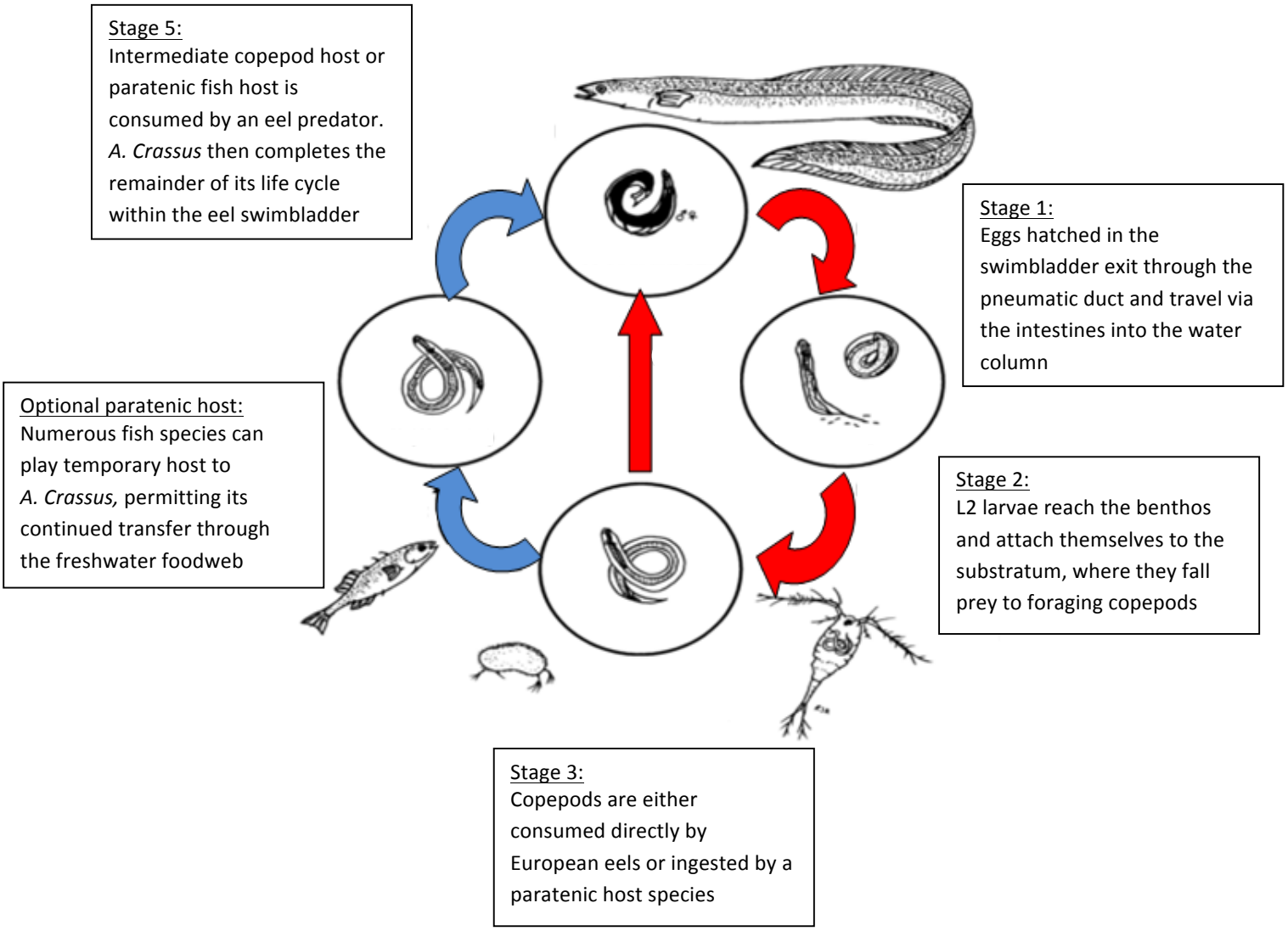

Figure 1: Trophic transfer of *A.crassus* (adapted from Kirk, 2003). Direct route of transfer shown in red and transfer via paratenic fish host indicated by blue arrows

There are four larval stages and a terminal adult stage in the life cycle of the *A. crassus* nematode. Eggs are hatched in the terminal host's swimbladder and migrate out through the pneumatic duct and intestines before their release into open water. These L2 larvae reach the benthos and attach themselves to the substratum, where they are preyed upon by their intermediate copepod host. It is unclear whether the active movement of L2 larvae attracts and instigates copepod predation during this stage (Thomas and Ollevier, 1993). Free living larvae can survive within the substratum in a dormant state for several days, particularly during periods of low water temperature (Kennedy and Fitch, 1990). Ingested L2 larvae then burrow through the copepod's intestinal wall and spill out into the haemocoel (Thomas and Ollevier, 1993). The second moult, from L2 to L3 larvae, is carried out in the haemocoel within 4-12 days (Lefebvre *et al*. 2012). The continued success of the L3 larvae relies upon its host falling prey to a paratenic host fish or European eel, the terminal host species.

The most direct route of transfer is therefore copepod to eel trophic transmission. In the natural environment however, food webs are complex and a number of different species can play host to *A. crassus*. These individuals will either ultimately fall prey to eels, thus allowing the nematode to complete its life cycle, or will act as dead-end hosts, harbouring the parasite but preventing it from further growth, maturation and reproductive success. A vast number of diverse host species, covering multiple taxonomic groups, have been described. Lefebvre *et al.* (2012) compiled a list of those presented in literature and described 50 paratenic host fish species, spanning 20 families. Reported levels of infection within theses hosts varies between species and across sample sites (e.g. Haenen and Banning 1990; Szekely 1993; Thomas and Ollevier, 1992). Szekely (1996) detailed the successful infection of a secondary paratenic fish host, suggesting that transfer of *A. crassus* is possible by multiple trophic pathways. This has also been reported in the work of Moravec (1996) and Moravec and Skorikova (1998), who document the presence of viable L3 *A. crassus* larvae in the aquatic snail *Galba carvus,* and various adult and larval aquatic insects, tadpoles and newts.

Exploiting a range of host species does not necessarily guarantee the success of *A. crassus* larvae. Many of these species have been found to mount an immune response to the invading parasite. A successful immune response would result in the encapsulation of L3 larvae, rendering them isolated and essentially no longer a threat to the host species. The drive to instigate an immune response, its speed and relative success vary between individuals. A detailed account is given by Szekely *et al.* in their 1996 study. The host reaction is characterised by the formation of a granuloma, created by mononuclear cells attaching themselves to the migrating L3 larvae present in tissues or within the abdominal cavity. The larvae and surrounding epitheliod cells then become necrotic as the granuloma is encased in connective tissue. In some cases the larvae can remain alive, although experiments have shown that encapsulated individuals ultimately lose their ability to infect any further host species (Szekely, 1996). A paratenic host can, however, contain both encapsulated and free living larvae, and

the widespread distribution of *A. crassus* within a given habitat will ultimately ensure the successful transmission of L3 larvae to the terminal eel host.

Once an infected intermediate or paratenic host is ingested by an eel, the L3 larvae carried by the host will migrate out through the intestinal wall and travel across the peritoneal cavity towards the swimbladder. This migration can be achieved in as little as one week following consumption (Haenen *et al.* 1989). L3 larvae then burrow through the swimbladder wall and into the lumen (Figure 2a and 2b). The lag time between reaching the lumen and the L3 to L4 larval stage metamorphosis has been reported as anywhere from 2-3 weeks (De Charleroy *et al*. 1990) to 3 months (Haenen *et al.* 1989). L4 larvae reside in the lumen, feeding off the hosts tissue and blood, until they mature to pre-adult and adult stage (Figure 2c). Heavily infected individuals can possess a significant quantity of L4 and adult larvae within the lumen (Figure 2d). Reproduction occurs in situ, with females reported to release up to 500, 000 eggs (Kennedy and Fitch, 1990). The L1 to L2 moult of new offspring occurs in utero and the females lay eggs of fully developed embryos ready to be released into the open water to complete the life cycle (Lefebvre *et al.* 2012).

The response of the European eel to *A. crassus* infection varies with exposure time. The physical presence of the parasitic nematode has been found to instigate antibody production in some individuals (Knopf *et al.* 2000). Little evidence exists, however, to show that eels are capable of mounting a successful attack on invading *A. crassus*. The majority of host affects documented in the literature detail physical changes to the swimbladder tissue. The swimbladder is a vital organ in any fish species, but is of critical importance to the European eel, which relies on efficient buoyancy control mechanisms to successfully complete the deep dives required for transatlantic migration and spawning in the Sargasso Sea. Alterations to the swimbladder's functionality therefore have species survival consequences.

A healthy eel is capable of maintaining neutral buoyancy on vertical migrations by sustaining a constant volume within the swimbladder. The mechanism for doing so relies on efficient deposition of stored gas whilst descending, and rapid resorption of gas whilst the individual makes their ascent. The rete mirabile is a counter-current capillary arrangement of arterioles and venules which functions as a gas reservoir, allowing the diffusion of gas between the swimbladder and capillary system (Evans, 1997). This is driven by high gas partial pressure, generated by the secretion of acid metabolites from gas gland cells lining the swimbladder and intensified by back diffusion and the counter-current concentration in the rete mirabile (Wurtz *et al.* 1996). The gas gland cells are also the location of the pentosphate shunt; a process which produces carbon dioxide and promotes its diffusion from gas gland cells into the lumen, thus contributing to the volume of air within the swimbladder. The health

of these structures and successful control of these mechanisms are essential to ensuring efficient swimbladder function.

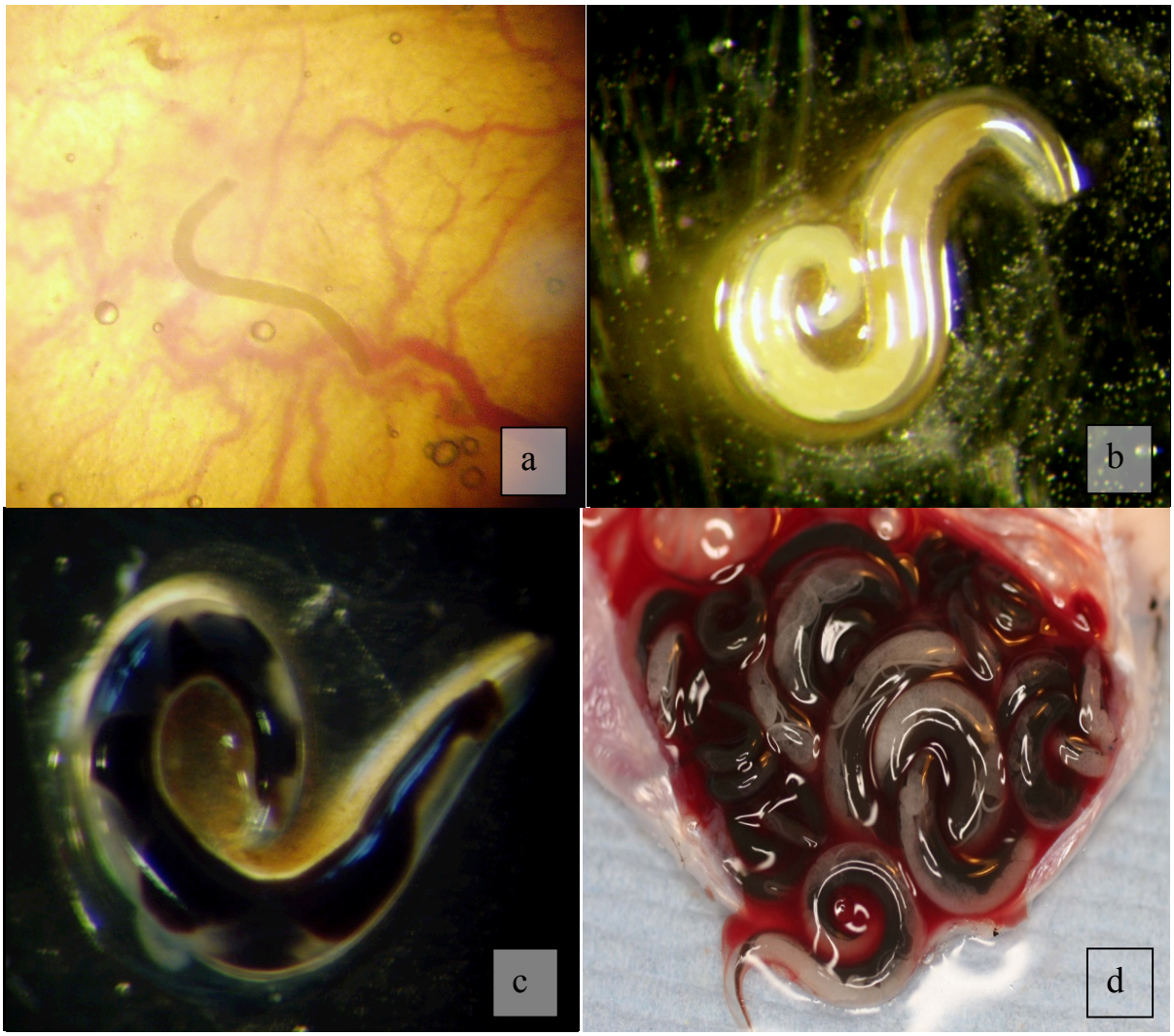

Figure 2: Stages of *A. crassus* larvae resident in terminal European eel host. a) L3 larvae embedded in swimbladder wall, b) resident L4 larvae removed from lumen of swimbladder, c) Isolated female adult *A. crassus, d)* Adult male and female *A. crassus in-situ*

L4 and adult larval stages of the *A. crassus* parasite are known to feed upon the swimbladder epithelium and conduct haematophagy. The epithelium is largely made up of gas gland cells and the mutilation caused by foraging *A. crassus* will likely render these cells unable to function. The damage caused to capillaries will also have similar consequences. Wurtz *et al*. examined changes in the gas composition of eel swimbladders with *A. crassus* infection in their 1996 study, and found a significant reduction in the percentage of oxygen present in the swimbladders of those harbouring the *A. crassus* parasite. They partially attributed this to loss of gas gland function, concluding that the mechanisms by which *A. crassus* feed and function within the swimbladder impair efficient gas deposition.

Physical changes to the structure of the swimbladder have also been reported. Individuals in areas of long-term parasitic exposure develop inflammation and increased thickness of the swimbladder wall (Molnar, 1994, Molnar *et al.* 1993). Shortening of the swimbladder has also been described in papers that have used this feature as an index of parasitic damage (e.g. Lefebvre *et al.* 2002). Changes at a cellular level have also been described. Wurtz and Taraschewski (2000) conducted a comparative histopathological study on infected and non-infected swimbladder tissue and, along with inflammation and marked swimbladder wall thickness, they noted migration of white blood cells, changes to the structure of epithelial cells, and areas of fibrosis. This suite of morphological changes described infers a likelihood that loss of functioning, further to the changes is gas composition described, can occur from long-term exposure to *A. crassus* infection. It also stands to reason that the compounding physical alterations and observable changes in swimbladder functionality may have long-term species survival implications.

The substantial decline in both standing stock and recruitment of the European eel has brought to light the threat of extinction and prompted the species to receive the classification of Critically Endangered on the IUCN Red List. Routine environmental sampling and analysis that produce fatalities should therefore be minimised, and methods that allow non-invasive diagnostics should be developed to assist in resource management decisions. The ability of *A. crassus* to invade a wide range of host species provides alternative reservoirs of infection from which samples can be obtained and the presence of the parasite investigated. Copepods are a necessary intermediate host and are therefore expected to show signs of infection upon routine sampling. Previous studies investigating the prevalence of *A. crassus* within copepods have been conducted under controlled laboratory setting and a study into the natural levels of infection would determine the efficacy of using copepod intermediates to assess the presence of *A. crassus* within a given habitat. It would be of further benefit to also examine the possibility of utilizing alternative paratenic hosts in the same manner. In managed eel stocks, a more accurate procedure that would allow for non-invasive determination of *A. crassus* infection directly within an individual eel would be extremely beneficial.

Despite its ability to rapidly spread throughout a catchment and beyond, *A.crassus* has not yet been officially recorded north of England on main land UK, although it has also been described throughout much of Wales, the Republic of Ireland and Northern Ireland. The parasite's ability to infect a wide range of mobile hosts makes it an imminent threat to Scottish eel stocks. Early detection would allow a unique opportunity to study its progression and the effects its presence will have on the ecosystem and resident eel population. The aims of this study are threefold: (1) to investigate non-invasive methods for the detection for *A. crassus* throughout a given catchment and directly within its European eel host, (2) to examine the morphological changes incurred by individuals exposed to *A. crassus* in a relatively recent and more established site of exposure, and (3) to determine whether

A. crassus infection can affect the physiological functioning of the host's swimbladder gas organ. In providing answers to these questions, it is hoped to add merit to the suggestion that the establishment and spread of *A. crassus* in wild European eel populations has facilitated the dramatic decline in population witnessed since the 1980's, and put forth viable non-invasive methods for the detection of *A. crassus* to allow for early detection and management intervention.

2 MATERIALS AND METHODS

Two Scottish and Two Northern Irish sample sites were selected for analysis; Baronscourt and Lough Neagh in Northern Ireland and Loch Lomond and Dubh Loch in the Loch Lomond and the Trossachs National Park of Scotland (figure 3). One site from each region was known to contain *A. crassus* within its catchment, whereas the presence of the parasite in the other site was undetermined.

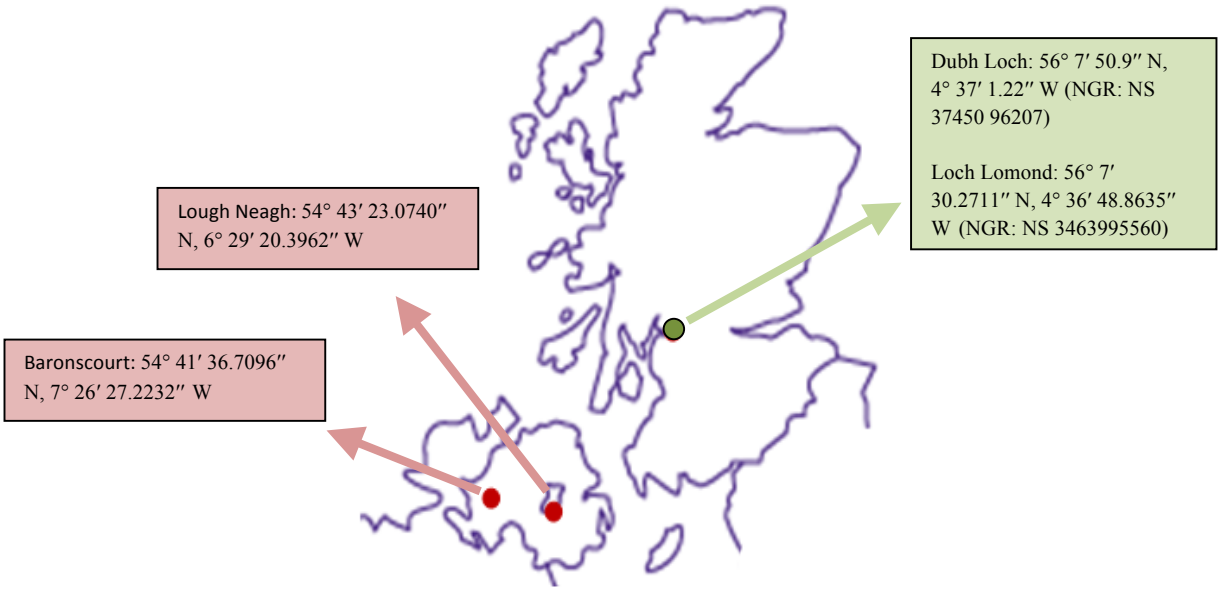

Figure 3: Locations of samples sites in (●) Northern Ireland and (●) Scotland

Baronscourt lakes comprise three large water bodies making up a total area of approximately 0.551 km². Very little previous research has been conducted in these highly productive waters, and it was not known whether *A. crassus* had yet managed to colonise these waters. Lough Neagh is the largest lake in the British Isles, comprising a total area of approximately 388 km². It is also the site of a highly prolific, long established eel fishery, sustained by the stocking of elvers which are fished out as yellow and silver eels and sold on to markets in London and Northern Europe. Lough Neagh has been infected with *A. crassus* since 2003 (D. Evans, pers. comm.).

Although *A. crassus* has not yet been officially recorded in Scotland, a pilot study showed it to be present in several resident eels sampled from Dubh Loch in 2008. This small body of water, filling a total area of 0.07 km², is located close to Loch Lomond and therefore provides a reservoir of parasite infection that can easily be transmitted by birds, small mammals or even high water levels from one water body to another. Loch Lomond is much larger in size, with a total area of 71km². The *A. crassus* swimbladder parasite has never been documented in these waters, although its close proximity to the Dubh Loch sample site suggests this merits further examination.

2.1 Non-invasive methods of *A. crassus* detection

Sampling of eels, perch and copepods from field-sites

Between the months of June and July, un-baited fyke nets were set in multiple locations throughout the study sites and left for 48 hours before collection. Each was positioned from a shallow shoreward location inwards towards the centre of the lake, covering a depth range of approximately 2m to 10m. A sample of ten eels, representing a range of lengths, were then collected from the nets and withheld for further laboratory analysis.

To further examine *A. crassus* infection rates within intermediate hosts, two plankton trawls, lasting approximately one minute each, were carried out during sampling sessions. Samples were stored in freshwater and analysed immediately upon return to the laboratory. Infection rates within paratenic hosts were also analysed by sampling perch (*Perca fluviatilis*) bycatch present in the fyke nets. Perch were selected on the basis of their widespread geographical distribution within the study area, suggesting bycatch samples would be easily obtained from each of the sample sites. Only individuals smaller than 14cm in length were selected for analysis, as this was deemed the maximum size of perch likely to succumb to eel predation, and therefore capable of completing the cycle of *A. crassus* infection.

This process was repeated at each of the Baronscourt, Loch Lomond and Dubh Loch sample sites.
Samples from Lough Neagh were obtained from the Lough Neagh eel fishery. Eels are caught by hook and line method and processed on-site daily. Only eels greater than 40cm are accepted for processing, resulting in each of our samples measuring 40cm or greater. The decision was made not to sample perch from this particular study site as none were taken as accidental bycatch and, as the vast majority of eels from this location are narrow headed, copepods are considered the primary food source. A two minute plankton trawl was carried out from shore at this location.

2.1.1 Intermediate and paratenic host analysis

Copepod processing

A thousand copepods from each sample site were isolated and investigated for signs of L2 or L3 *A. crassus* infection using microscopic examination.

Paratenic host laboratory examination

The selection of perch for paratenic source sampling was restricted to those under 14cm in order to represent the natural size of perch consumed by eels in the wild, and therefore ensure that those sampled were capable of completing the *A. crassus* life cycle. Each was dissected and examined for any signs of *A. crassus* infection within the swimbladder or surrounding tissues.

2.1.2 Eel ultrasound for *A. crassus* diagnostics

Prior to dissection, eels were examined for the presence of *A. crassus* within the swimbladder using ultrasonography. Characteristic horizontal striations are produced by swimbladder tissue, with dark grey breaks in these bands providing evidence of a foreign mass present within the body of the swimbladder. Any such irregularities in the ultrasound picture were recorded and photographed, and a judgement made as to the presence or absence of *A. crassus* in each individual.

2.2 Morphological changes associated with *A. crassus* infection

Eels were anaesthetised with a solution of clove oil dissolved in ethanol and pithed to ensure loss of innervation prior to dissection. Weight and body length measurements were taken and used to calculate overall body condition based on Fulton's condition factor K ($100W\ L_T^{-3}$). Following a midline incision, swimbladders were located and examined in-situ for visible signs of *A. crassus*. Any morphological distortions or gross pathologies were noted and each swimbladder assigned a value of 1-5, based on a ranking system of overall condition (table 1). The swimbladder and gonads were isolated and examined microscopically to allow for sex determination and a closer examination of tissue for the presence of any L3 larvae embedded in the swimbladder wall. The gonads, viscera and connective tissue were then removed, and the isolated swimbladders weighed and measured from cranial to caudal tip.

Table 1: Criteria for assigned values of overall swimbladder condition score

Condition Score	Criteria
1	Elongated shape, transparent swimbladder wall
2	Elongated shape, slight opacity in swimbladder wall
3	Shortened tips and more rounded shape, Increased opacity
4	Shortened and rounded shape, opaque, signs of haematophagy (increased blood vessels and bloody marking on swimbladder wall), internal fluid
5	Shortened and rounded shape, opaque, signs of haematophagy, large amounts of viscous fluid, presence of blood and metabolites in lumen

2.2.1 Histological preparation

A swimbladder was taken from each of the sites in Scotland and used for histological examination. It was not possible to obtain samples from Ireland so a sample from an area of long-term *A. crassus* exposure in England, similar to Lough Neagh, was used to provide comparison. Samples were fixed using a haematoxylin and eosin (H&E) and DFA sirius red (DFASR) staining method to show fibrotic tissue presence. Transverse and longitudinal section slides were produced, to allow for comparison of swimbladder wall thickness and fibrotic tissue density between sample areas.

2.2.2 Physiological changes associated with *A. crassus* infection

To test the potential effects *A. crassus* infection had on the physical integrity of the swimbladder, the extensibility of infected and uninfected swimbladders were compared. An experimental design, comprising an air pump attached to a sealed set of Buchner flasks and manometer, was set-up to achieve an air-tight system in which changes in internal pressure could be easily measured (figure 4). An eel swimbladder was attached to the inward air supply using a tight ligature and placed inside the first Buchner flask. The air pump was then switched on, and pressure allowed to build up within the system as the swimbladder tissue inflated, until rupturing of the outer membrane occurred and the swimbladder burst. Swimbladder inflation and manometer readings over time were recorded on a video recording device with in-built timer. An application of Boyle's Law allowed pressure within the swimbladder upon rupturing to be calculated, based on the volume of the inflated swimbladder, volume of air within the swimbladder and the fixed volume of experimental apparatus.

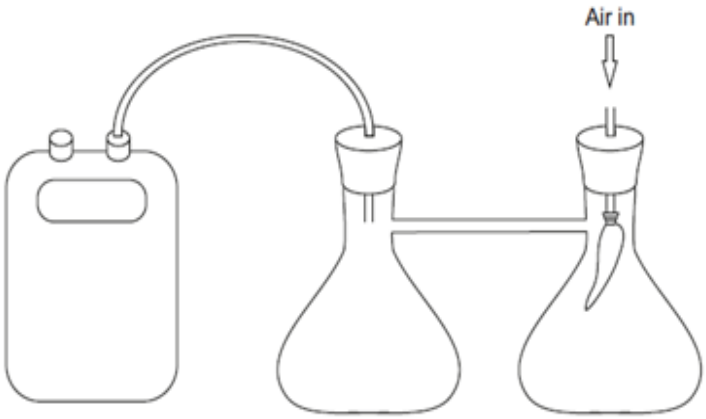

Figure 4: Pressure test apparatus and experimental set-up

To ensure consistent and comparable measurements were obtained, a calibration was carried out with the air pump apparatus. This allowed the natural build-up of pressure within the system to be accounted for prior to the addition of swimbladder tissue. This was achieved by running the air pump through the experimental apparatus for 60 seconds, recording obtained pressure readings at two second intervals and plotting these values in a time series graph. Once readings on the manometer provided a steady curve of pressure change over time for three consecutive trials, the equipment was ready for use.

Statistical Analysis

Results of the pressure test were used for statistical analysis to assess the impact on *A. crassus* infection on swimbladder extensibility and internal pressure tolerance. To conform to the assumptions of ANOVA, all data was checked for normality using an Anderson-Darling normality test. When data were not normal, a Log10 transformation was applied.

Eel condition was examined with respect to sample site in a one-way ANOVA with Fisher's LSD post hoc testing. Swimbladder condition was then examined with respect to the infection status of the sample site. A Spearman's rank order correlation test was first used to test the data, as swimbladder condition scoring was subjective and the data was not normally distributed. This was followed by a one-way ANOVA and Fisher's LSD post hoc testing. To examine the effects of *A. crassus* infection on swimbladder condition, a regression analysis of swimbladder length with respect to body length was conducted for grouped data from infected and uninfected sample sites. An ANCOVA test was then used to further explore this relationship. Swimbladder pressure at burst with respect to sample site was examined using a one-way ANOVA with Fisher's LSD post hoc testing. Data obtained for swimbladder pressure at burst was also examined with respect to swimbladder weight using the same

statistical testing. To determine whether the effects of swimbladder weight on pressure change yielded a true cause/effect relationship, a regression analysis was also conducted. The absolute pressure within the swimbladder lumen upon rupture was examined with respect to both the infection status of the sample site and each site independently, using a one-way ANOVA.

3 RESULTS

3.1 Non-invasive methods of *A. crassus* detection

3.1.1 Intermediate and paratenic host analysis

The technique of sampling copepod intermediate hosts and perch paratenic hosts to determine the presence of *A. crassus* in a system proved ineffective. Methods of larval detection within these hosts can be difficult, requiring laborious and detailed microscopic examination, and prevalence numbers appear to be too low to allow for efficient detection (table 2).

Table 2: Levels of A. crassus infection in perch and copepod samples

Location	Number of copepods sampled	% Affected with A. crassus	% Without A. crassus	Number of perch sampled	% Affected with A. crassus	% Without A. crassus
Baronscourt	1000	0	100	10	0	100
Lough Neagh	1000	0	100	0	0	100
Loch Lomond	1000	0	100	6	0	100
Dubh Loch	1000	0	100	6	0	100

3.1.2 Non-invasive methods of *A. crassus* detection – Ultrasound diagnostics

The use of ultrasound as a diagnostic tool was far more effective. Each individual examined was correctly diagnosed as infected or non-infected with *A. crassus*, verified by post-mortem internal examination (figure 5a to d). In some cases, inconsistencies in the swimbladder wall were noticeable, and were later confirmed to be L3 *A. crassus* larvae burrowing through the swimbladder wall from the body cavity. Some individuals also had bunching of L4 *A. crassus* at either the cranial or caudal end

of the swimbladder, diagnosed by an abrupt termination of the reflection pattern characteristic of the swimbladder (figure 5e and 5f).

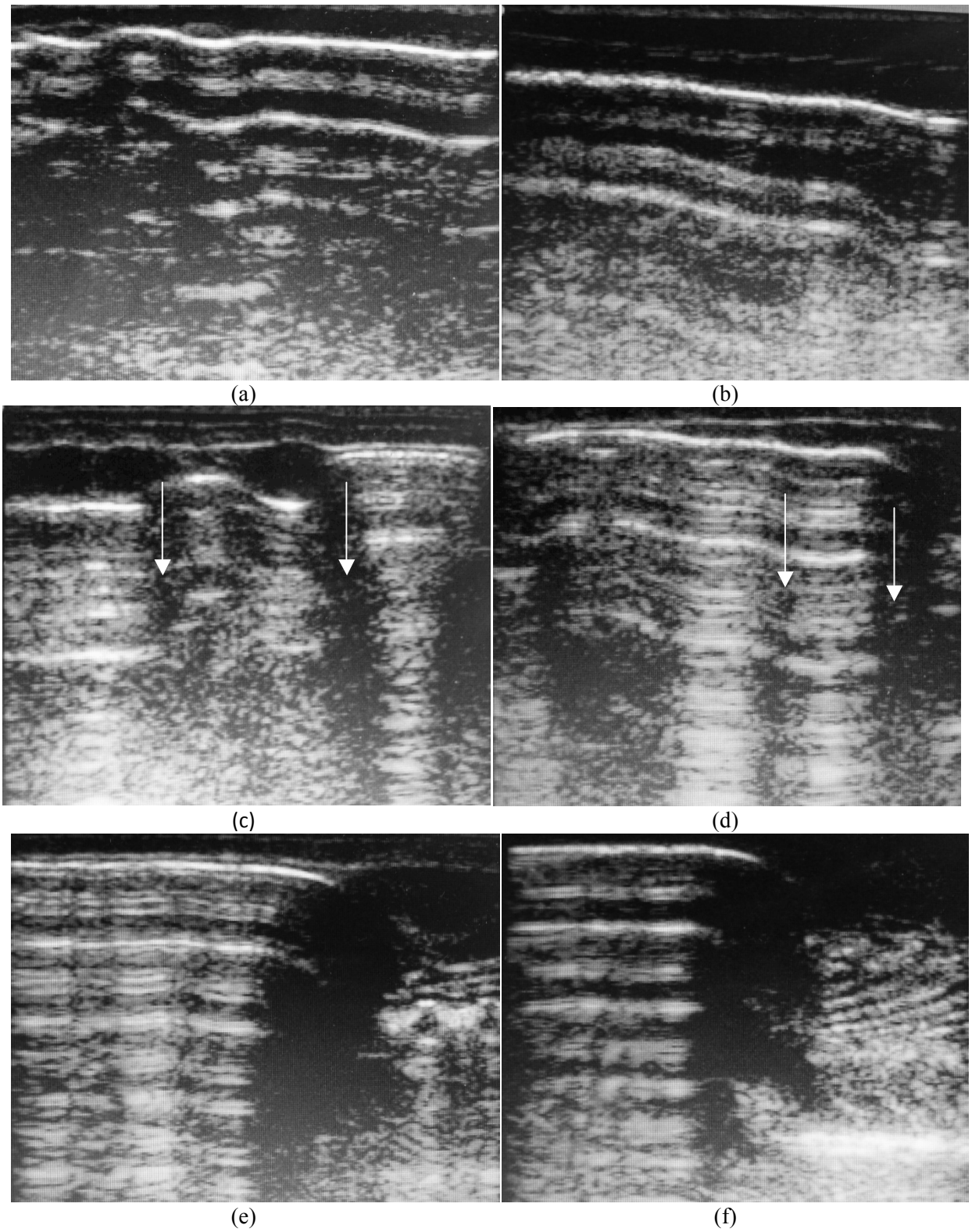

Figure 5: Diagnostic ultrasound images: (a) and (b) uninfected swimbladder; (c) and (d) swimbladder with A. crassus infection, nematode presence shown by breaks in horizontal striations indicated by white arrow; (e) and (f) bunching of L4 A. crassus at caudal tip of swimbladder

3.2 Morphological Changes Associated with *A. crassus* Infection

To ensure all data was normalised, results for eel weight, Fulton's condition factor and pressure change upon swimbladder burst were log transformed using Log_{10}.

Results for eel condition, measured using Fulton's condition factor, were examined for a location effect. Condition factor was found to differ significantly between locations ($F_{(3,32)}=6.21$, $p=0.002$). Post Hoc comparisons showed that the comparative groupings Lough Neagh and Baronscourt, Lough Neagh and Loch Lomond, and Dubh Loch and Baronscourt yielded statistically significant results between groups (figure 6). Those with no significant difference in condition were the groupings of the Lough Neagh and Dubh Loch infected sites, Baronscourt and Loch Lomond uninfected sites, and the recently-infected Dubh Loch and uninfected Loch Lomond sample regions.

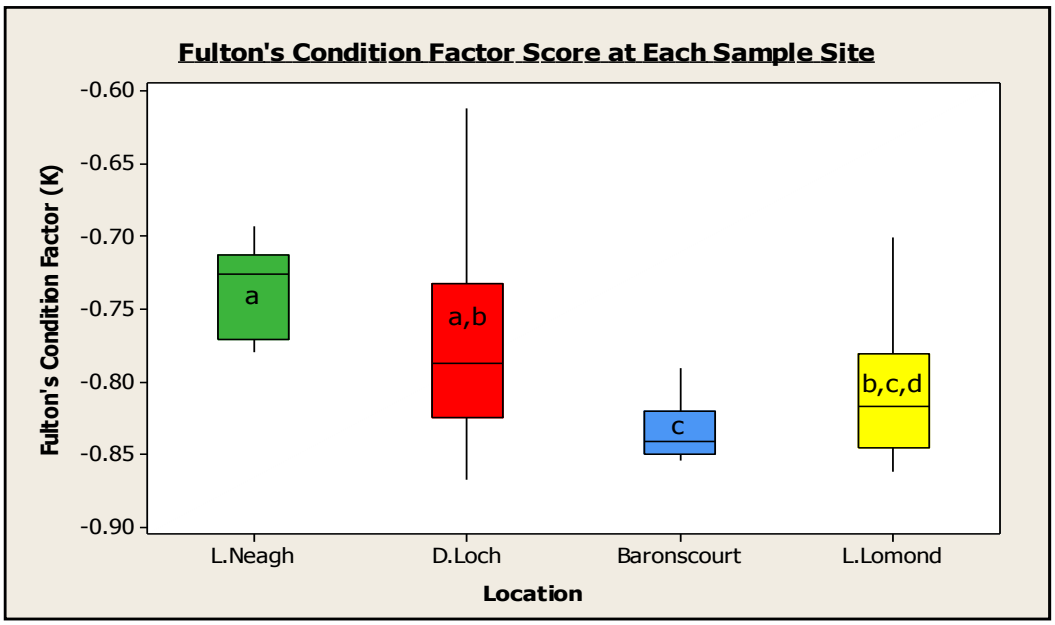

Figure 6: Fulton's condition score summary statistics at each of the four sample sites (different alpha characters indicate statistical difference at $p<0.05$ level)

Location results were grouped into infected and uninfected sites. These pooled results were then tested to explore the relationship between swimbladder condition and exposure to *A. crassus* infection. A strong correlation was found to exist between swimbladder condition and location ($r=0.907$, $p<0.001$). These results were then further quantified using ANOVA testing ($F_{(1,70)}=47.63$, $p<0.001$) and Post-Hoc analysis to determine where these similarities lay within the data. Results indicated that the uninfected Baronscourt and Loch Lomond grouping, along with the infected Dubh Loch and Lough Neagh pairings, did not differ significantly from each other in terms of swimbladder condition score.

The reported shortening in swimbladder length that occurs in individuals exposed to long-term *A. crassus* infection levels was examined using regression analysis. Total body length and swimbladder length were compared in sites of no *A. crassus* exposure and sites where *A. crassus* had become established. Data from uninfected individuals from the Loch Lomond and Baronscourt sites showed a strongly positive significant linear relationship between eel body length and swimbladder length (β=0.174, t(15)= 4.06, p=0.001). At the infected Lough Neagh and Dubh Loch sites a positive linear relationship was also shown, but was not found to have statistical significance (β=0.0468, t(19)= 0.99, p=0.336) (figure 7).

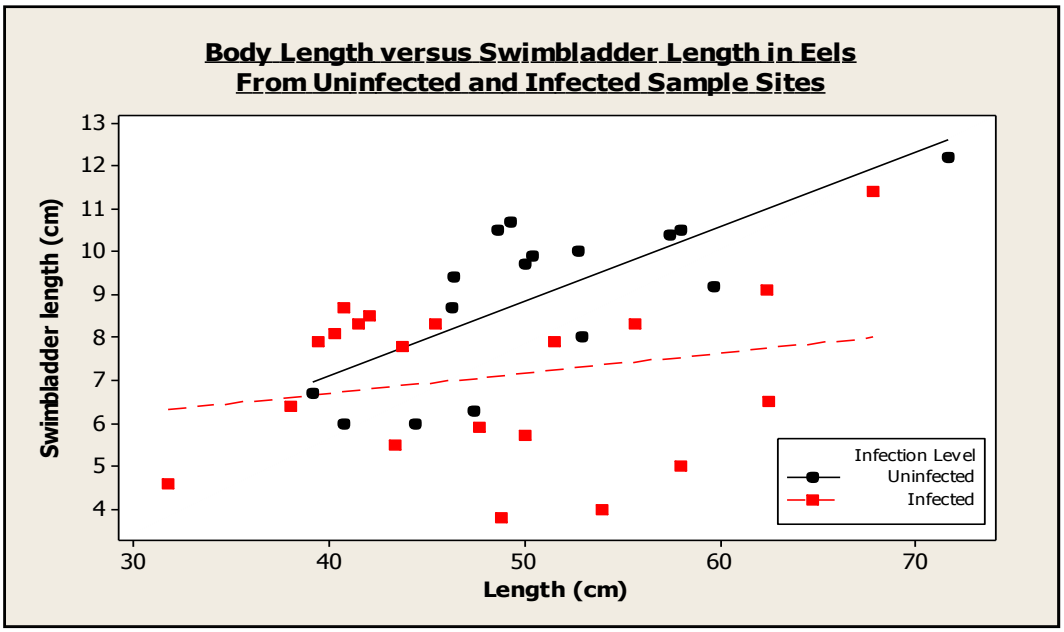

Figure 7: Scatterplot of linear regression showing total body length versus swimbladder length for infected and uninfected sample sites

This relationship was further explored using an ANCOVA test to look at the effects of site infection status and body length on swimbladder length. Both factors were found to have a significant effect on swimbladder length, although the influence of site ($F_{(1,35)}$=7.8, p= 0.009) was greater than body length ($F_{(1,35)}$=7.33, p=0.011).

3.2.1 Histological examination

Histological examination showed substantial folding of the inner epithelium of the swimbladder taken from a site of long-term *A. crassus* exposure. Noticeable thickening in the inner epithelial and muscularis mucosa cells was also apparent, when compared with both the Dubh Loch and Loch Lomond samples (figure 8). A pattern of epithelial cell shape distortion and elongation can be seen when comparing the uninfected swimbladder of Loch Lomond with the relatively recent infection in Dubh Loch and long-term infection of the English sample site (figure 8f, d and b, respectively).

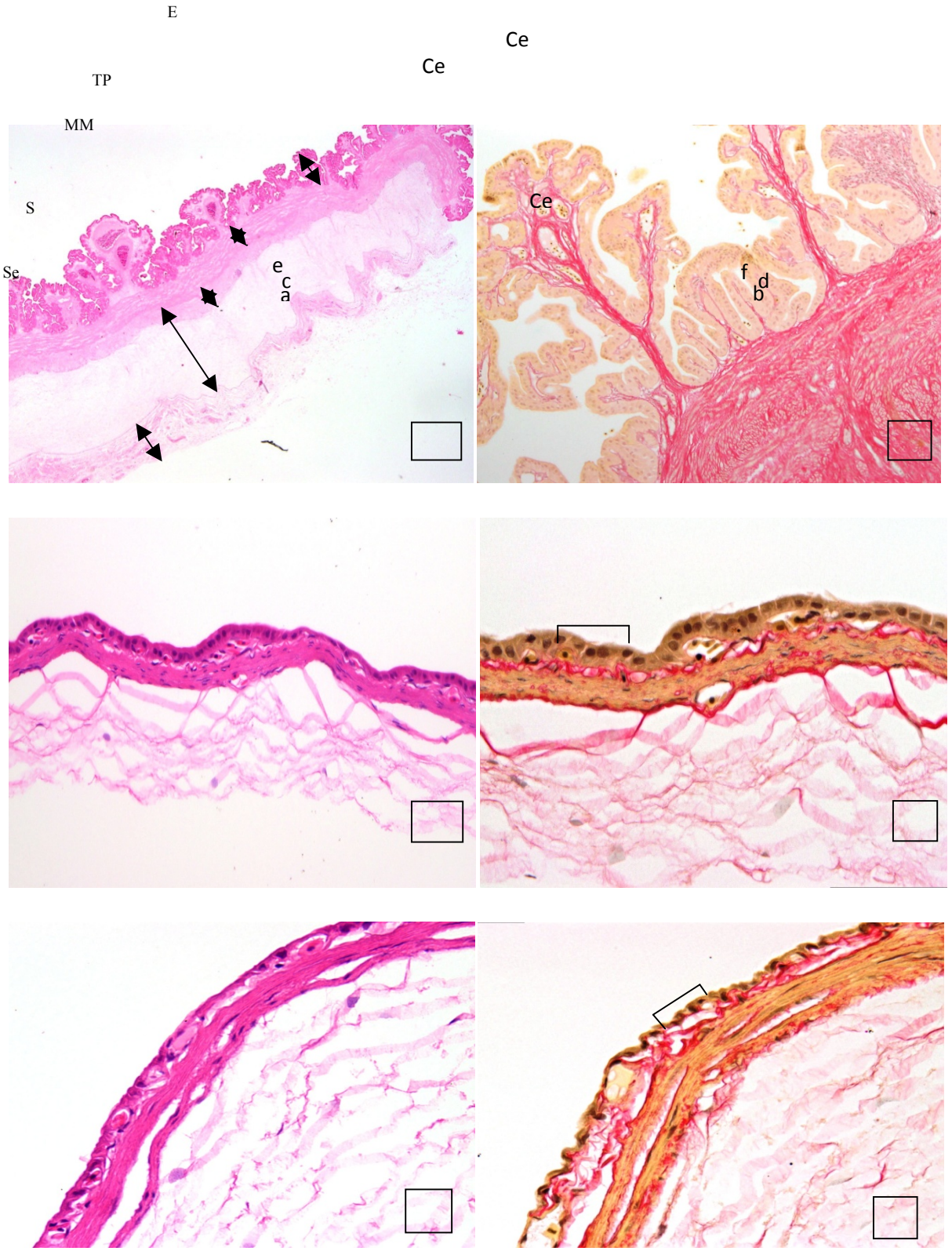

Figure 8: a) A. crassus infected sample showing folded epithelium (E), tunica propria (TP), muscularis mucosa (MM), submucosa (S) and serosa (Se); b) A. crassus infected sample showing folded inner surface and columnar epithelial cells (Ce); c) Dubh Loch infected sample showing linearly ordered epithelium and thin layers of tunica propria and muscularis mucosa; d) Dubh Loch sample showing columnar epithelial cells (Ce); e) Loch Lomond uninfected sample showing thin epithelial, tunica propria and muscularis mucosa layers; f) Dubh Loch sample showing reduced, cubic epithelial cells (Ce)

3.2.2 Pressure test

The pressure reading immediately before the swimbladder burst was subtracted from the pressure in the system following swimbladder rupture, and used to give a value for pressure change within the system. ANOVA of pressure change upon burst and location revealed that these two factors were closely related ($F_{(3,34)}=11.97$, $p<0.001$). Significantly different results were produced by the direct comparison of Lough Neagh and Dubh Loch, Lough Neagh and Loch Lomond, Dubh Loch and Baronscourt, and Dubh Loch and Loch Lomond (figure 9).

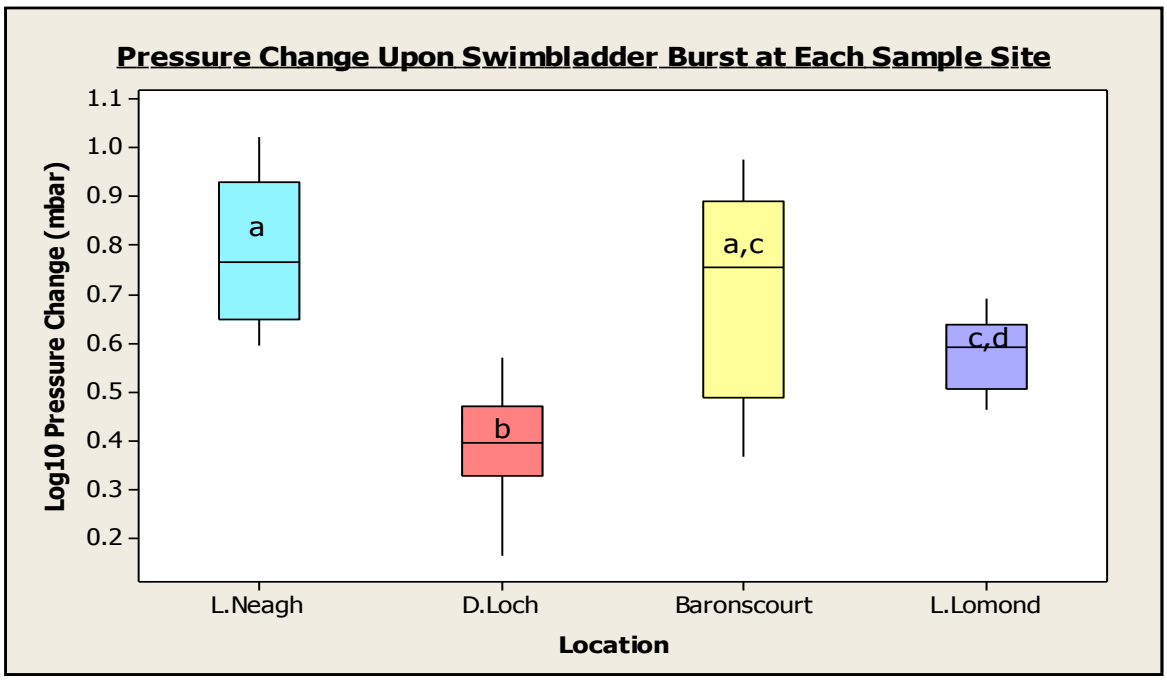

Figure 9: Pressure change upon swimbladder burst summary statistics by sample site

Pressure change data was also investigated in relation to swimbladder weight, to determine if swimbladder weight influenced the pressure an individual swimbladder was able to sustain. ANOVA testing showed this to be a valid hypothesis ($F_{(15,34)}=3.17$,$p=0.01$). Regression analysis of the untransformed pressure data on swimbladder weight showed, however, that there was no correlation ($\beta=0.099$, $t(35)=0.13$, $p=0.901$), as would have be expected if swimbladder weight and pressure change at burst truly had a cause/effect relationship.

The absolute pressure within the swimbladder lumen immediately preceding swimbladder rupture was calculated using an application of Boyle's Law. ANOVA results showed that this test was not able to discriminate between swimbladders from samples sites grouped by infection status at the significance level of $p=0.05$ ($F_{(1,28)}=3.65$, $p=0.067$), however there is a relationship. A significant difference in internal pressure tolerance was seen when comparing the results of each location separately ($F_{(3,28)}=3.08$, $p=0.046$). Results obtained for individual pressure tolerance showed that eels sampled

could tolerate a range of atmospheric pressures, all within a depth range that would allow them to complete their migration out to the Sargasso Sea (table 3).

Table 3: Internal swimbladder pressure range immediately preceding rupture, grouped by location and correlated to water depth (m)

Location	Pressure Range (mbar)	Pressure Range (atm)	Water Depth (m)
Lough Neagh	0.069-1.245	69.542-1245.2	690-12450
Dubh Loch	0.3486-3.386	348.7-3385.7	3480-33857
Baronscourt	0.376-0.445	376-445.5	3760-44550
Loch Lomond	0.1301-0.8658	130.1-865.8	1302-8658

4 DISCUSSION

Results for methods of non-invasive *A. crassus* detection were varied. The use of intermediate and paratenic host species does not appear to provide a viable method of detection. The sample sizes used in this study were relatively small, which may have influenced the ability to detect nematodal larvae. Previous research investigating *A. crassus* prevalence in copepods has primarily been conducted in a controlled laboratory setting (e.g. Ashworth *et al*. 1996). Results do not, therefore, reflect natural conditions. A study into the natural infection levels of *Philometra sanguinea*, a freshwater nematode parasite infecting multiple fish species, yielded prevalence values at a maximum rate of 0.25% in June, but 0% prevalence in April (Yaschuk, 1974). The author examined 65, 000 copepods to determine these values, suggesting our sample size of 1,000 may be too small for *A. crassus* determination purposes. This also therefore indicates that the use of copepods as an indicator for *A. crassus* infection within a habitat is not a realistic management option. The quantity of copepods that would need to be examined, coupled with the seasonal discrepancies which may also influence infection rates, make this a time-consuming and unreliable exercise.

The use of perch for paratenic host sampling yielded similar results. No infected individuals were found, even in sample sites known to contain *A. crassus*. Although small, sample sizes were similar to those of eels, all of which were found to harbour the parasite. This therefore suggests prevalence to be much lower in paratenic host species. Infection rates are a direct consequence of habitat species composition, seasonality and food web dynamics so location effects are likely, but previous studies have reported *A. crassus* in as few as two out of five perch sampled (Szekely, 1995). Other species such as ruffe were found to yield 100% prevalence (Szekely, 1993) and may prove a more useful species for the purposes of determining parasitic presence, either exclusively or in combination with

other fish species such as perch. Evidence from the literature suggests that sampling a range of paratenic host fish species simultaneously should unveil the presence of *A. crassus* larvae if it is present in the habitat. Although this method did not work in our pilot study, it would be expected that an increase in sample size and species selected for analysis would provide more accurate results and allow for *A. crassus* detection without the necessity to net and handle any European eels.

The most useful of all non-invasive diagnostic tools proved to be the application of ultrasonography. Testing for *A. crassus* on an individual level allows for the determination of precise infection rates within a population and provides the opportunity for the isolation and treatment of infected individuals. The ultrasound method yielded 100% accuracy in the diagnosis of parasitic infection, and further allowed for the identification of distinct morphological distortions related to swimbladder shape and size in infected individuals. This method is time-consuming and intrusive, requiring the eels to be trapped by fyke net, stored in a holding tank and anaesthetised prior to sampling, during which time eels produced copious amounts of slime and became visibly stressed. Although eels are extremely resilient, care must be taken to ensure minimal stress is inflicted upon individuals and the lowest possible anaesthetic dose is administered. Quantification of parasite density by this method is also not possible. Breaks in the pattern of reflection can represent a single nematode or several bunched together. The use of ultrasonography is therefore limited in its ability to provide subsequent information regarding infection level, but is ideal as a presence/absence management tool.

Alternative methods of *A. crassus* detection have been proposed. Similar to the use of ultrasound, Bergi *et al.* (1998) used a radio-diagnostic method to assess swimbladder inflammation and diagnose the presence of *A. crassus* parasites. This technique provided a more detailed analysis of swimbladder health by indicating the severity of pathological changes incurred. Information on air, worm and exudate content, along with the extent of shortening and overall shape change, was retrievable. The only limitation in using this method was the lack of information concerning swimbladder thickness and, as with ultrasonography, difficulty in quantifying *A. crassus* infection. Buchmann *et al*. (1991) and Hoglund and Pilstrom (1995) isolated and characterised *A. crassus* antigens from the cuticle, gonads, intestinal wall and intestinal contents of larval samples. Theses antigens can be detected using ELISA (Hoglund and Pilstrom, 1995; Knopf *et al*. 2000) and would allow for individual parasite detection without producing eel mortalities. This method is, however, invasive and would require the scientific skill necessary to obtain samples and interpret ELISA results for each individual assessed. A simple method of screening was proposed by Crean *et al.* (2003), who noted the presence of anal reddening in infected individuals and suggested its use as a visual diagnostic. Stages of anal reddening also allowed for the quantification of infection levels, with a more intense colour indicating a heavier parasitic load. Whilst this technique could be of use in some areas, and yielded a successful diagnosis for our Lough Neagh samples, anal redness was not apparent in all infected eels sampled from the

Dubh Loch site. This suggests anal redness to be a compounding effect of long-term exposure to the *A. crassus* parasite, possibly a result of the repeated expulsion of L2 larvae. This technique therefore has the potential to falsely diagnose more recently infected individuals as *A. crassus* free and is not a viable method if information on individual infection rates are required.

This study tested the merit of using external parameters to gain insight into overall fish health. Fulton's condition factor uses non-invasive measurements of body length and weight to calculate a value for overall body condition in fish, eliminating the need for internal examination or other invasive techniques such as fat content analysis. This method was applied to our samples and showed Lough Neagh and Dubh Loch eels, those that were infected with *A. crassus*, to score highest in overall body condition. Post Hoc analysis also showed that the groupings of infected and non-infected sample sites, along with the Loch Lomond and Dubh Loch grouping, were not significantly different in their condition scores. Lough Neagh and Dubh Loch are both highly productive water bodies, and improved condition may simply represent a location effect rather than a direct consequence of *A. crassus* infection. The similarity between infected and non-infected site grouping, coupled with the recently infected Dubh Loch and uninfected Loch Lomond sites, do suggest that there may be a link between incidences of *A. crassus* infection and overall body condition. It should be noted, however, that body condition is a function of weight and length, and a heavier weight value (yielding a higher condition score) could also suggest that an individual is consuming more prey, thus increasing their chances of ingesting infective intermediate or paratenic hosts. Larger sample sizes would facilitate the further examination of this relationship.

Detailed information concerning overall swimbladder condition and parasite load can ultimately only be gained through necropsy. Histological examination allows any morphological changes that occur in infected tissue to be studied at a cellular level. Histopathologies resulting from A. crassus exposure have been previously described in the literature and include overall swimbladder wall thickening, folding of the internal epithelial membrane, changes to the structure of gas gland cells, fibrosis and inflammation (Wurtz and Taraschewski 2000; Molnar *et al*. 1993). A comparison was possible between the swimbladder tissues from the uninfected Loch Lomond site, the relatively recently infected Dubh Loch site and the English site used to represent long-term exposure to *A. crassus*. The tissue showed a graduated shift in the overall shape and positioning of epithelial gas gland cells, becoming less linear and cuboidal in favour of a more rounded and columnar cellular structure. A substantial amount of folding of this layer was clearly visible in the swimbladder that has been exposed to parasitic infection for a number of years. The DFA Sirius Red staining method will stain blue in the presence of fibrotic tissue, but no fibrosis was visible in the samples obtained. Overall thickening of the swimbladder wall was highly noticeable in the swimbladder tissue obtained from the

English sample site, although not obvious in the sample taken from Dubh Loch. This suggests swimbladder thickness to be a direct consequence of long-term exposure to the *A. crassus* parasite.

The suite of morphological changes that occur as a result of parasitic infection make comparison between individuals difficult. An index of swimbladder health was proposed by Lefebvre *et al.* (2002), who utilised macroscopic features to determine the overall health state of examined swimbladders. In a similar technique, a list of assessment criteria was compiled for the eel swimbladders examined in this study, and ranked in order of decreasing overall condition. Individuals from Loch Lomond and Baronscourt were all assigned a value of one, reflecting total transparency and a healthy, elongated swimbladder. Results from the Spearman's rank correlation verified a close correlation between location and swimbladder condition, which was confirmed by ANOVA and Post Hoc analysis. The latter showed close similarities between the paired data groups from infected and uninfected sites. Criteria used to categorise swimbladder condition were simply based on morphological characteristics and did not include the physical presence or absence of *A. crassus* nematodes. This removed any error associated with previously infected individuals who were not host to *A. crassus* larvae at the time of experimental analysis. Although subjective, the results for swimbladder condition ranking therefore show a close association between historical exposure to *A. crassus* and overall decrease in swimbladder condition.

Further investigation into morphological changes was conducted using swimbladder length to body length ratios, to establish whether overall shortening of the swimbladder was a direct function of exposure to *A. crassus*. Samples were grouped into exposed (Dubh Loch and Lough Neagh) and unexposed (Loch Lomond and Baronscourt) locations. Results showed a high correlation between swimbladder length and body length in samples from the uninfected group and no direct correlation between swimbladder length and body length in the individuals from sites harbouring the *A. crassus* parasite. Overall swimbladder shortening has been described by a number of authors (e.g. Wurtz and Taraschewski, 2000) and is of significant importance from a life history perspective. By decreasing the volume of air an eel can contain within its swimbladder, shortening and rounding of the cranial and caudal tips act to compromise the functionality of this important buoyancy control organ.

Changes to swimbladder structure have important physiological applications. To examine this relationship, the influence of *A. crassus* infection on swimbladder function was investigated using a laboratory set-up designed to test the response of an inflated swimbladder to increased internal pressure. The results of this pressure test were used to investigate the potential relationship between location (as a proxy for *A. crassus* exposure) and the internal pressure an individual's swimbladder was able to sustain. Based on the changing morphology that results from parasitic infection, particularly a build up of fibrotic tissue, it was expected that samples taken from infected individuals

would not be able to sustain increased internal pressure for as long as those that were deemed healthy. The pressure at which a given swimbladder burst was found to be related to location. Results differed for all areas studied except the pairing of Baronscourt and Loch Lomond, both unexposed to *A. crassus* infection, and Lough Neagh and Baronscourt. The Lough Neagh and Baronscourt pairing is an interesting result given the long-term presence of *A. crassus* documented in the Lough Neagh catchment. Analysis of the results in more detail therefore suggest that the pressure test described did not have the ability to fully discriminate between infected and uninfected individuals at a $p=0.05$ significance level, although a relationship was present. This result is verified by the internal pressure calculations, in which no discrimination was achieved between the internal pressure tolerated in infected individuals against those who were *A. crassus* free. The range of pressures obtained in these results suggested that all swimbladders sampled would tolerate the pressure change that occurs with depth when completing their deep dives in the open ocean. The similarity in results from infected and uninfected sites, in samples with known pathologies, means the accurate application of these findings requires further investigation.

Results of this test were further analysed alongside swimbladder weight, to determine whether the physical weight of the tissue was a factor in determining the pressure it was able to sustain prior to rupturing. Although these factors did appear to be connected, results of the regression analysis showed that this was not a strongly positive linear relationship, as would be expected if this was a true cause/effect pairing. Previous research does suggest that infection compromises the ability to control overall air pressure within the swimbladder. Wurtz and Taraschewski (1996) clearly showed that *A. crassus* impairs the mechanism of internal gas deposition by altering the cellular structure and functional efficiency of the required swimbladder tissue. The experimental design used was similar to that of a study by Alexander (1959), which did manage to show differing levels of extensibility between his cyprinid samples. The need to calibrate the equipment prior to the beginning of every set of samples indicates that the air pump used was inconsistent in its output, which may have had an incidental effect on the results achieved. Further experimental analysis, ideally using a more consistent air input would validate these results.

Finding from this study echo those of others in detailing a catalogue of morphological changes induced by long-term exposure to the *A. crassus* parasite. Further analysis of the potential physiological effects incurred as a result of parasitic infection yielded inconclusive results with this experimental design. Other studies have suggested that the pathology and associated physiological effects that result from *A. crassus* infection will impair the ability of European eels to undertake the lengthy migrations necessary for reproduction (Kirk, 2003). With the widespread distribution of this efficient parasite and its continued spread, as witnessed by its discovery in previously undocumented

Scottish water, there is clearly a need to examine the treatment and management options for infected stock.

Several *A. crassus* management and treatment options have been suggested although none have proved a viable long-term solution. The use of anti-helminth drugs is likely the most effective treatment method, although requires direct application to infected individuals. Taraschewski *et al*. (1988) tested numerous nematocidal medications and found the application of levamisole HCl and metrifonate to be the most effective, inducing paralysis, death and expulsion of A. crassus worms. Neither treatment had the ability to destroy larvae embedded in the swimbladder wall and therefore required repeated applications to ensure all traces of the parasite were removed. Oral administration of the antibacterial drugs flumequine and oxytetracycline had the capacity to treat all stages of *A. crassus* infection, but did not induce antibody production or acquired immunity (Van der Hayden *et al*. 1996). Administration of these treatments are therefore also limited to repeated dosage as necessary, and do not provide a long-term solution to parasitic infection. Boon *et al.* (1989) suggested the use of dietary modification, based on experimental findings related to erythrocyte consumption by *A. crassus* L4 larvae. It is thought that a diet rich in special fatty acid composition has the ability to strengthen erythrocyte membranes, rendering it difficult for the *A. crassus* larvae to feed, and therefore mature and reproduce. Whilst this approach has merits in providing a more long-term solution, it relies on a controlled feeding environment only applicable to managed stocks maintained in closed systems. There have been conflicting results based on the ability of *A. crassus* larvae to tolerate increased salinity. Kennedy and Fitch (1990) and Kirk *et al.* (2000) showed that egg hatching and infectivity decreased with increasing salinity levels, although some individuals were able to survive and reproduce for up to six months in full seawater. The ability of some *A. crassus* individuals to osmoconform was later documented by Kirk (2002), suggesting that, whilst it may decrease infection rates, the movement of managed eel stocks to saline conditions would be ultimately ineffective in preventing repeated infection.

A. Crassus is an extremely efficient and effective parasitic nematode. The only limitations to continued spread and survival are the availability of suitable copepod intermediate hosts and low environmental temperatures. There has been increasing evidence to suggest that the global European eel population has reached a critically low level throughout much of its natural range. Although the cause of such large scale stock loss is likely a result of numerous compounding factors, the results of this study suggest that persistent exposure to *A. crassus* can significantly alter the morphology and potentially functionality of the swimbladder. These findings mirror those documented in the literature and give stock to the suggestion that the successful colonization of the parasitic swimbladder nematode *A. crassus* has, at the very least, been an important contributor to the reduction of the European eel standing stock. Treatment options are limited and restricted to managed stocks. The use

of non-invasive diagnostic techniques such as ultrasound and radiography can at least provide the identification of infected individuals and contaminated habitats, such that these options can be considered. Recent restrictions placed on European eel fisheries and continued stocking efforts may function to alleviate the significant pressure placed on European eel stocks, compounded by the effects of *A. crassus* parasitic infection, and aid the recovery of this critically endangered species.

5 REFERENCES

Alexander, R. M. (1959). The Physical Properties of the Isolated Swimbladder in Cyprinidae. *Journal of Experimental Biology*, *36 (*June), 341–346.

Ashworth, S. T., Kennedy, C. R., & Blanc, G. (1996). Density-dependent effects of *Anguillicola crassus* (Nematoda) within and on its copepod intermediate hosts. *Parasitology*, *113 Pt 3*, 303–9.

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. H., Lokin, C. J. A., Ceusters, R., & Ollevier, F. (1989). Some Properties of the Blood of European Eel (*Anguilla anguilla*) and the Possible Relationship with *Anguillicola crassus* Infestations. *Aquaculture*, *76*, 203–208.

Buchmann, K., Pedersen, L. O., & Glamann, J. (1991). Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms*, *12*(1), 55–57.

Castonguay, M., Hodson, P. V., Moriarty, C., Drinkwater, K. F., & Jessop, B. M. (1994). Is there a role of ocean environment in American and European eel decline? *Fisheries Oceanography*, *3*(3), 197–203.

Crean, S. R., Dick, J. T. A., Evans, D. W., Elwood, R. W., & Rosell, R. S. (2003). Anal redness in European eels as an indicator of infection by the swimbladder nematode, *Anguillicola crassus*. *Journal of Fish Biology*, *62*, 482–485.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., & Ollevier, F. (1990). The life cycle of Anguillicola crassus. *Diseases of Aquatic Organisms*, *8*(1990), 77–84.

Dekker, W. (2003). Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? *Fisheries Management and Ecology*, (10), 365–376.

Desaunay, Y., & Guerault, D. (1997). Seasonal and long-term changes in biometrics of eel larvae : a possible relationship between recruitment variation and North Atlantic ecosystem productivity. *Journal of Fish Biology*, *51*(Supplement A), 317–339.

Evans, D. 1997. The Physiology of Fishes (2nd edition). CRC Press LLC. USA

Feunteun, E. (2002). Management and restoration of European eel population (*Anguilla anguilla*): An impossible bargain. *Ecological Engineering*, *18*(5), 575–591.

Freyhof, J. & Kottelat, M. 2010. *Anguilla anguilla*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. <www.iucnredlist.org>. Downloaded on 07 August 2012.

Haenen, O. L. M., & Banning, P. V. (1990). Detection of Larvae of *Anguillicola crassus* (an Eel Swimbladder Nematode) in Freshwater Fish Species. *Aquaculture*, *87*, 103–109.

Haenen, O. L. M., Grisez, L., De Charleroy, D., Belpaire, C., & Ollevier, F. (1989). Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Diseases of Aquatic Organisms*, *7*, 97–101.

Hoglund, J., & Pilstrom, L. (1995). Mechanical isolation and characterization of antigens from adult *Anguillicola crassus*. *Fish and Shellfish Immunology*, *5*(1), 51–60.

Kennedy, C. R., & Fitch, D. J. (1990). Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal of Fish Biology*, (36), 117–131.

Kirk, R. S., Lewis, J. W., & Kennedy, C. R. (2000). Survival and transmission of *Anguillicola crassus* Kuwahara , Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology*, *120*(3), 289–295.

Kirk, R. S., Morritt, D., Lewis, J. W., & Kennedy, C. R. (2002). The osmotic relationship of the swimbladder nematode *Anguillicola crassus* with seawater eels. *Parasitology*, *124*(Pt 3), 339–47.

Kirk, R. S. (2003). The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology*, *10*, 385–394.

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. *The Science of the Total Environment*, *310*(1-3), 237–44.

Knopf, K., Naser, K., Van Der Heijden, M. H., & Taraschewski, H. (2000). Humoral immune response of European eel *Anguilla anguilla* experimentally infected with *Anguillicola crassus*. *Diseases Of Aquatic Organisms*, *42*(1), 61–69.

Koops, H., & Hartmann, F. (1989). Anguillicola-infestations in Germany and in German eel imports. *Journal of Applied Ichthyology*, *1* (January), 41–45.

Lefebvre, F., Contournet, P., & Crivelli, A J. (2002). The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology*, *124* (Pt 4), 457–63.

Lefebvre, F. Fazio, G. & Crivelli, A. J. (2012). *Anguillicoloides crassus*. In: Woo, P., T. & Buchmann, K. (Eds.), Fish Parasites: Pathobiology and Protection (pp.310-326). CAB International, UK.

Lough Neagh Partnership. 2007. Discover Lough Neagh: Lough Neagh Visitor Guide. <http://www.discoverloughneagh.com/Portals/1/brochures/LoughNeagh_Visitors07.pdf>. Downloaded on 20 August 2012.

Lucas, M. C. and Baras, E. 2001. Migration of Freshwater Fishes. Blackwell Science, Great Britain.

Maitland, P. 2007. Scotland's Freshwater Fish. Trafford Publishing, Canada.

Molnar, K. (1994). Formation of parasitic nodules in the swimbladder and intestinal walls of the eel *Anguilla anguilla* due to infections with larval stages of *Anguillicola crassus*. *Diseases of Aquatic Organisms*, *20*, 163–170.

Molnar, K., Baska, F., Csaba, G., & Szekely, C. (1993). Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda : Dracunculoidea). *Diseases of Aquatic Organisms*, *15*(1990), 41–50.

Moriarty, C., Dekker, W., & (Eds). (1997). Management of the European Eel. *Irish Fisheries Bulletin*, *15*, 108.

Szekely, C. S. (1993). Paratenic hosts for the parasitic nematode *Anguillicola crassus* in Lake Balaton, Hungary. *Diseases of aquatic organisms*, *18*, 11–20.

Szekely, C. S. (1995). Dynamics of *Anguillicola Crassus* (Nematoda: Dracunculoidea) Larval Infection in Paratenic Host Fishes of Lake Balaton, Hungary. *Acta Veterinaria Hungarica*, *43*(4), 401–422.

Szekely, C. S. (1996). Experimental studies on the infectivity of *Anguillicola crassus* third-stage larvae (Nematoda) from paratenic hosts. *Folia Parasitologica*, *443*, 305–311.

Szekely, C. S., Pazooki, J., & Molnar, K. (1996). Host reaction in paratenic fish hosts against 3rd stage larvae of *Anguillicola crassus*. *Diseases of aquatic organisms*, *26*, 173–180.

Taraschewski, H., Renner, C., & Mehlhorn, H. (1988). Treatment of fish parasites 3. Effects of levamisole HCl, metrifonate, fenbendazole, and ivermectin on *Anguillicola crassus* (nematodes) pathogenic in the air bladder of eels. *Parasitology Research*, *74*, 281–289.

Thomas, K and Ollevier, F. (1992). Paratenic hosts of the swimbladder nematode *Anguillicola crassus*. *Diseases of aquatic organisms*, *13*, 165–174.

Thomas, K., & Ollevier, F. (2009). Hatching, survival, activity and penetration efficiency of second-stage larvae of *Anguillicola crassus* (Nematoda). *Parasitology*, *107* (02), 211.

Van Der Heijden, M. H., Helders, G. M., Booms, G. H., Huisman, E. A., Rombout, J. H., & Boon, J. H. (1996). Influence of flumequine and oxytetracycline on the resistance of the European eel against the parasitic swimbladder nematode *Anguillicola crassus*. *Veterinary Immunology and Immunopathology*, *52*(1-2), 127–134.

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), 121–34.

Würtz, J., Taraschewski, H., & Pelster, B. (1996). Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology*, *112 (Pt 2)*, 233–8.

Yaschuk, V. D. (1974). Dynamics of the infestation of the intermediate host of Philometroides sanguine. *Veterinariya*. Moscow, No 6, pp 67-71 (In Russian).