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The Evolutionary Ecology of Lampreys (Petromyzontiformes)

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“A parasite for instance, is a shocking and a baneful monster, yet still Nature has infused into his blandishments a not unpolished charm.”

Plato

ABSTRACT

Lampreys (Petromyzontiformes) are an ancient vertebrate group, comprising 40 currently recognised species that range throughout the Northern and Southern Hemispheres. Despite a conserved morphology, lampreys nevertheless express a diverse range of life history strategies. Unusually for vertebrates larval lampreys are filter-feeding organisms prior to undergoing an extensive anatomical reorganisation, and the adoption of either a parasitic or a non-parasitic adult life. Parasitic lampreys consume the flesh and blood of actinopterygian fishes, either in marine or freshwater environments, while non-parasitic lampreys do not feed following their metamorphosis from the larval form.

Morphological and genetic similarities between pairs of parasitic and non-parasitic lampreys have led to taxonomic confusion regarding the specific status of many non-parasitic forms, and the suggestion that the loss of the trophic adult phenotype is the result of a single species capable of producing alternative life history strategies. In this thesis it is argued that at least some paired species of lampreys do not comprise two distinct evolutionary lineages; rather, that non-parasitic lampreys represent one extreme in a continuum of life history variation expressed by a parasitic species.

Some lamprey species, such as the European river lamprey *Lampetra fluviatilis*, are morphologically variable, exhibiting divergent phenotypes in response to ecological pressures, such as alternative foraging environments. Loch Lomond, Scotland contains a population of *L. fluviatilis* that feeds exclusively in the lake and exhibits a reduced body size and an overall morphology distinct from the typical anadromous form. Its foraging strategy indicates that it may be capable of switching hosts in the face of declining numbers of a presumed favoured and formerly abundant host, suggesting a certain amount of plasticity in its trophic ecology that may have ensured its survival in this freshwater lake.

This freshwater-resident form, as well as anadromous *L. fluviatilis* and the non-parasitic species *L. planeri*, were found to spawn in a single river system within the Loch Lomond basin, and this site is crucial for the continued presence of this life history variant in Loch Lomond. The appearance of sexually mature specimens of three discrete phenotypes in this river, each representing an alternative life history strategy that may, or may not, belong to a single species, provides a crucial opportunity to test the strength of assortative mating between lamprey species pairs. Within this system the strength of assortative mating was found to be weak, and points to the possibility that freshwater-resident *L. fluviatilis* are mitigating gene flow between large anadromous parasitic *L. fluviatilis*, and small, non-parasitic *L. planeri*.

As well as weak behavioural isolation, inter-specific sneak male mating tactics were documented among these populations, and represents the first time this phenomenon has been observed between paired lamprey species. Such behaviour indicates a lack of species-specific cues acting between *L. fluviatilis* and *L. planeri,* and suggests that hybrid offspring could be common in some systems. Testing hybrid viability (survivorship) between Loch Lomond's two *L. fluviatilis* life history strategies and the sympatric *L. planeri* revealed no post-zygotic barriers to gene flow, at least in the form of gamete incompatibility.

Perhaps more convincingly though, when comparing traditional morphometrics and body shape variation, as well as mitochondrial DNA sequences, between *L. fluviatilis* expressing different foraging strategies with populations of *L. planeri,* no robust species specific differentiation was observed. In fact, species delimitation between *L. fluviatilis* and *L. planeri* appears to be related solely to overall body size, which is itself a function of life history strategy. However, life history strategy was not correlated with current species designation as relationships among mtDNA haplotypes indicate non-parasitic populations have evolved independently multiple times throughout the geographic range of *L. fluviatilis* in Europe. Therefore, *L. planeri* should not be considered as a distinct species, either morphologically or genetically. Instead, *L. fluviatilis* appears capable of expressing a range of life history strategies; from parasitic anadromous populations through to non-parasitic stream-resident populations.

The overall research approach employed in this thesis, i.e., the combination of ecological, behavioural, taxonomic and molecular studies, could be used to robustly examine the evolutionary ecology of parasitic and non-parasitic lampreys elsewhere.

CONTENTS

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

CHAPTER SEVEN

Resolving the taxonomy of European river (Lampetra fluviatilis) and brook (L. planeri) lampreys – a synthesised approach…...…………………………………139

CHAPTER EIGHT

Implications for the conservation and management of Lampetra fluviatilis populations in the U.K.…………………………………………………………197

List of Tables

List of Figures

List of Publications

CHAPTER TWO has been accepted for publication in the Journal of Fish Biology [Hume, J. B., Adams, C. E., Bean, C. W. & Maitland, P. S. (*in press*). Evidence of a recent decline in lamprey parasitism of a nationally rare whitefish *Coregonus lavaretus* in Loch Lomond, Scotland: is there a diamond in the ruffe?]. The chapter text contains the accepted manuscript in its original form, with the exception of minor editing for Thesis format.

CHAPTER THREE has been part-published in a report to Scottish Natural Heritage [Hume, J. B. (2011). Adult lamprey survey of the Endrick Water SSSI & SAC 2009-2010. *SNH Commissioned Report* No. 480].

CHAPTER FIVE has been accepted for publication in the Journal of Fish Biology [Hume, J. B., Adams, C. E., Mable, B. & Bean, C. W. (*in press*). Sneak male mating tactics between lampreys exhibiting alternative life history strategies.]. The chapter text contains the accepted manuscript in its original form, with the exception of minor editing for Thesis format.

CHAPTER SIX has been accepted for publication in the Biological Journal of the Linnean Society [Hume, J. B., Adams, C. E., Mable, B. & Bean, C. (2012). Post-zygotic hybrid viability in sympatric species pairs: a case study from European lampreys.]. The chapter text contains the accepted manuscript in its original form, with the exception of minor editing for Thesis format.

APPENDIX 7.2 has been accepted for publication in The Glasgow Naturalist [Hume, J. B. & Adams, C. E. (2012). First record of larval sea lamprey *Petromyzon marinus* L. in the Endrick Water, Loch Lomond.]. The Appendix text contains the accepted manuscript in its original form, with the exception of minor editing for Thesis format.

Appendices

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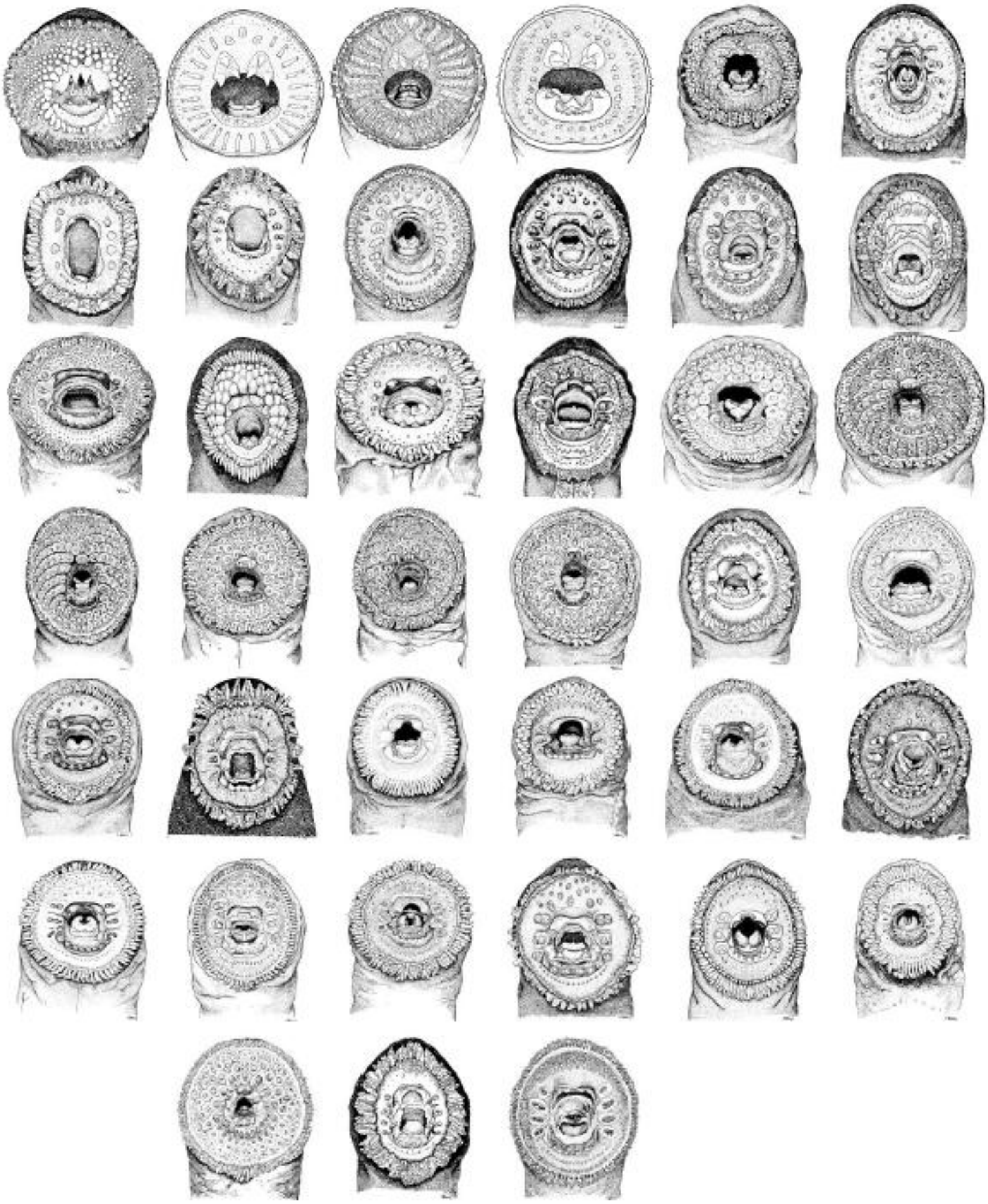
Author's Declaration

The material presented in this thesis is the result of original research, conducted between October 2009 and October 2012, under the supervision of Professor Colin E. Adams, Dr Colin W. Bean and Dr Barbara Mable. This work has not been submitted, in whole or in part, towards the fulfilment of any other degree. This work is based solely on data collected and analysed by myself, with the exception of Chapter Two, where additional raw data was provided by Professor Peter S. Maitland and duly noted in the text. Any published and unpublished material not of my own is acknowledged in the text.



Line drawings of the oral discs of 39 of the 40 extant species of lampreys. Modified from Renaud (2011)

“Buried in scattered companies in the soft soil, it may be said to lead the life of the mole; and it is there he finds all it wants of food, in search of which by taste or scent, it moves through its tracks as appetite or disposition prompts; and from observation it may be judged that, except in search of a new feeding ground, it never willing exposes itself to the dangers of a rapid stream, the strength of which it may scarcely be able to stem, or to the appetite of any prowling inhabitant of the river, from which its powers would not enable it to escape."

Ammocoetes branchialis, Couch (1877), *A History of the Fishes of the British Islands*

“From every economic standpoint it would appear to be advantageous to rid the world entirely of the lampreys. It would certainly be greatly to the advantage of the fisheries of the State of New York if all were destroyed. Naturally, however, the student of biology must mourn the loss of a form so interesting and so instructive. The questions naturally arise: how can the fish be protected from the lampreys; and is it possible to remove the lampreys from our lakes?”

H. A. Surface, (1897)

Chapter One

An introduction to the biology of lampreys (Petromyzontiformes), with particular reference to paired species

1.1 GENERAL INTRODUCTION

Lampreys (Petromyzontiformes) represent an evolutionary success story, yet for those with a general zoological interest they remain an obscure group to this day. They are sometimes referred to as "living fossils" as evidenced by their presence in ancient rocks of *c.* 365 million years ago (Janvier *et al.*, 2004; Gess *et al.*, 2006), and modern species display many of the primitive characteristics associated with other fossil animals of this distant period. Chief among these characteristics is the absence of a hinged jaw and the presence of a circular disc that surrounds the mouth, a feature the lampreys share with their closest living relations, the hagfishes. Lampreys and hagfishes (cyclostomes) are the only extant groups that lack true jaws, and together with the first fossil fishes – the ostracoderms – are grouped as agnathans (without jaws).

The round mouth, or oral disc, of lampreys exhibits many small keratin-capped teeth and the mouth itself contains a piston-like "tongue" organ, which bears similar teeth. Lampreys have only a single nostril that leads to a blind-ended tube containing the olfactory bulb located above the gills. The top of the head also bears a translucent patch of skin, below which lies the pineal organ with a light-sensitive retina, in addition to two eyes located on either side of the head. The balancing organs of the inner-ear contain only two semi-circular canals as opposed to the three found in other vertebrates (Richardson *et al.*, 2010). Like many aquatic organisms lampreys are able to detect changes in pressure *via* the lateral line system, which consists of individual neuromasts and appear as a series of fine pits located along the surface of the head and body (Bodznick & Northcutt, 1995). The gill chambers open to the outside *via* seven rounded, port-hole like structures and breathing occurs in a tidal motion, in which water is alternately drawn in and pumped out of the gills (Lewis, 1980). The skeleton of lampreys lacks any ossification, and is entirely cartilaginous (Sterba, 1962). Lampreys qualify as members of the vertebrate lineage based on the presence small pieces of cartilage (arcualia) located on either side of the dorsal nerve cord, representing rudimentary vertebral joints.

In place of a jointed and rigid spinal column lampreys have a gelatinous notochord that is both flexible but rigid enough for the swimming muscles to anchor to. The swimming motion of lampreys is sinuous and eel-like, with waves of muscular contraction travelling along the body from the head to the tail (Islam & Zelenin, 2008). Lampreys lack pectoral and pelvic fins and have only one or two dorsal fins, the posterior-most of which extends around the tail and becomes the caudal fin. A cloaca is located where the trunk and tail regions meet, and is the common opening for both the urogenital and intestinal ducts (Richardson *et al*., 2010).

One characteristic that has likely contributed more to their long evolutionary history and continued presence is that the majority of the lamprey life-cycle is spent as a burrowing larval form known as an ammocoete (Appendix 1.1). The ammocoete is a nocturnal microphagous filter-feeding stage that buries within mud and sand banks along the sides and bottom of streams and rivers for several years (Hardisty, 1944). During metamorphosis into the adult form the ammocoete remains nocturnal, once again burying itself in sediment or hiding beneath cover (Manion & Stauffer, 1970). Following the completion of metamorphosis the adult life history may progress in several directions. Of the 40 lamprey species currently described 22 never feed again, instead they mature sexually in natal streams within six to nine months of metamorphosis and then die following reproduction. As a result of their life history strategy these species, commonly termed “brook lampreys”, are no larger than the fully grown larval stage. The remaining 18 species go on to a parasitic life history, where they attach to host species using the oral disc and feed on their blood and tissue. This adult trophic period may last from a few months to several years, after which the lamprey matures sexually and spawns just once before it dies. All lampreys spawn in freshwater and lay eggs in gravel nests called redds.

Lampreys have a temperate distribution (Hardisty & Potter, 1971), principally because early development is restricted to water temperatures below 30°C (Macey & Potter, 1978). As a result of this there is a notable evolutionary division between lampreys found in the Northern and Southern Hemispheres, where 36 species belong to one family, Petromyzontidae, in the north, and four species belong to two families, Geotriidae and Mordaciidae, in the south. The deep divergence time of the two major lamprey groups is likely to have been a result of the widening of the tropical Tethys Sea *c*. 200 million years ago, which bounded the equator at this time, as the supercontinents of Gondwana and Laurasia separated (Gill *et al*., 2003).

One of the most intriguing aspects of the evolution of lampreys concerns the presence of what has been termed “paired species” (Zanandrea, 1959) in seven of the ten lamprey genera inhabiting Europe, North America and Australia (Appendix 1.2). These pairs comprise a non-parasitic lamprey that is believed to be derived from a parasitic and often migratory species, and although the adults are ecologically and phenotypically different, the larvae of these pairs are often morphologically inseparable (Zanandrea, 1959). In addition, in several cases more than one non-parasitic lamprey has been derived from a wide-ranging parasitic species, and so there are several “stem-satellite” derivatives (Vladykov & Kott, 1979). However, although many lamprey taxonomists consider life history to be species specific, contention - and indeed refutation of this theory - arises when one considers the repeated and parallel evolution of non-parasitic lamprey populations from a sympatric parasitic species where both are genetically indistinguishable (Espanhol *et al*., 2007; Boguski *et al*., 2012; Docker *et al*., 2012). This evidence indicates that in many cases, though not all, non-parasitic lampreys do not in fact encompass a single evolutionary lineage, but instead represent alternative life-history strategies of a single polymorphic species.

1.2 LARVAL STAGE

The prolonged existence of lampreys as an evolutionary lineage, as well as their relative obscurity, could be attributed to their extensive and cryptic larval stage. A brief examination of the ammocoete anatomy reveals an organism lacking in many distinctive features, most notably eyes. In fact, rudimentary eyes are present yet remain buried beneath the skin and so their function is largely replaced by the light-sensitive pineal organ located on the dorsal surface of the brain, beneath a translucent patch of skin (Richardson *et al*., 2010). A low and continuous dorsal-caudal fin extends around most of the trunk and tail, which appears rounded. Instead of the characteristic adult oral disc, ammocoetes have a horseshoe-shaped oral hood that is formed from an overhanging upper-lip and partially enclosed by a lower lip. The mouth itself is preceded by a basket-like mesh of fibres that fill the oral hood and acts to trap larger particles before they reach the mouth (Hardisty & Potter, 1971). Behind the mouth lies the velum, a pair of muscular paddles that act to create breathing currents by rhythmically pulsing forwards and backwards (Mallat, 1981). This apparatus also ensures the movement of water through the mouth only occurs in one direction – into the mouth and out through the gills.

Contrary to the tidal-breathing mechanism of adult lampreys, where water is alternately drawn in and pumped out of the gill chambers, ammocoetes respire much like other fishes, with water travelling in a single direction across the gills. Externally there are differences in the branchial region as well, where ammocoetes display a series of seven triangular slits within a groove, whereas adult lampreys have a row of seven round openings. This likely reflects the internal anatomy where adult gills open into individual water tubes, while in ammocoetes they lead directly into the pharynx (Richardson *et al.*, 2010). Particles of food that enter the ammocoete mouth along with the water current are strained off here, to prevent them escaping across the gills, and passed to the end of the pharynx where the gullet leads into a long, straight intestine (Gage, 1928). The intestine runs through the trunk of the ammocoete where it opens at the cloaca, located at the base of the tail. Beneath the gills lies the endostyle, a gland that produces digestive enzymes and secretes them through a duct into the pharynx (Richardson *et al.*, 2010). Later in its development the endostyle more closely resembles the typical vertebrate thyroid gland, which produces hormones and circulates them within the blood.

It is perhaps of little surprise then that it was not until 1856, when August Müller deduced ammocoetes to be the larval stage of adult lampreys, that the ammocoete was recognised as not being a chordate species in its own right (Scholtz, 2008). Prior to this there were are at least seven species belonging to the genus *Ammocoetes* (Duméril, 1816). However, 200 years prior to Müller's description Sir Izaak Walton wrote the following: "*The Eel may be caught especially with a little, a very little Lamprey which some call a Pride, and may in the hot months be found many of them in the River Thames, and in many mud-heaps in other rivers, yea, almost as usually one finds worms in a dunghill."* (The Compleat Angler, 1653, p172). The word "pride" is likely derived from the medieval Latin name for lamprey "lamprid", and the fact that Walton described finding these within "mud heaps" particularly in summer months perfectly fits the ecology of ammocoetes.

1.2.1 *Early Development*

The developmental period of fertilised lamprey eggs is strongly correlated with temperature, and so the time it takes larvae to hatch out varies (Manion & McLain, 1971; Manion & Hanson, 1980). The temperature threshold for the successful embryonic development of *Petromyzon marinus* falls between 11 and 15°C (Rodríguez-Muñoz *et al*., 2001) and typically hatching occurs some two to four weeks after fertilisation, with a reduced developmental period resulting from higher temperatures (Bayer *et al*., 2000). Mortality of young ammocoetes during high spring temperatures is common, and an incipient lethal temperature of > 22°C has been suggested for anadromous *Entosphenus tridentatus* and non-parasitic *Lampetra richardsoni* (Bayer *et al*., 2000), and > 25°C for *Lethenteron appendix, P. marinus* and the Southern Hemisphere species *Geotria australis* (Potter & Beamish, 1975; Macey & Potter, 1978).

Newly hatched lampreys (pro-larvae) are not particularly variable in size, for example anadromous *Lampetra fluviatilis* are 7 - 8 mm long (Hardisty, 1961; Tuikkala, 1971) as are non-parasitic *Lampetra planeri* (Maitland, 2003). Very small ammocoetes emerge from the nest *c*. 30 days after hatching and begin drifting downstream at night (Piavis, 1961; Manion & McLain, 1971; Jones & Derosier, 2001). The larvae of freshwater-resident *P. marinus* from the Laurentian Great Lakes have been recorded leaving the nest 18 - 21 days after hatching, and it was estimated that 0.4 – 1.1% of eggs within each nest successfully hatched and drifted downstream (Applegate, 1950). These larvae ranged in size from 6 – 9 mm. Manion (1968) recorded hatching success of this species as 5.3 – 7.8%, but there were no estimates of successful dispersal.

Mortality rates appear relatively low and constant throughout the larval period (Okkelberg, 1922; Hardisty, 1961; Kelso & Todd, 1993), but are likely to be especially high during the earliest months where predation is highest, particularly by other fishes (Hardisty, 1961; Heard, 1966; Manion, 1968; Tuunainen *et al*., 1980). Larger *P. marinus* larvae were found to be predated by a diving beetle, *Dysticus* sp. (Manion & McLain, 1971) but given that ammocoetes spend the majority of their time within the sediment it is difficult to observe mortality directly (Smith *et al*., 2011). High initial mortality of the eggs has been proposed as the result of fungal infection or suffocation by sediment (Manion & McLain, 1971; Jones & Derosier, 2001). However, it is likely that once they settle out and begin burrowing mortality is reduced rapidly. Zerrenner & Marsden (2001) estimated *P. marinus* survival as 85% between year 1 and 2, and 89% between year 2 and 3.

1.2.2 *Burrowing*

The pineal gland situated near the brain is important in driving larval behaviour as it stimulates the ammocoete to exhibit negative phototaxis and restless swimming behaviour when illuminated (Rovainen, 1980). This is aided by additional light sensitive cells on the skin, particularly concentrated around the tail region, and results in the ammocoete actively moving away from bright areas to areas of reduced light (Ullén *et al*., 1993). Ammocoetes also exhibit positive thigmotaxis, whereby if they cannot find a suitable substrate in which to burrow they will rest quietly on their side, but which under normal circumstances induces them to remain burrowed. Ammocoetes tend to exhibit burrowing behaviour only in areas of slow flow; otherwise they drift passively with the current (Applegate, 1950). This presumably enables them to select for optimal areas of substrate, as they can only burrow in soft sediments that typically accumulate in areas of slow flow (Hardisty, 1944). All of these behaviours ensure that when the ammocoete makes contact with suitable substrate the instinct to burrow is strong, and it begins with corkscrew-like thrashes of the tail driving the head downward into the substrate. When the head and branchial regions are hidden the body is laid flat across the substrate and localised contractions of muscle blocks pull the rest of the ammocoete into the substrate, with the oral hood acting as a probe or anchor while moving (Sawyer, 1959). Once completely covered the ammocoete moves back towards the surface of the substrate where it settles in a position of roughly 70° to the vertical, and often upside down (Mallatt, 1982).

Ammocoetes do not create U-shaped burrows; instead water drawn into the mouth is expelled from the gills directly into the surrounding sediment. Typically, burrows appear as small indentations on the surface of the sediment, with a single ammocoete resting below each. It is not clear whether ammocoetes excrete a substance in order to prevent the walls of the burrow collapsing (Beamish & Lowartz, 1995), but this seems unlikely given that they do not appear to fully form burrows, or indeed require such, instead they cover their body with only the oral hood breaking the surface. Ammocoetes typically burrow in the surface layers of substrate. Larger ammocoetes (90 – 160 mm) will burrow approximately 75 – 150 mm below the surface, but are capable of rapidly burrowing deeper if disturbed, and recently hatched ammocoetes (*c*. 20 mm) will settle in very fine surface sediment (13 mm), often only deep enough to cover themselves (Maskell, 1929; Applegate, 1950; Dendy & Scott, 1958; Lee, 1989). The depth at which ammocoetes burrow is possibly a function of oxygen tension in these areas as this has been shown to be a major factor in the maintenance of burrowing behaviour in captive ammocoetes (Galloway *et al*., 1987). Ammocoetes can tolerate low oxygen content (Hill & Potter, 1970; Schoonoord & Maitland, 1983) but survive in anoxic conditions for only a few hours (Potter *et al*., 1970).

1.2.3 *Habitat*

The physical nature of the substrate is of critical importance to ammocoetes, and the distribution and character of those substrates is to a large extent a function of river hydrology and the complexity of a river or stream. In general, ammocoete habitats fall broadly into three categories: type I, dominated by soft, organic sediments; type II, dominated by sandy sediments with low organic content; and type III, dominated by gravel or larger particles (Klar & Weise, 1994; Yap & Bowen, 1998). Based on this system one would expect to find ammocoetes within all three habitat types, though where present, type I habitats will be preferred over type II, and type II over III (Sugiyama & Goto, 2002). The use of terminology such as “optimal” or “sub-optimal” in regards to habitat is particularly prevalent in ammocoete surveys for management purposes, where ‘optimal’ is defined as: stable fine sediment or sand ≥ 15 cm deep, low water velocity and the presence of organic detritus (e.g., Bull, 2004).

Inspection of environments found to contain ammocoetes reveals a multitude of abiotic and biotic factors that are common among sites (Goodwin *et al*., 2008). Flow rate above larval habitats is highly dependent on stream gradient (Baxter, 1954; Schroll, 1959; Neeson *et al*., 2006), but in general falls between 0.01 m s^{-1} in shallow areas (Kainua & Valtonen, 1980; Malmqvist, 1980; Mundahl *et al*., 2006), up to 0.8 m s^{-1} in deeper pools (Thomas, 1962). Substrate porosity, a measure of the space among particles relative to the total sample volume, can range from < 10% - > 70% voids (Lee, 1989; Beamish & Lowartz, 1995). Conductivity in these areas can range from < 1 – 580 µL cm^{-2} s^{-1} (Young *et al*., 1990; Beamish & Lowartz, 1995; Stone & Barndt, 2005) and pH appears to be a useful descriptor, with a higher pH generally favoured (Goodwin *et al*., 2008). Similarly, particle size of the substrates can vary widely, typically ranging from silt-clay (< 0.05 mm) to gravel (> 2 mm)

(Hardisty, 1944; Manion & McLain, 1971; Malmqvist, 1980; Lee, 1989; Koonce, 1990; Kelso, 1993; Kelso & Todd, 1993; Beamish & Lowartz, 1995; Mundahl *et al*., 2006; Goodwin *et al*., 2008; Gruber *et al*., 2012). The organic content of substrates can be as high as 20% of dry weight in some larval habitats (Hardisty, 1944; Potter *et al*., 1986; Beamish & Lowartz, 1995; Waterstraat & Krappe, 1998).

Ammocoetes are most often encountered in relatively shallow water, usually ≤ 1 m in depth (Dendy & Scott, 1953; Malmqvist, 1978; Potter *et al*., 1986; Kelso & Todd, 1993; Jellyman & Glova, 2002; Stone & Barndt, 2005; Mundahl *et al*., 2006), but they may be more common in deeper areas (> 2 m) than previously assumed (Taverny *et al*., 2011). For example, ammocoetes are regularly collected from lentic habitats where they tend to be found on abrupt drop offs in relatively deep water (1 – 19 m) (Smith *et al*., 1974; Morman, 1979; Lee & Weise, 1989). These can include sites far from stream and river mouths, up to 1.6 km in some cases (Thomas, 1962; Wagner & Stauffer, 1962). Thomas (1962) suggested that lentic-living larvae could be found on far from typical substrates, instead lying beneath "various articles" such as strips of bark. Ammocoetes of anadromous *E. tridentatus* have been collected from a pollution abatement pond for a fish hatchery, where they were found in high numbers (36,450 estimated population size; density 21.8 m^{-2}), suggesting ammocoetes may be able to colonise atypical habitats (Nelson & Nelle, 2007).

1.2.4 *Density*

Ammocoetes can often be found in variable densities among suitable habitats throughout an extensive range (ERA, 2005; Goodwin *et al*., 2009), or even within streams (Thomas, 1963; Malmqvist, 1980; Fluri & Beamish, 1991). In Scotland, for example, in the River Spey *Lampetra* spp. ammocoetes formed densities of 5 – 15 m^{-2} (APEM, 2004), yet in the Endrick Water and the River Teith these densities were as high as 195 m^{-2} (Gardiner *et al*., 1995; Bull, 2004; Forth Fisheries Foundation, 2004; Watt *et al*., 2007). Ammocoetes of the larger *P. marinus*, which is known to spawn frequently in these systems, represented < 1 % of the population (Laughton & Burns, 2003; Ravenscroft & Seed, 2008). This is a pattern regularly seen in Europe, where *P. marinus* typically has low densities and represents 4 - 5 % of the total ammocoete population, despite adults penetrating far upstream (Maitland & Lyle, 2000; APEM, 2004). Applegate (1950) however found densities of freshwater-resident *P. marinus* in tributaries of the Laurentian Great Lakes to be approximately 100 m^{-2}, typically though these range from 1 – 13 m^{-2} (Morman, 1987; Kelso & O'Conner, 2001). Estimates of total population size from eight streams, all entering Lake Ontario and Lake Michigan, and ranging in length from < 0.5 – 9 miles, calculated *P. marinus* ammocoete populations ranging from 4,300 – 336,700 $stream^{-1}$ (Applegate, 1950; Smith & McLain, 1962).

The density of larvae in particular habitats varies in accordance with a suite of environmental conditions as well as the physical structure of the substrate. Most studies have indicated that soft sediments, organic matter, water velocity, water depth, and shade are key factors in predicting ammocoete density at any given site, although their relative importance varies among sites. Medium-fine sand (0.125 – 0.5 mm) and organic matter content were strongly correlated with high densities (up to 25 m^{-2}) of *L. appendix* (Beamish & Lowartz, 1995) and *Ichthyomyzon fossor* ammocoetes (Yap & Bowen, 1998), both non-parasitic species from North America. Similarly, Potter *et al.* (1986) found organic matter content to be correlated with the density of parasitic *G. australis* in an Australian stream. The presence of fine to more course particle sizes (0.05 – 1.94 mm) has been shown to explain *Lampetra* spp. ammocoete densities (Kainua & Valtonen, 1980; Kelso, 1993; Goodwin *et al*., 2008) as well as freshwater-resident *P. marinus* (Young *et al*., 1990).

Not all studies agree however as Mundahl *et al.* (2006) did not find patterns of larval density of *L. appendix* correlated with sediment particle size, and Malmqvist (1980) found that organic matter content could not predict *L. planeri* ammocoete density in a Swedish stream, instead chlorophyll *a* concentration was more important. Water depth was not found to be important by Beamish & Lowartz (1995), but their study sampled at sites < 30 cm deep, while Potter *et al.* (1986) found the density of *G. australis* ammocoetes to be negatively correlated with depth. Mundahl *et al.* (2006) found that the density of larval *L. appendix* did not correlate with water depth, current velocity or organic content. The importance of shade in predicting larval densities is similarly variable; where Malmqvist (1980) did not find it to be a useful predictor, yet Potter *et al.* (1986) and Waterstraat & Krappe (1998) did.

Densities also vary across larger spatial scales, and in general will tend to increase in areas lower in catchments (Fluri & Beamish, 1991; Waterstraat & Krappe, 1998; Torgersen & Close, 2004; Goodwin *et al*., 2008; Gruber *et al*., 2012). This is possibly due to the increased likelihood of finding suitable habitat in these areas as a result of reduced flow rates, and also a result of continued downstream drift of ammocoetes (Sjöberg 1980; Kainua & Valtonen, 1988; Ojutkangas & Jussila, 1988). A dramatic example of the importance of lower reaches of rivers to ammocoetes was evidenced by the drastic decline in anadromous *L. fluviatilis* ammocoete abundance in a Finnish system, where river regulation disrupted the flow rates and reduced the larval population from an estimated 1.4 million individuals to 6000 in the eleven years between 1982 and 1993 (Ojutkangas *et al*., 1995). At broader regional scales pH (Goodwin *et al*., 2008) and water depth (Torgersen & Close, 2004) were found to be the most important variables in explaining variation in larval density for the anadromous parasitic species *L. fluviatilis* and *E. tridentatus* respectively.

Larval density is often greater at a given site when a wide range of ammocoete sizes are present, particularly where small larvae are abundant (Beamish & Lowartz, 1995). As larval densities have been shown to be greater in areas of well-sorted sediments, it is possible that these areas provide better conditions for burrowing for a range of ammocoete body sizes. Smaller individuals are often associated with finer sediments (Almeida & Quintella, 2002; Sugiyama & Goto, 2002), as small ammocoetes have difficulty in burrowing into coarse substrates (Quintella *et al*., 2007). An interesting effect of larval density is that it may alter sex ratio in local populations, as the proportion of males has been found to increase significantly with relative density in some streams (Docker & Beamish, 1994), and as a result could impact on recruitment to the adult population (Hardisty, 1961). However, as there are no consistent sex-specific differences in the size of larvae at the time of gonadal differentiation among different sites, other environmental factors likely have a modifying effect (Docker & Beamish, 1994). Density does not have a significant impact on the proportion of ammocoetes that undergo metamorphosis (Morman, 1987; Holmes & Youson, 1997), but it can impact on their overall rate of growth (Mallat, 1983).

1.2.5 *Movement*

It is evident in most systems that ammocoetes are distributed along the length of rivers in a non-random pattern (Stone & Barndt, 2005; Sullivan *et al*., 2008; Neeson *et al*., 2011), largely as a response to spawning typically occurring in the upper reaches of rivers. But there is some indication that in the period immediately following dispersal from the nest, ammocoetes are positively attracted to pheromones, particularly petromyzonol sulfate (Zielinski 1996*a*), which signals the ammocoete to settle in areas colonised by conspecifics of a similar age (Zielinski 1996*b*). It is probable though that most of this distribution is explained by passive downstream movement, particularly during periods of flooding

(Hardisty, 1944; Manion & McLain, 1971; Manion & Smith, 1978), but also during times of reduced flow (Hardisty & Potter, 1971). Increasing density and physical disturbance of larval habitat have also been implicated in mass downstream movement of ammocoetes (Manion & Smith, 1978). Estimates of downstream drifting suggest ammocoetes travel at similar speeds to other larval fishes in rivers (White & Harvey, 2003), although it is likely ammocoetes move much more slowly in time through the system as they regularly stop to burrow and feed (Manion & McLain, 1971; Manion & Smith, 1978). In one tributary of Lake Erie, North America ammocoetes of freshwater-resident *P. marinus* were estimated to travel less than 3.5 km per year (Morman, 1979).

There is a tendency towards gradation in the size of ammocoetes distributed throughout a river system, with larger, older ammocoetes predominately found in downstream regions (Leach, 1940; Hardisty, 1944; Baxter, 1954; Potter, 1970; Hardisty & Potter, 1980; Waterstraat & Krappe, 1998). Although *G. australis* populations in the lower stream reaches of New Zealand included very small to the largest ammocoetes (Potter *et al*., 1986; Todd & Kelso, 1993) this is likely due to the short lengths and steep gradients in these stream systems. This relationship between river length and ammocoete size may be confused by the presence of multiple spawning sites at differing distances from the mouth of the river. Ammocoetes may however actively seek out new habitat (Potter, 1980; Smith *et al*., 2011), although for what purpose is not clear as many ammocoetes remain in the same patch for several years (Manion & McLain, 1971). Despite the poor active swimming ability of ammocoetes in comparison with other larval fishes (Sutphin & Hueth, 2010) it has been suggested that ammocoetes adopt a free swimming mode during the hours of darkness (Enequist, 1937; Kelso, 1993), and this is likely reflected in the greater catches of ammocoetes from traps at night (Manion & McLain, 1971).

1.2.6 *Diet*

Ammocoetes have variously been described as being a microphagous filter-, suspension-, detritus- or deposit-feeder, with their diet consisting mostly of single-celled plants and animals, and a variety of organic detritus (Moore & Potter, 1976; Sutton & Bowen, 1994; Yap & Bowen, 1998). Hardisty (2006) suggested that smaller ammocoetes are filter-feeders, collecting edible particles from the water column as they respire, while larger ammocoetes rely more on grazing from the sediment surface. Larger individuals are likely to collect higher concentrations of food by feeding in this manner, as opposed to the more passive method of filter-feeding. Recently settled freshwater-resident *P. marinus* ammocoetes begin to feed at sizes 7 – 8 mm (Manion & McLain, 1971). Ammocoetes collect food particles by creating a feeding current with the velar apparatus, which simultaneously supplies oxygen to the gills, trapping particles on a mucus membrane within the oral hood, then passed periodically to the gut in the form of a string for digestion (Randall, 1971; Mallatt, 1981; Yap & Bowen, 1998). This system has one notable drawback in that when an ammocoete's metabolism slows down during cold temperatures, for example in winter months, the demand for oxygen is similarly reduced. As a result, the rate of feeding follows an annual cycle, slowing in the winter. The origins of the mucus produced during feeding are not clear, having been attributed to cells in the gills or pharynx, and even the endostyle (Mallatt, 1981).

The pumping rate of the ammocoete varies around 3 – 13 ml g^{-1} body weight hr^{-1} and suggests they are capable of pumping a volume of water 10 – 20 times their body weight per hour (Malmqvist & Brönmark, 1981). Experimental work has estimated that ammocoetes are able to capture up to 80% of food particles (yeast cells) presented in an enclosed through-flow chamber (Hardisty, 2006) indicating it is an efficient strategy. As larvae are limited only by size in terms of the particles they can capture (< 0.3 mm) the ammocoete gut has been found to contain almost all major groups of microscopic organisms in streams and rivers, but bacterial and algal groups tend to dominate (Manion, 1967; Moore & Beamish, 1973; Sutton & Bowen, 1994). Diatoms in particular appear to be the most numerous cells identified in ammocoete gut contents, followed by desmids and blue-green algae (Manion & McLain, 1971; Bowen, 2003; Mundahl *et al*., 2005). Protozoans appear to be of less importance to the ammocoete diet, although this is likely to be obscured given that they are more readily digestible than algal cells. Rotifers and cladocerans have occasionally been recorded, but these are thought to be accidentally ingested or to have entered through the gill pores (Hardisty, 2006). By volume however, organic detritus would appear to be the most important element in the ammocoete diet, especially in winter months (Sutton & Bowen, 1994; Hollet, 1995; Bowen, 2003; Mundahl *et al*., 2005).

1.2.7 *Growth*

The growth of ammocoetes throughout the larval period does not follow a linear trajectory; instead, maximum growth is achieved in the first two years, followed by a reduction in growth rate as the ammocoete increases in length, but increasing once again in the final stages (Hardisty, 1944; Manion & McLain, 1971; Beamish & Austin, 1985; Quintella *et al*., 2003). Ammocoetes also appear to undertake what has been termed a "resting phase" in the final year of larval life, where growth ceases altogether in a portion of the population (Leach, 1940; Applegate, 1950; Potter, 1970; Lowe *et al*., 2003). Yet during this period greater levels of lipids are stored than at any other point in larval life (Lowe *et al*., 1973; Potter, 1980). The reason for a lack of growth by older, therefore larger, ammocoetes could be seen as reduction in intestinal surface area relative to body weight as the ammocoete grows, therefore limiting the efficiency with which the ammocoete is able to assimilate any captured food particles. In addition to this there is also evidence to suggest ammocoetes reduce in length during winter months (Thomas, 1962; Malmqvist, 1983; Beamish & Austin, 1985), and may be the result of reduced digestive efficiency at low temperatures (Moore & Potter, 1976). The assimilation efficiency of food entering the ammocoete gut has been estimated as being 72% between May – October, and 53% between November – March (Sutton & Bowen, 1994), although a seasonal effect is not apparent between spring and summer (Mundahl *et al*., 2005). However, growth rates for *Ichthyomyzon gagei* were found to be constant despite seasonal temperatures varying 8.5 to 26°C (Beamish, 1982).

Differences in growth rates between sexes are common in some populations, and have been noted in *P. marinus* (Applegate & Thomas, 1965), *Lampetra* spp. (Bird & Potter, 1979; Malmqvist, 1980), and *I. fossor* (Purvis, 1970). But sex specific differences were not noted among ammocoetes of *I. gagei* (Beamish, 1982) or *Ichthyomyzon greeleyi* (Beamish & Austin, 1985), and in *Lampetra aepyptera* these differences varied among streams and age classes (Docker & Beamish, 1994). Experimental work suggests that ammocoete growth rate is higher where filter-feeding bivalves are present nearby or within larval habitats (Limm & Power, 2011), or caddis-fly larvae (Trichoptera) are added to ammocoete holding tanks (Allegret *et al*., 1977). These results are likely caused by the concentration and deposition of organic-rich material by these organisms into surrounding sediments and may act to increase ammocoete growth in localised areas, leading to differential growth rates among streams (Purvis, 1979).

The relationship between density and the growth rate of ammocoetes is complex, and appears to be dependent on the type of study employed in testing its effect. Growth is density dependent when studied in aquaria and enclosures (Hanson *et al*., 1974; Mallatt, 1983; Morman, 1987; Murdoch *et al*., 1992) but inconclusive in field trials (Manion & McLain, 1971; Purvis, 1979; Jones *et al*., 2001). It is likely that as closed systems prevent ammocoete dispersal away from areas of high density, that any effects are being confounded and possibly amplified by the study design (Jones & Derosier, 2001). Mallat (1983) for example showed that ammocoetes of *E. tridentatus* could be successfully grown on a diet of yeast cells at a range of temperatures (4 – 16 °C), but that density within experimental aquaria had a negative effect on growth even when food concentration remained constant. Similarly, Malmqvist (1983) suggested that *L. planeri* ammocoetes held in cages within a stream exhibited slower growth at higher densities, as did Morman (1987) who tested freshwater-resident *P. marinus* ammocoetes. A population of freshwater-resident *P. marinus* within the middle sections of a large experimental stream, but not held in cages, was shown to exhibit depressed growth as a result of downstream drift being offset by recruitment from upstream sections (Manion & McLain, 1971). Ammocoetes of *L. aepyptera* were found to show modal reductions in length and weight within several streams, but only for particular age classes (Docker & Beamish, 1994), suggesting density-dependent effects may be limited to individuals of a particular size.

These findings are hard to explain given that food is unlikely to be a limiting factor within streams (Moore, 1972; Moore & Beamish, 1973), and so it has been suggested ammocoetes may excrete growth inhibitors into the immediate environment surrounding burrows (Mallat, 1983). Swink (1995) tested this theory explicitly by exposing control subjects held at a variety of densities to water conditioned by ammocoetes held in extremely high densities (equivalent to 800 m^{-2}) throughout a protracted period, and found that within a given population larval growth did not differ between the test or control groups. This would suggest there is no evidence for a waterborne growth inhibitor. Weise & Pajos (1998) suggested that there may be intra-specific competition between younger age classes and any that attempt to colonise in later years, although there does not appear to be any evidence supporting inter-specific competition between larvae from different species (Lamsa *et al*., 1980; Murdoch *et al*., 1991).

1.2.8 *Larval Duration*

The ability to accurately estimate ages of ammocoetes within a given population, and therefore make some assumptions as to the duration of the larval period, is hampered by the lack of a suitable alternative technique to length-frequency distributions. This methodology relies on a restricted and well-defined spawning period, which although certainly true for lampreys; it also assumes that the progeny of any given year will be well separated in size from ammocoetes hatching in any other year. However, such differences in size between these subsequent age classes depend on the vagaries of growth for each individual and consistency with cohorts, and this technique is hampered particularly in older age classes that may overlap in length. It also requires a large sample size (Holmes, 1991), which includes representatives from all possible age classes that may be well separated in space due to larval drift. The use of this technique can lead to wide-ranging estimates of larval life in some species, particularly for non-parasitic brook lampreys, which may have a larval period of between 3.5 and 6.5 years depending on geographical location (Beamish & Medland, 1993).

There have been attempts to validate length-frequency curves by ageing individual ammocoetes using the only mineralised part of their bodies, the statoliths, located within the inner-ear. The statoliths are small structures (350 µm) composed of calcium phosphate that is deposited at the base of the structure as the ammocoete grows (Brothers, 2003; Avallone *et al*., 2007). Once removed, they can be mounted in oil and examined for the presence of annuli (growth rings). These appear as dark bands during the winter when growth is depressed and as opaque broader bands during rapid periods of growth in warmer months (Volk, 1984). This can lead to difficulty where winters are mild, as growth will be more or less continuous and annuli may not develop, such as experienced by *I. gagei* (Beamish & Medland, 1988). The presence of annuli also appears to be correlated with ambient calcium ion concentration, particularly during periods of growth, and can lead to ambiguity in the estimation of ammocoete age (Barker *et al*., 1998). Growth rate appears to have a strong impact on the applicability of this technique, as in populations experiencing rapid or slow growth, the use of statoliths both over- and underestimated the ages of individuals (Dawson *et al*., 2009). In other trials it has however proven to be a useful technique, accurately assigning age classes to a variety of fast and slow growing ammocoetes of various lengths for freshwater-resident *P. marinus* (Beamish, 1987; Holmes, 1991).

In non-parasitic *L. planeri* populations larval duration was believed to be 3.5 – 4.5 years in English streams (Hardisty, 1944), but in other studies this was re-estimated as between 4.5 – 6.5 years (Hardisty, 1961), with most individuals remaining as ammocoetes for 6.5 years (Hardisty & Huggins, 1970). In other non-parasitic species, such as *Entosphenus hubbsi* and *L. appendix*, larval duration is also typically 5.5 – 6.5 years (Potter & Bailey, 1972; Seagle & Nagel, 1982). In English rivers anadromous *L. fluviatilis* are believed to have a larval duration of 3.5 – 4.5 years (Hardisty & Huggins, 1970). Anadromous *P. marinus* populations also from the U.K. have larval durations of approximately five years (Hardisty, 1969) but more southerly populations in Portugal remain as ammocoetes for just four years (Quintella *et al*., 2003) suggesting a latitudinal trend may exist. However, freshwater-resident *P. marinus* populations from the Laurentian Great Lakes average six years (Lowe *et al*., 1973), yet this may be as high as 19 years for some individuals (Manion & Smith, 1978).

In some ammocoete populations there are individuals that have longer larval durations than their cohorts but similar lengths, indicating the existence of a resting phase prior to metamorphosis, and during which subsequent growth is halted but lipid is accumulated (Leach, 1940). This has been suggested as occurring in a wide range of species, including: *Ichthyomyzon* spp. (Potter & Bailey, 1972; Beamish, 1982), *Lampetra* spp. (Hardisty & Huggins, 1970); *P. marinus* (Lowe *et al*., 1973) and *Mordacia* spp. (Potter, 1970). There does not appear to be evidence of a resting phase in all individuals, as freshwater-resident *P. marinus* larvae can remain at the same size, or fluctuate up or down in length, for as long as five additional years after their cohorts have metamorphosed (Manion & Smith, 1978).

1.2.9 *Teratology*

In several species a distinctive "golden form" of ammocoete has been collected, and is assumed to be a case of xanthochroism, a genetic abnormality resulting in an unusually yellow phenotype. These include: *L. planeri* (Zanandrea, 1961; Maitland *et al*., 1994), *L. richardsoni* (Pletcher, 1963), *Lethenteron zanandreai* (Zanandrea, 1961), *L. appendix, Ichthyomyzon* spp. and *P. marinus* (Manion, 1972). This may be what is referred to by Vladykov (1960) as "light colour phase" ammocoetes, and what Beamish & Medland (1988) referred to when they mentioned < 1% of the *I. greeleyi* population "exhibited very little pigmentation". Around 6% of *P. marinus* ammocoetes examined from Big Garlic River in North America were described as being "yellowish" (Manion & McLain, 1971) where the melanocytes were small with poorly developed processes. Melanistic larvae ranging from blue to a deep black have also been described in *L. zanandreai* (Zanandrea, 1956).

Ammocoetes with additional tails have been described in *P. marinus, L. appendix, Ichthyomyzon* spp. and *L. planeri* (Manion, 1967; Bird & Potter, 1979). Several normal sets of twins have been produced from *P. marinus* eggs that followed a typical developmental trajectory and were not conjoined (Hanson, 1985). An isolated case of neoteny was described from a small collection of *L. zanandreai* ammocoetes from the Fibbio River, Italy where a single ammocoete contained fully developed eggs and was said to have secondary sexual characteristics, while eleven other individuals had visible but less mature eggs (Zanandrea, 1956). It seems apparent from the site description of this river (Zanandrea, 1956) that this was the result of industrial pollutants entering the system from a nearby tannery. Neoteny has also been suggested to occur in *I. fossor* (Leach, 1940), *L. aepyptera* (Vladykov & Kott, 1978), *L. planeri* (Hardisty & Potter, 1971*a*) and *Entosphenus lethophagus* (Hubbs, 1971) but all cases have been disputed by V. D. Vladykov who cited a lack of evidence (Vladykov, 1985).

1.3 METAMORPHOSIS

The phenomenon of metamorphosis, whereby the phenotype of an organism becomes reorganised out-with the embryonic stage, is typically a response to a new mode of life. The difference in structure and function of the ammocoete body plan compared with that required for an active and sometimes parasitic adult stage cannot be bridged by a gradual transition, and instead requires an extensive and prolonged period of metamorphosis. Adult structures originate from clusters of stem-cells activated to begin their designated developmental pathways by the presence of hormones circulated throughout the ammocoete’s body. The changes that take place during metamorphosis could have taken place within the egg of lamprey ancestors, leading to a direct developmental trajectory such as seen in the marine hagfishes. In this light it could be suggested that lamprey metamorphosis represents the continuation of an interrupted embryonic development, during which time the ammocoete stage grows larger but not does appreciably alter its gross morphology (Leach, 1944).

Ammocoetes can be seen to be an adaptation to a purely freshwater existence, and so changes in the function of the kidneys, skin and gills as well as to the gut, are required during the transition to the migratory adult stage if they are to enter the marine environment (Hardisty, 1956; Beamish *et al.*, 1978). Modifications to the respiratory system allow the adult lamprey to make better use of well-oxygenated water, and involve changes to the heart and circulatory system as well altering the type of haemoglobin contained in red blood cells (Percy & Potter, 1988). Extensive modifications to the architecture and musculature of the head enables the development of the oral disc and subsequent development of the tongue, teeth and salivary glands able to secrete enzymes used in the digestion of tissues.

1.3.1 *Anatomical Reorganisation*

Some authors refer to lampreys during this period in their life cycle as either macrophthalmia or transformers, but the general process is common to all species where it has been described to date, including *P. marinus, Lampetra* spp., *Lethenteron* spp., *Ichthyomyzon* spp., *Entosphenus* spp. and *G. australis*. Much of what happens externally can be characterised in six stages, each containing relatively well defined morphological alterations to the ammocoete body plan (Leach, 1940; Manion & Stauffer, 1970; Bird & Potter, 1979; Potter *et al*., 1980; Bird *et al*., 1982; Beamish & Medland, 1988).

In Stage 1 a darkening of the patch of skin on the ammocoete head, below which lies the eye, becomes more elliptical and a depression appears at this site. At the same time the lips of the oral hood thicken and the branching of the fibres within the oral hood itself becomes less complex. The lower lip then contracts in Stage 2 and the mouth appears smaller as the lips continue to thicken, while the head narrows and a distinctive bulge forms anterior to the branchial region. In Stage 3 the head and anterior branchial region shrinks further as cartilage is lost, and the gill pores begin to appear rounded. The lips join up creating a circle around the mouth, forming the oral disc in Stage 4. Most of the fibrous mesh will have disappeared and the tongue may begin to become visible, and the eyes will be well developed. Stage 5 is characterised by the protrusion of the eyes and the appearance of tooth bearing plates (lamina) on the oral disc. The branchial region will also lose the groove typical of ammocoetes. The teeth will erupt in Stage 6, and the ammocoete colouration will be replaced by a more silvery appearance.

Internal changes appear to be far more variable in their timing. For example during Stage 5, when much of the reorganisation of the gills has taken place, the blood will contain a mixture of adult and larval haemoglobins. This is no doubt a consequence of the need to maintain a minimal level of respiration throughout the restructuring of the branchial regions, and a switch to tidal breathing once the velum stops beating. But changes to structures involved in feeding occur more slowly, such as the tongue and lamina, which do not develop until Stages 5 & 6. The adult foregut, a newly developed structure in metamorphosing individuals, joins the mouth to the intestine. This forms during Stage 3 when a cylindrical mass of cells forms above the ammocoete pharynx and gradually becomes hollow to produce a tube (Leach, 1940; Hilliard *et al.*, 1983). The timing of the completion of this step varies among and within species, and can take 3.5 – 10 months in *L. fluviatilis*. The diameter and surface area of the intestine rapidly increases during Stages 5 & 6, which acts to increase the surface area available for the absorption of food (Hilliard *et al.*, 1983). During metamorphosis feeding does not take place (Youson, 2003). The degeneration of the larval endostyle presumably begins very early in metamorphosis, or even during the ammocoete resting phase (Leach, 1940), and is well advanced by Stage 3. It is no longer apparent at Stage 6, and the adult thyroid, which develops concomitantly with the destruction of the endostyle, is fully formed by the completion of Stage 6.

1.3.2 *Timing & Causes*

For the majority of species, metamorphosis begins in the summer months (Appendix 1.3), such as in anadromous *L. fluviatilis* and non-parasitic *L. planeri* from the U.K., where the earliest metamorphosing stages are likely to be found in June and July. It takes between two and three months for gross anatomical and physiological changes to take place. Interestingly, it is those species living at extreme latitudes that tend to deviate from the usual summer time period. *Tetrapleurodon* spp., freshwater lampreys that inhabit the Mexican highlands at the southern extreme of the Petromyzontidae range, can begin metamorphosis as early as April. A general trend seems to indicate that the onset of metamorphosis is correlated with latitude, where more northerly latitudes result in an earlier onset (Bird & Potter, 1979; Beamish & Austin, 1985; Potter, 1980). For example, *Caspiomyzon wagneri* begins transforming in July at 47°, August - September at 42° and October - December at 35° latitude (Vladykov *et al.*, 1986). Within ammocoete populations the onset of metamorphosis appears to be highly synchronised, with the majority of individuals attaining the same Stage at similar times. In the non-parasitic *I. gagei* over 70% of individuals were in Stage 1 when collected on September 4-5th, and five days later 80% had entered Stage 2 (Beamish, 1982). Synchronicity in localised populations would suggest then that whatever the signal determining the onset of metamorphosis it acts equally on all individuals that are susceptible to it, in terms of their age, size and lipid content (Potter, 1970).

As photoperiod is strongly correlated with latitude, as opposed to temperature which may be affected by altitude, water depth, etc., it may be that day length has a stronger influence on the timing of metamorphosis than other factors. Experimental ammocoetes that were expected to begin metamorphosis, but had their pineal glands removed in the months prior to onset, did not transform (Cole & Youson, 1981). It could be that temperature triggers the activation of the pineal gland-hormonal axis causing the onset of metamorphosis during warmer months, and co-ordinating all individuals receptive to hormonal cues from the pineal, and exposed to those temperatures, to begin transformation during the same period (Potter, 1970; Purvis, 1980; Cole & Youson, 1981; Holmes *et al*., 1994). This would help to explain apparently counter-intuitive experimental results from ammocoetes kept at different, but constant, temperatures and during which earlier metamorphosis occurred in populations maintained at higher temperatures (Moore & Potter, 1970; Potter, 1980; Holmes *et al*., 1994). Although these data appear to contrast the effects of latitude, it would suggest that metamorphosis is initiated by a rise in temperature as opposed to being triggered by an absolute value (Holmes & Youson, 1994, 1997). For example, ammocoete populations that are exposed to low winter temperatures (i.e., at high latitudes) will be likely to respond to a slight rise in water temperature during early spring. While populations experiencing mild winters (i.e., at low latitudes) will require a greater rise of temperature typically seen in summer. This could help explain the apparent effect of latitude on the onset of metamorphosis within a single wide-ranging species.

Given the importance of thyroid hormones to the metamorphosis of tadpoles into frogs, which superficially resembles the transformation of the ammocoete stage to adult lamprey (Hardisty, 2006), it was anticipated that they would also play a key role in the transformation of petromyzontids (Horton, 1933; Leach, 1944). But it is not easy to deduce the role of thyroid hormones in lamprey metamorphosis given that they are present at high concentrations in ammocoete blood (Wright & Youson, 1977). These concentrations vary throughout the year, but peak immediately prior to the onset of metamorphosis (Leatherland *et al*., 1990*b*). Additionally, it is the endostyle that produces thyroid hormones, but which is itself replaced by the adult thyroid gland during transformation. When Stage 1 of metamorphosis begins thyroid hormone concentration is below that in the period prior to onset, declining rapidly and remaining low thereafter (Wright & Youson, 1977; Holmes & Youson, 1993; Holmes *et al*., 1994). As the endostyle degenerates rapidly at onset, it may well be the case that as its production of thyroid hormones decreases during metamorphosis, the endostyle itself maintains the ammocoete body plan for many years through its continuous output of thyroid hormones. The application of a goitrogen (potassium perchlorate), that acts to inhibit thyroid hormone production, was seen to induce metamorphosis in *P. marinus* ammocoetes that were not expected to undergo transformation (Holmes & Youson, 1993), indicating the thyroid hormones play some role in the maintenance of the ammocoete body plan.

A key indicator of impending metamorphosis in individual ammocoetes is the amount of lipid that they have stored during their final year, and which will subsequently be mobilised after environmental and hormonal cues have triggered the onset of transformation (Youson & Holmes, 1993; Youson, 2003). In freshwater-resident *P. marinus* this threshold level is indicated by a condition factor of > 1.5, which equates to a length of at least 120 mm and a weight of 3 g (Youson *et al*., 1993; Zerrenner & Marsden, 2001). This condition factor will decline throughout the period of metamorphosis (Potter *et al*., 1978; Potter *et al*., 1980) indicating that lipids are being consumed as the adult body plan develops.

For those species that go on to feed parasitically there is a period of starvation following the completion of metamorphosis that may last six months or more (Potter & Beamish, 1977), and during which they largely depend on the lipids stored during the final year of the larval period (Lowe *et al*., 1973). Lipid is deposited in a step-wise fashion as the ammocoete ages, increasing in the spring and summer, so that a 2+ ammocoete will have a greater lipid content than a 1+ individual, 3+ more than 2+, and so on. Individuals about to undergo metamorphosis may have lipid content comprising up to 17% of their wet weight (Hardisty, 2006). Despite the differences in size at which metamorphosis occurs, non-parasitic *L. planeri*, and parasitic *L. fluviatilis* and *P. marinus* all have an average fat content of 13.5 – 14.5% prior to transformation (Lowe *et al*., 1973; Moore & Potter, 1973). Following completion of metamorphosis the lipid content of *L. fluviatilis* and *L. planeri* is approximately 8% (Moore & Potter, 1976*b*) but in *P. marinus* may be as low as 1.3% (Beamish *et al*., 1979).

Between species there is great variability in the size at which metamorphosis begins. Some species, such as the parasitic *L. fluviatilis*, begin transforming at very narrow size ranges e.g., 97 – 103 mm from four separate Welsh rivers (Hardisty, 2006), while sizes of non-parasitic *L. planeri* transformers from elsewhere in the U.K. can range 100 – 165 mm

(Potter & Huggins, 1973; Bird & Potter, 1979) at onset. This is likely the result of the greater variability in the age structure of *L. planeri* ammocoetes compared with *L. fluviatilis*.

1.3.3 *Migration*

Metamorphosing individuals begin to move away from the typical ammocoete habitats into mid-channel areas that tend to have higher flow-rates and coarser sediments (Beamish, 1980; Beamish & Medland, 1988; Kelso & Todd, 1993). This is likely in response to a greater oxygen requirement during the reorganisation of the respiratory system (Galloway *et al*., 1987). In general, the downstream migration of recently transformed lampreys extends for several months, and for those species such as *P. marinus* that begin metamorphosis in the summer, movement away from larval habitats will begin in autumn and may carry on through the entire winter depending on prevailing conditions (Applegate, 1950; Bird & Potter, 1979). Similar to the patterns of movement exhibited by the ammocoete population, newly metamorphosed lampreys sometimes drift passively with the current, although they are capable of short bursts of active swimming (Dauble *et al*., 2006). The greater free-swimming ability of these newly transformed lampreys, and a reduced burrowing behaviour, results in a more rapid transport downstream (Manion & Smith, 1978).

Migration peaks during periods of increased flow (Applegate, 1961; Potter, 1980) yet still occurs mostly at night (Potter & Huggins, 1973; Potter *et al*., 1980; Dauble *et al*., 2006). Freshwater-resident *P. marinus* from the Laurentian Great Lakes that have completed metamorphosis are approximately 140 mm in length (Applegate, 1950; Applegate, 1961); while anadromous *L. fluviatilis* may be as small as 80 mm (Maitland *et al*., 1984). In an apparently extreme case, the anadromous parasitic Western river lamprey *Lampetra ayresii* is said to leave rivers of British Columbia at sizes ranging from 40 – 190 mm (Beamish, 1980).

For parasitic species, the time at which post-metamorphic migration begins is limited by the time it takes for the foregut to become patent. This is especially important for those species that will go on to enter the marine environment as they will begin swallowing sea water, excreting the excess salts from chloride cells located in the gills (Beamish, 1980; Bartels & Potter, 2004). The gills of adult petromyzontids, as in other fishes, play an important role in osmoregulation. The gills of ammocoetes of both parasitic and non-parasitic lampreys contain ammocoete mitochondrion-rich cells (AMRCs), intercalated mitochondrion-rich cells (IMRCs) and pavement cells (Bartels & Potter, 2004). In anadromous parasitic species i.e., those that will feed at sea, AMRCs are lost during metamorphosis and chloride cells develop, which are then subsequently lost during the return freshwater migration to spawn (Morris & Pickering, 1976). Essentially, these cells are restricted to the marine stage of these species' life-cycle, and are believed to play a role in secreting excess salts from the body (Bartels & Potter, 1991). However, in recently metamorphosed *L. appendix*, a non-parasitic lamprey, chloride cells were also found in the gill epithelium (Bartels *et al*., 2011). The development of these cells in a lamprey that will never enter a marine environment indicates the retention of a cell type that would be present in an ancestral lamprey species.

Elsewhere, freshwater-resident parasitic *P. marinus* in the Laurentian Great Lakes continue to develop chloride cells following metamorphosis (Youson & Freeman, 1976) despite residency in Lake Ontario for at least 10,000 years (Bryan *et al*., 2005). This makes the retention of chloride cells in *L. appendix* even more striking given that molecular evidence suggests this species split from the anadromous parasitic *Lethenteron camtschaticum* > 130,000 years ago (Docker *et al*., 2009; Lang *et al*., 2009). This could explain the findings of Hardisty (1956) and Holmes *et al.* (1999) who discovered that during metamorphosis *L. planeri*, a recently derived (< 10,000 years) non-parasitic species from the anadromous *L. fluviatilis*, was able to osmoregulate in up to 70% seawater for a short time.

1.4 POST-METAMORPHIC FEEDING

Following the completion of metamorphosis, 18 species of lamprey are known to begin feeding in a more directed and active manner in comparison to the sedentary life of the ammocoete stage. Such is the diversity of life histories amongst these species it is difficult to encapsulate this variation in all-encompassing phrases, as many species are known to exhibit a wide range of foraging strategies and behaviours. However, in broad terms nine of these species feed exclusively in freshwater, while the other half feed in marine or brackish water. Of the nine that can feed at sea, at least four species are known to contain populations restricted to freshwater. One other species of lamprey that feeds following metamorphosis (*Eudontomyzon* sp. n "migratory Black Sea lamprey") is believed to be extinct, and no museum material can be located (Kottelat *et al*., 2005; Naseka & Diripasko, 2008). For those newly metamorphosed lampreys that will go on to feed, as opposed to non-parasitic lampreys that do not, they are referred to as juveniles during this period of their life cycle as they have still not matured sexually.

Juvenile lampreys employ four modes of feeding: blood-feeding, tissue-feeding, blood/tissue-feeding and carrion-feeding (scavenger). Blood-feeding is the ancestral mode for modern-day petromyzontids, and tissue-feeding is a more recently derived strategy (Renaud *et al*., 2009). Scavenging probably played some part in the development of a more predatory mode of life, although its evolutionary origins and continued existence remain unclear. Some species that feed on tissue continue to consume their prey after it has died, such as *L. ayresii* (Beamish, 1980) and *Entosphenus minimus* (Bond & Kan, 1973) so perhaps scavenging was a common strategy in ancestral petromyzontids. Additionally, several species have been recorded cannibalising other lampreys; such as *E. minimus* (Kan & Bond, 1981); *P. marinus* (Davis, 1967); and *L. ayresii* that feeds on conspecifics as well as *E. tridentatus* (Beamish, 1980).

These different diets are reflected in the morphology of the head region, especially the arrangement of teeth on the oral disc and tongue, and the relative size of the buccal glands (Potter & Hilliard, 1987; Renaud *et al*., 2009). These features can be used to crudely separate petromyzontids at the generic level (Gill *et al*., 2003), where; *Ichthyomyzon, Petromyzon* and *Mordacia* feed on blood; *Eudontomyzon, Lampetra, Lethenteron* and *Geotria* feed on tissue; and *Entosphenus* and *Tetrapleurodon* consume both blood and tissue. The monotypic *Caspiomyzon wagneri* is a presumed scavenger as no animal material has been detected in its gut contents, although their parasites have (Renaud, 2011). It lacks the dentition typical of those species that feed on tissue, and no host species have been identified. In general terms, blood-feeding species exhibit the highest number of teeth on the oral disc, almost completely covering the surface. They also have high numbers of small projections known as oral fimbriae and papillae surrounding the disc, and large buccal glands that deliver saliva to the mouth. This saliva contains a powerful mixture of proteolytic enzymes and anticoagulants known as lamphredin that keeps the blood meal flowing. In addition, the oral papillae are innervated and so potentially play some role in detecting a suitable site for the attachment of the juvenile lamprey, such as near a large vein.

Tissue-feeding species exhibit reduced numbers of teeth on the oral disc, but have a prominent cusp located on the tongue. They possess fewer oral papillae than blood feeders and smaller buccal glands, but the velar apparatus bears many tentacles that are longer than in blood-feeding species. It seems likely that these play some role in preventing particles of tissue, scraped off by the large cusp on the tongue, from entering the branchial chambers. Those species that feed on both blood and tissue exhibit some mixture of these characteristics and indicate a transitional status from the ancestral blood-feeding parasitic mode, to the more recently derived tissue-feeding predatory mode. *Caspiomyzon wagneri* has many blunt teeth on both the oral disc and tongue, yet it has moderately large buccal glands, which could compensate for a reduced ability to remove larger pieces of tissue. *Lampetra ayresii*, which has the dentition typical of tissue-feeding petromyzontids, is said to be attracted to the dead bait of recreational fishermen, where they consume large amounts of flesh before it can be retrieved (Beamish, 1980), and so perhaps scavenging is a mode of feeding open to all lampreys that feed following metamorphosis.

1.4.1 *Feeding Location on Hosts*

The location on the body of prey species, to which juvenile lampreys attach and begin feeding (Gradwell, 1972; Hilliard *et al*., 1985), is likely to indicate the relative importance of those regions to that species' nutritional requirements. For example, in a generalist tissue feeder such as *L. ayresii* they may consume the entire body of their prey, excepting the head and tail, particularly if the prey species is < 150 mm long (Beamish, 1980). However, on larger prey species they will mostly attach to the anterior-dorsal region and create deep, rounded wounds. *Entosphenus tridentatus*, which consumes both blood and tissue, tends to attach in the anterior-ventral region, creating holes 1 – 3 cm in diameter (Beamish, 1980). In blood-feeding species, such as *P. marinus*, it is assumed that attachment location should correlate with blood availability. Certainly most attachments by this species are sited anterior-ventrally behind the pectoral fins, which would give access to large abdominal veins (Farmer & Beamish, 1973; Cochran, 1986*b*). Bergstedt *et al.* (2001) suggested that such a location was due to a lower amplification of lateral movement by the host that would be less likely to dislodge the lamprey during its feeding period, yet this feeding location is not common to all species of lamprey. Large numbers (10 – 25) of *Ichthyomyzon* spp. have been recovered from the gill cavities of paddlefish *Polyodon spathula*, and these blood-feeding lampreys may be taking advantage of an ample supply of food delivered under pressure, as well as avoiding being dislodged by the regular breaching behaviour of this host species (Cochran & Lyons, 2010).

Lampreys that feed in in deep water, either in marine or lacustrine environments, (e.g., *P. marinus*, *E. tridentatus*) tend to attach ventrally, while those in shallower waters (e.g., *Ichthyomyzon* spp., *L. ayresii*) tend to attach dorsally (Cochran, 1986). It seems likely that in shallow areas this prevents the lampreys from being dislodged by hosts if they scrape along the substrate (Farmer & Beamish, 1973). But, for species such as *L. ayresii* that feeds on tissue rather than blood, site selection on the dorsal aspect of hosts would give them access to greater muscle mass. *Ichthyomyzon* spp. are known to remain attached to, or initiate new attachments, to large hosts such as sturgeon *Acipenser* spp. and paddlefish during winter in North America (Cochran *et al.*, 2003). It is unlikely these lampreys feed during this time; instead they may be taking advantage of the host's avoidance strategies for conditions such as anoxia or ice scour, as well as reducing their own predation risk. When a lamprey initiates feeding it quickly destroys the epidermis and can penetrate into the muscle tissue in anywhere between 4 hours and two days, leaving a wound that can take > 3 months to heal (Kinnunen & Johnson, 1986).

Some lampreys appear to preferentially feed on one side of their host. This lateralisation has been observed in anadromous *E. tridentatus*, which prefers to feed on the left-side of walleye pollock *Theragra chalcogramma*, but the right-side of sockeye salmon *Oncorhynchus nerka* (Beamish, 1980). Anadromous *P. marinus* are also said to feed preferentially on the right-side of salmon in the St. John River system (Potter & Beamish, 1977).

1.4.2 *Duration of Feeding Attachments*

The length of time an individual lamprey remains attached to a host differs widely according to a range of factors, but it is ultimately a direct consequence of the variation in feeding rate between different lamprey species or individuals, the mode of feeding employed, and the ability of the host to survive the interaction (Bence, 2003). The vast majority of such studies refer to freshwater-resident *P. marinus* populations from the Laurentian Great Lakes, not least because of their drastic impact on local fisheries, but also as a consequence of the blood-feeding mode they employ. Such feeding behaviour is equivalent to other host-parasite interactions, and is more easily modelled than the essentially predatory mode of feeding employed by tissue-feeding petromyzontids. In this species the duration of feeding is ultimately limited by the number of days that the host can remain alive for, and most models have assumed host mortality to equate with the cessation of feeding. Using this logic Farmer (1980) calculated that *P. marinus* could remove up to 10% of a host's blood per day, but a feeding rate in excess of this threshold would result in host mortality, and therefore cut short the attachment time. These results indicate a significant negative correlation between the number of days to host death and the percentage of host blood removed each day (Farmer *et al*., 1975). Under experimental conditions, recently metamorphosed *E. tridentatus* that had initiated their first feed attached for seven days before the host died (Richards & Beamish, 1981).

An interesting problem that arises when considering the duration of feeding among adult petromyzontids is the possibility that host availability may alter the foraging strategy of an individual. In this instance if host density is equated with availability, then an experimental design incorporating a varying numbers of hosts, or exposing lampreys to additional hosts during a feeding event, could mimic this effect. No significant differences in attachment times were noted by Cochran & Kitchell (1989) when testing freshwater-resident *P. marinus* with either one or two hosts, but the duration of a second feeding period was shorter if that same lamprey had fed on a previous host. It is not clear what effect lamprey feeding has on the blood chemistry of hosts, and whether or not a host that has been attacked is somehow distasteful to another lamprey (Edsall & Swink, 2001). Additionally, no research seems to have described the effects of appetite or satiation in petromyzontids.

1.4.3 *Host Size*

Lampreys are highly selective in their choice of hosts, particularly in regards to host size. In the most basic terms, a high host:lamprey body weight ratio is more likely to result in host survival (Farmer *et al*., 1975), and therefore would present the lamprey with a greater opportunity to feed before having to locate a new host. Kitchell (1990) estimated that in Lake Michigan lake trout *Salvelinus namaycush*, hosts > 3 kg would be able to resist mortality caused by foraging freshwater-resident *P. marinus*. In contrast to this though, Schneider *et al.* (1996) suggested there was no difference in size between dead lake trout or live specimens with healed lamprey wounds, indicating that host body size is not the only factor influencing survival. The majority of experimental and field studies suggest that larger hosts are attacked more frequently than smaller individuals (Hall & Elliot, 1954; Farmer & Beamish, 1973; Swink, 2003), but that in some systems there is greater relative importance to individual lampreys selecting for host surface area (Cochran, 1985; Swink, 1991), or even host weight (Cochran & Jenkins, 1994). It is not clear whether this apparent selection for individual hosts (based on length, surface area or weight) is in fact avoidance of smaller individuals (Swink, 1991). Certainly some species of lamprey actively forage on very small hosts (Cochran & Jenkins, 1994) as they themselves are diminutive (e.g., *Eudontomyzon danfordi, Tetrapleurodon spadiceus, E. minimus*), and there is some evidence of an ontogenetic shift to larger host sizes as individual lampreys grow (Davis, 1967; Maitland *et al*., 1984; Harvey *et al*., 2006).

1.4.4 *Diversity of Host Species*

A wide range of fishes are preyed upon by lampreys, both in the marine environment and in freshwater. In a broad sense, those species with smaller scales or naked skin are more likely to be fed upon by lampreys compared to those with heavy scales (Cochran, 1994). For example, the majority of petromyzontids appear to favour salmonids or coregonids over percids, even when both are available in high concentrations. However, foraging lampreys will preferentially feed on the most abundant host species when given the choice between salmonids or coregonids (Bence, 2003). Some species of lamprey are capable of exploiting novel hosts, exemplified by the effect of *P. marinus* in the Laurentian Great Lakes, but also where exotic hosts have been introduced into systems already containing native lampreys (Cochran & Jenkins, 1994; Inger *et al*., 2010). Where available some lamprey species will feed on apparently unsuitable or even dangerous hosts, such as *I. unicuspis* that feeds heavily on muskellunge *Esox masquinongy* in the Ottawa River (Renaud, 2002).

Alternative host groups may be exploited if available, although their suitability as a nutritive source remains unproven. There are authenticated reports of *T. spadiceus* attached to manatees *Trichechus manatus* in Mexico (Cochran *et al*., 1996), and *P. marinus* attached to a Greenland shark *Somniosus microcephalus* (Gallant *et al*., 2006), killer whales *Orcinus orca* (Samarra *et al*., 2012), North Atlantic right whales *Eubalaena glacialis* (Nichols & Hamilton, 2004) and minke whales *Balaenoptera acutorostrata* (Nichols & Tscherter, 2011).

1.4.5 *Locating Hosts*

Little direct data exists on the extent of feeding migrations in most lampreys, but it is reasonable to assume that they are carried great distances by their hosts after they attach and commence feeding, thus facilitating their dispersal during the juvenile feeding phase (Nursall & Buchwald, 1972; Moore *et al*., 1974; Marsden *et al*., 2004; Howe *et al*., 2006). For example, anadromous *P. marinus* have been captured up to 400 km from shore (Kelly & King, 2001), while *G. australis* probably travels > 1000 km from its natal streams (Potter *et al*., 1979). Presumably petromyzontids are therefore able to locate and continue to feed on suitable hosts throughout the sometimes lengthy juvenile feeding period. Evidence suggests that *P. marinus* is attracted to host species *via* an olfactory response (Kleerekoper & Mogensen, 1963), and so it would be expected that larger concentrations of hosts are more easily located by foraging lampreys. Indeed, many lampreys seem to prefer feeding on shoaling species, potentially actively associating themselves with prey and feeding on them while the school of fishes moves around. For example, *L. ayresii* is commonly associated with large shoals of Pacific herring *Clupea pallasii* in the Strait of Georgia (Beamish, 1980). In the Laurentian Great Lakes potential host species for freshwater-resident *P. marinus* do not appear to either avoid conspecifics with lampreys attached to them, or attempt to avoid lamprey themselves (Farmer & Beamish, 1973) and this could act to prolong the association with any given shoal.

Different lamprey species forage at a range of depths, and presumably do so in those areas most likely to contain their preferred hosts. Freshwater-resident *P. marinus* in the Laurentian Great Lakes have been recorded feeding at depths ranging from < 1 – 165 m (Applegate, 1950) and anadromous *E. tridentatus* is usually captured at depths of 100 – 250 m (Beamish, 1980) indicating they can utilise a range of host species. *Lampetra ayresii* however has only been recovered in surface waters in the Strait of Georgia, usually < 50 m where it associates with its shallow water hosts (Beamish, 1980; Bond *et al*., 1983). There also appears to be seasonal movement of freshwater-resident *P. marinus* during the juvenile feeding phase, with most individuals feeding in deeper waters in winter and early spring, moving to shallower areas and into bays in late summer, and these lamprey may be tracking host movement (Applegate, 1950). Anecdotal and experimental evidence suggests that some petromyzontids forage most actively at night, and that in those species that feed in shallow waters (e.g., *Ichthyomyzon* spp., *Lampetra* spp.) this may be more important in avoiding predation than any other factor (Cochran, 1986). For *P. marinus*, which tends to feed in deep waters, this may be less important, and could be used instead to approach hosts when they are resting.

Varying proportions of prey populations will bear either fresh or healed lamprey-induced wounds and act as indicators as to how actively lampreys are foraging in an area. *Entosphenus tridentatus* was shown to have fed on 0.6 – 10% of the walleye pollock, and 27% of the sockeye salmon examined in the Strait of Georgia (Beamish, 1980). *Entosphenus macrostomus* was believed to have fed on > 50% of the salmonids in Mesachie Lake, Vancouver Island (Beamish, 1982; Beamish & Wade, 2008). Some populations of hosts are heavily preyed upon. For example, in Love Lake, Maine 85% of salmon bore some evidence of lamprey attachment (David, 1967), and Pacific salmon *Oncorhynchus* spp. in Kamchatka are subject to both *E. tridentatus* and *L. camtschaticum* feeding in the same season, resulting in up to 74% of individuals from six species bearing lamprey-induced scars (Shevlyakov & Parensky, 2010).

The effect of such extensive feeding by juvenile petromyzontids can be extreme, as in Lake Oneida where freshwater-resident *P. marinus* killed *c*. 31, 000 fish in a four-week period (Forney, 1986). In Lake Ontario, where there is some suggestion local strains of lake trout are less susceptible to mortality caused by *P. marinus* feeding, 17, 000 – 121, 000 hosts are believed to be killed in *c*. six-week feeding period (Schneider *et al*., 1996). The lethality of lamprey feeding depends on so many factors already outlined that it is almost impractical to calculate except on an individual lamprey-host basis. Host species of the freshwater-resident *P. marinus* in Lake Superior have a probability of surviving lamprey feeding of just 0.14 (Koonce & Pycha, 1985), yet in Lakes Huron and Champlain it is 0.66 and 0.74 respectively (Madenjian *et al*., 2008). This may reflect the relatively later appearance of *P. marinus* in Lake Superior, and indicate that host species in Lakes Huron and Champlain have had enough time for the evolution of traits leading to greater survivorship.

1.4.6 *Growth*

Growth during the juvenile feeding period is a function of the host:lamprey size ratio, as well as the duration of attachment (Cochran & Kitchell, 1989). In freshwater-resident *P. marinus* growth may be rapid and extensive in spring and summer (Forney, 1986; Kitchell, 1990), particularly if the attachment duration of a feeding bout is long (> 18 days). But there is great variability in the rate of feeding between individuals and so an accurate model of growth rate for different species is generally not possible (Cochran *et al*., 2001). The lipid content of the lamprey increases significantly during the juvenile phase (Beamish *et al*., 1979), and there is a related increase in energy density (Cochran *et al*., 1999). Under experimental conditions, where temperatures ranged 5 – 20°C, growth rates declined with an increase in lamprey weight (Farmer & Beamish, 1973). The rapid growth rates of freshwater-resident *P. marinus* in the Laurentian Great Lakes are potentially a result of the exceptional feeding opportunities created by vast numbers of hosts, limiting the need for exhaustive foraging periods during which they attempt to locate hosts. They may increase in length from 130 to 400 mm in just five months (Potter *et al*., 1979), and yet anadromous populations of *L. fluviatilis* increase from 100 to 300 mm in 18 months (Zanandrea, 1959) indicating the latter species derives less nutrition from its host, or expends more energy foraging.

Populations of wide-ranging species vary in the eventual mean size they will reach following the juvenile feeding phase. For example, anadromous *L. fluviatilis* from the River Severn, U.K. are usually 300 mm long (Hardisty & Huggins, 1973; Abou-Seedo & Potter, 1979); in the River Neva, Finland they are 325 mm (Berg, 1948); yet in Lithuania they may grow as large as 408 mm (Gaygalas & Matskevichyus, 1968). Anadromous populations of *P. marinus* attain the largest sizes of all petromyzontids. In Quebec they may reach sizes in excess of 780 mm (Beamish & Potter, 1975) and in Portugal > 900 mm (Andrade *et al*., 2007). In the Laurentian Great Lakes, where *P. marinus* has been studied extensively, some evidence points to adaptive growth responses by lamprey populations in reaction to the availability of hosts (Jorgensen & Kitchell, 2005). In Lakes Huron and Ontario *P. marinus* has increased in both length and weight since the restocking of the lakes with potential hosts that had previously been in decline (Houston & Kelso, 1991), indicating that if hosts were scarce the adult population was maturing at a smaller size.

1.4.7 *Duration of the Juvenile Period*

Accurate estimates of the duration of the juvenile period are only available for nine species, and great variation exists among populations of even these few. Some species, such as the freshwater *E. macrostomus* may feed for a few months (Kan & Bond, 1981), while anadromous *G. australis* and *E. tridentatus* may feed for as long as four years (Beamish, 1980; James, 2008). But this disparity in feeding duration between freshwater and marine species is not a general rule, as the freshwater *T. spadiceus* could feed for as long as two years (Alvarez del Villar, 1966), while anadromous *L. ayresii* feed for just four months (Beamish, 1980). *Lampetra fluviatilis*, which tends to feed in estuaries, typically spends 18 months feeding (Zanandrea, 1959) and freshwater-resident *P. marinus* populations feed for 12 to 18 months in the Laurentian Great Lakes (Applegate, 1950).

1.4.8 *Alternative Foraging Strategies*

Some species of lamprey contain populations that exhibit atypical foraging strategies. In *L. fluviatilis*, which predominately feeds in inshore waters, there is evidence for so-called "praecox" variants (Berg, 1948). These populations are smaller in length than typical individuals, and it is assumed that they spend a reduced period of time feeding within estuaries. In the River Severn, U.K. one such praecox population ceases feeding at mean lengths of 240 mm, and it is estimated they feed for 12 months as opposed to typical individuals of *c*. 300 mm that have fed in the estuary for 18 months (Abou-Seedo & Potter, 1979). *Lampetra fluviatilis* has also produced several populations that feed exclusively within large bodies of freshwater (Valovirta, 1950; Abakumov, 1960; Tuunainen *et al*., 1980; Maitland *et al*., 1994; Inger *et al*., 2010), and which can be either typical in size (Goodwin *et al*., 2006) or much smaller at the conclusion of the feeding period (Adams *et al*., 2008; Hume, 2011).

Similarly, *L. camtschaticum* another marine foraging species, has produced a complex of freshwater-resident and praecox variants (Heard, 1966; Nursall & Buchwald, 1972; Sidorov, 2005; Artamonova *et al*., 2011), as has *E. tridentatus* that exhibits several resident and enigmatic populations (Coots, 1954; Beamish, 1980*b*; Kostow, 2002; Taylor *et al*., 2012). Although most notable for the invasion and subsequent colonisation of the upper Laurentian Great Lakes *P. marinus* has also produced several other freshwater-resident populations during its evolutionary history, including Lakes Ontario (Eshenroder, 2009), Champlain (Wilson, 1955), Cayuga and the other Finger Lakes of New York State (Wigley, 1959). Not all petromyzontids however appear able to tolerate impoundment in freshwater, as evidenced by the extinction of *E. tridentatus* in Dworshak Reservoir, Idaho following the construction of a dam (Wallace & Ball, 1978). In *P. marinus*, which typically feeds in marine waters, there are instances when populations will begin feeding during the downstream migration following metamorphosis (Davis, 1967; Potter & Beamish, 1977; Bird *et al*., 1994;

F. Igoe, *pers. com.*), particularly where they must pass through large lakes. Similar behaviour has been recorded in *Mordacia mordax* in Australia, where populations may feed for < 6 months in brackish lakes before entering the sea to feed for a further 18 months (Potter *et al.*, 1968).

In more extreme cases typically non-parasitic lamprey species may exhibit an evolutionary atavism, a throw-back to their evolutionary past, by being able to actively feed following metamorphosis. The best understood example of this was seen in a population of *L. richardsoni* from Morrison Creek, Vancouver Island that was said to be able to feed parasitically in freshwater, and was named as *L. richardsoni* var. "*marifuga*" (Beamish, 1987). However, no evidence of feeding within this river system has yet been observed (NRT, 2007). Unusually large non-parasitic *L. appendix* (range 260 – 354 mm) have been recovered on occasion, which have the dentition capable of permitting at least a scavenging mode of feeding, although again no evidence of such activity has been presented (Manion & Purvis, 1971; Cochran, 2008).

1.5 ADULT STAGE

With the conclusion of the juvenile period, those lamprey species that feed following metamorphosis are ready to enter the adult stage. This corresponds to the state at which the non-parasitic brook lampreys find themselves immediately following the completion of their metamorphosis, and once again both major petromyzontid life history strategies are at similar stages in their overall life cycle. This period in the lamprey life cycle is characterised by sexual maturation and the urge to reproduce, an act that always takes place in freshwater. For all species this will require an upstream migration away from larval habitat or juvenile feeding grounds, and towards areas suitable for the construction of nests. For those species that have been feeding in marine environments this often entails extensive migrations, and requires an ability to detect and successfully enter freshwater environments. The final stages of sexual maturation take place during these upstream migrations as the lamprey once again undertakes a lengthy period of starvation where resources are redirected away from growth towards the production of gametes. Then, once prevailing conditions and the availability of conspecifics dictates, the spawning act itself begins. Typically this occurs in restricted periods of time and space, condensing the lamprey's life-long exertions into just a few days of exhaustive and ultimately fatal reproductive effort.

1.5.1 *Spawning Migration*

For non-parasitic species, it could be said that the adult period begins at the completion of metamorphosis, yet these lampreys generally remain for some time buried within the substrate prior to beginning their spawning migration. The time at which the spawning migration commences in anadromous species can vary widely. In *L. fluviatilis*, for example, some populations begin to enter freshwater in the autumn (Hardisty, 1973; Abou-Seedo & Potter, 1979; Maitland *et al*., 1984), others in the spring (Berg, 1948; Maitland *et al*., 1994), and yet others will continue to do so throughout the winter period (Sjöberg, 1980; Hume, 2011). A similar strategy is seen in *C. wagneri*, which has discrete autumn and spring migrations (Nazari & Abdoli, 2010), and also in *L. ayresii* (Beamish, 1980). *Petromyzon marinus* appears more tightly coordinated, with most individuals commencing migration in December, and peaking between February and April (Applegate, 1950; Beamish & Potter, 1975, 1977; Almeida *et al*., 2000). *Geotria australis* begins entering rivers in both Australia and New Zealand in winter (Potter *et al*., 1983; Kelso, 1996). Those lamprey species that remain in fluvial environments throughout the juvenile period tend to exhibit more restricted migration timing, such as *I. unicuspis*, which almost always moves upstream in April (Cochran & Marks, 1995). However, the enigmatic freshwater Mexican lampreys (*Tetrapleurodon* spp.) may not migrate to any notable extent, as their spawning period is believed to be as long as six months given the low latitude of their geographic range (Cochran *et al*., 1996).

Non-parasitic lampreys, typically living their lives within natal streams, are often described as being non-migratory and most authors fail to note any upstream movement in their populations. This cannot be strictly true though, as some measure of upstream movement must be initiated in order for them to reach suitable spawning grounds, although admittedly such movement is usually of limited extent. Many *L. planeri* populations in European streams for example may migrate < 2 km, and do so in a restricted three to four week period immediately prior to spawning (Hardisty, 1944; Malmqvist, 1980*b*), yet others may undertake more protracted migrations of > 5 km over a six month period (Hume, 2011).

The duration of spawning migrations therefore differs between individuals as well as species, with some lampreys overwintering in rivers prior to spawning in spring. For *L. fluviatilis*, *E. tridentatus* and *C. wagneri*, all of which spawn in spring, this second period of fluvial residency may last anywhere from < 1 to 12 months (Abou-Seedo & Potter, 1979;

Beamish, 1980; Ahmadi *et al*., 2011; Hume, 2011). The spawning migration of *G. australis* appears to be the most extensive, and they may reside in rivers for up to 18 months after leaving their marine feeding grounds (Kelso, 1996). Even at times of year where lampreys are actively moving upstream, this may be punctuated with long periods of inactivity. For example, anadromous *P. marinus* entering Portuguese rivers may rest for up to 42 days in some locations (Almeida *et al*., 2002). The average rate at which a lamprey moves upstream largely depends on the species' body size and prevailing water conditions. For large petromyzontids, such as anadromous *P. marinus*, average speeds of 0.5 to 3.2 km h^{-1} have been recorded (Hardisty, 1979) during their spawning migrations, and *G. australis* in New Zealand has been found to travel up to 12 km day^{-1} (Jellyman *et al*., 2002).

Some of the larger species are capable of extensive spawning migrations, travelling huge distances to attain suitable spawning grounds. Freshwater-resident *P. marinus* for example penetrate up to 49 miles into Lake Huron tributaries (Applegate, 1950) but anadromous populations may travel > 125 miles upstream (Beamish, 1979). Anadromous populations of *E. tridentatus* can travel > 250 miles upstream between May and August (Beamish, 1980). Even smaller estuarine species, such as *L. fluviatilis*, can travel up to 80 miles upstream (Gaudron & Lucas, 2006). Those lampreys that begin migration earlier in the season do not migrate further than those that follow later (Clemens *et al*., 2012).

Lampreys entering freshwater following a period of feeding in marine environments rapidly alter their physiology to do so. *Lampetra fluviatilis* captured in estuaries one month after beginning their upstream migration survived for three days when transferred to 70% sea water but were chronically dehydrated (Abou-Seedo & Potter, 1979), suggesting they were already losing the ability to osmoregulate in salt water. Similar findings for *L. ayresii* suggest they cannot be retained in salt water during the period when their cohorts are migrating upstream, although some individuals can seemingly osmoregulate longer than others (Beamish, 1980).

The mechanisms that encourage maturing lampreys to enter rivers appear to be related, to one extent or another, on the levels of discharge from those rivers. For example, *L. fluviatilis* is known to enter rivers earlier in autumn during years where discharge rates were high, as opposed to years where reduced rainfall in the autumn postponed river entry until as late as November or December (Abou-Seedo & Potter, 1979). Similarly, anadromous *P. marinus* have been shown to be stimulated to resume upstream migration when discharge over dams increases (Almeida *et al*., 2002). Adults of freshwater-resident *P. marinus* in the Laurentian Great Lakes congregate in the fluvial fan of river mouths prior to the spawning run, dropping back into the lakes each day (Applegate, 1950). This may be a negative response to low levels of discharge during this period. In contrast, *E. tridentatus* begin their upstream migration earlier in low-discharge years, and later in years with high flow rates (Keefer *et al*., 2009). It may well be that periods of high discharge create velocity barriers to migrating lampreys in some river systems.

Although direct evidence is lacking it would appear that the phases of the moon also play their part in initiating and maintaining the upstream migration. In the Severn Estuary, U.K. reduced numbers of *L. fluviatilis* were noted on nights where the full moon was present (Abou-Seedo & Potter, 1979). It is probable that any influence of the moon relates to ambient light intensity, as many species appear to preferentially migrate only in hours of darkness, including anadromous *P. marinus* (Almeida *et al*., 2002, Andrade *et al*., 2007) as well as freshwater-resident populations (Applegate, 1950). *Caspiomyzon wagneri* is believed to migrate upstream in surface waters when it is dark, yet drop towards the bottom of rivers when the moon is bright (Nazari & Abdoli, 2010). The respiratory physiology of migrating lampreys alters during this period, exhibiting greater levels of respiration and an increased heart rate during dark hours (Claridge *et al*., 1973), and this is possibly controlled by the pineal gland.

Petromyzontids appear most sensitive to light during the early stages of the spawning migration, but as sexual maturity increases the negative phototactic response decreases (Applegate, 1950; Sjöberg, 1977). Experimental work with artificial lighting has proven inconclusive, with some suggestion that lampreys are in fact positively attracted to lighted traps (Purvis *et al*., 1985; Fredricks *et al*., 1996). The eyes do not play a major role during this time, as experimentally blinded lampreys exhibit the same diel activity pattern as control animals (Binder & McDonald, 2007). Instead, dermal photoreceptors clustered in the caudal region are believed to be important in directing the lampreys to seek shelter during daylight hours (Binder & McDonald, 2008*a*). Light avoidance behaviour is strongly linked with ambient temperatures experienced by lampreys, as evidenced by the fact that in warmer conditions the extent of the negative phototactic response decreases, and individuals continue migrating during daylight hours (Binder & McDonald, 2008*b*). If we couple this finding with the knowledge that, at least in some petromyzontids, sexual maturity is initiated and more rapid under warmer water temperatures (Clemens *et al*., 2009), then it is reasonable to suggest that, both the loss of negative phototaxis and the onset of rapid sexual maturity, acts to ensure lampreys arrive on spawning grounds at such a time when embryonic development will be optimised.

1.5.2 *Pheromones*

The olfactory system of petromyzontids is highly developed and suggests that odours play a key role in their life history (Kleerekoper, 1972). In addition to the mechanical factors of discharge rate and temperature, pheromones are important in directing adult lampreys back into rivers suitable for spawning (Vrieze *et al*., 2010). There is no evidence however that petromyzontids home to natal streams (Waldman *et al*., 2008; Spice *et al*., 2012), instead maturing adults are initially attracted to rivers based on hydrological cues, such as flow rate (Sorensen, 2003; Vrieze *et al*., 2011), but the presence of conspecifics maintains their directional swimming into the river itself (Sorensen, 1998; Vrieze & Sorensen, 2001). This was indicated by Moore & Schleen (1980) who noted that when rivers containing large numbers of freshwater-resident *P. marinus* were treated by the application of a larvicide, that the numbers of adults returning to that river the following spring were generally much lower. This suggested that *P. marinus* ammocoetes played a role in attracting adults, and was confirmed to be the case when water conditioned by ammocoetes was seen to preferentially attract adult lamprey, and that the response was stronger when greater numbers of ammocoetes were present (Teeter, 1980; Sorensen & Gallaher, 1994).

When tested explicitly it was found that pheromones produced by *P. marinus* ammocoetes were responsible for directing adult lampreys into rivers and maintaining their upstream movement (Bjerselieus *et al*., 2000). These olfactory cues were subsequently found to contain the ammocoete bile acids petromyzonol sulphate (PS), petromyzonamine disulphate (PADS) and petromyzosterol disulphate (PSDS) (Collodi, 1998, 2000; Sorensen, 2004; Fine *et al*., 2004; Fine & Sorensen, 2008), none of which are species specific. These compounds are produced by and induce the same behavioural response in a wide range of migratory petromyzontids (Sorensen, 1998; Fine *et al*., 2004; Gaudron & Lucas, 2006; Yun *et al*., 2011; Stewart & Baker, 2012). Results of extensive trials indicate that some mixture of these compounds acts as a migratory pheromone produced by the larval stages of lampreys, and that not only is it evolutionarily conserved in the petromyzontid lineage, but it represents the first migratory pheromone identified in a vertebrate (Sorensen *et al*., 2004).

1.5.3 *Barriers to Migration*

Lampreys have a limited ability to surmount obstructions, both natural and man-made, on their passage upstream, and some species are more able to tackle such obstructions than others. This has led to drastic reductions in the availability of river habitat for spawning populations of both parasitic and non-parasitic species (Nunn *et al*., 2008; Mateus *et al*., 2012). Chief amongst such natural barriers are falls, and these are presumably an obstruction faced by petromyzontids throughout their freshwater existence. It is of no real surprise then that many species are capable of finding their way to the very headwaters of vast river systems (Beamish, 1980). The jumping ability of petromyzontids is relatively limited, and in *P. marinus* it is probably only 60 cm, though there are reports of them clearing 120 cm (Applegate, 1950). Instead, when faced with vertical waterfalls lampreys will attach to the face with their oral disc and throw themselves upwards a short distance before reattaching and resting for a period (Youngs, 1979; Kelso, 1996; Zhu *et al*., 2008). Under certain circumstances, such as witnessed in the Ocqueoc River, the sheer mass of migrating lampreys attempting to clear the falls results in some individuals being heaved over the top by the action of their cohorts (Applegate, 1950). There are substantiated reports of *G. australis* in New Zealand leaving the water and travelling along the dampened edge of a river to circumnavigate a hydroelectric dam (Potter *et al*., 1983).

Anadromous *P. marinus* may however find it difficult to surmount weirs in several Portuguese rivers, where it takes them several hours to weeks in order to bypass the obstructions (Andrade *et al*., 2007), and during which time they are subject to significant levels of poaching. Successive up-stream barriers have a cumulative effect on the abundance of upstream migrants, and this is well illustrated by *E. tridentatus* in the Columbia River, U.S.A. which must overcome a battery of hydroelectric dams as well as natural falls. One study that examined the numbers of migrating individuals continuing upstream after surmounting the Bonneville Dam found that sample sizes rapidly decreased in three subsequent upstream reaches, from 31 to 18, and then to 5% of those that bypassed Bonneville (Keefer *et al*., 2009). Flow rates at such obstructions are a serious impediment to petromyzontid migration, and when faced with high flows they will tend to remain attached to structures for long periods and not attempt to bypass weirs (McLaughlin *et al*., 2003; Quintella *et al*., 2004; Mesa *et al*., 2010; Kemp *et al*., 2011). Some species, such as *L. fluviatilis*, do not make best use of standard fish passes (Laine *et al*., 1998; Kemp & O'Hanley, 2010) and instead require more specific structures to aid their passage across in-stream barriers (Moser *et al*., 2005; Russon & Kemp, 2011). Even under moderate flow regimes lampreys can struggle to approach and overcome even small barriers, particularly in shallow water conditions (Youngs, 1979; Russon *et al*., 2011).

1.5.4 *Energetics*

The energetic requirements of lampreys undertaking the spawning migration and subsequently sexual maturation and spawning itself, are largely met by the lipids assimilated during the post-metamorphic feeding phase (Moore & Potter, 1976), or in the case of non-parasitic lampreys, during the extensive larval period. It has been shown that during the earliest stages of the upstream migration lampreys derive their energy from the anaerobic metabolism of glycogen stored in the muscles, which they are able to replenish very rapidly (Patton *et al*., 2011). Subsequently as starvation proceeds they switch to aerobic metabolic strategies in order to conserve their glycogen stores. Lampreys first beginning the upstream migration have the greatest body mass, and this decreases steadily as the migratory period progresses. In anadromous *P. marinus*, for example, individuals can weight 896 g when first re-entering freshwater, but 645 g following the completion of spawning (Beamish, 1979). A similar decline in body weight is seen in the non-parasitic *I. gagei* between the end of metamorphosis and the end of the spawning period (Beamish & Legrow, 1983). Approximately 10% of the wet weight at this stage is lipid, declining to 4% in spent individuals (Beamish *et al*., 1979), or as low as 2% in spent *I. gagei* (Beamish & Legrow, 1983). Energy content follows a similar downward trajectory, with early migrants estimated as having 6.607 kcal g^{-1} dry weight compared to 5.607 kcal g^{-1} dry weight in spent individuals (Beamish, 1979). During a 60 km upstream migration, anadromous *P. marinus* are estimated to expend *c*. 190 kcal, and *c*. 300 kcal for a 140 km upstream migration (Beamish, 1979).

1.5.5 *Sexual Maturation*

As lampreys undertake their spawning migration the gonads begin to mature and the gametes enter the final stages of development (Dziewulska & Domagala, 2009). This is indicated by an increase in the gonadosomatic ratio, and in females, an increase in the diameter of the oocytes (Abou-Seedo & Potter, 1979; Beamish *et al*., 1979; Potter *et al*., 1983; Nazari & Abdoli, 2010; Ahmadi *et al*., 2011). Mature female petromyzontids contain a single elongate ovary extending from posterior of the last branchial chamber to the cloaca, and this can constitute up to 30% of the total body weight in *P. marinus* (Applegate, 1950). Once they have matured fully the eggs are released into the body cavity (Yorke & McMillan, 1980). Lamprey eggs are generally ovoid in shape and white to orange in colour (Larsen, 1970). In anadromous parasitic species, such as *P. marinus*, *G. australis*, *C. wagneri* and *L. fluviatilis*, ripened ova are *c*. 1 mm in diameter (Applegate, 1950; Potter *et al*., 1983; Dziewulska & Domagala, 2009; Ahmadi *et al*., 2011), while those of *E. tridentatus* from Japan are somewhat larger at 1.2 mm (Yamazaki *et al*., 2003). The non-parasitic *I. fossor* also has eggs *c*. 1.2 mm in diameter (Leach, 1940). There is some indication that egg size is environmentally regulated, at least in small non-parasitic lamprey populations (Beamish *et al*., 1994; Yamazaki *et al*., 2001). For example, in female *L. aepyptera* that spawn at small body sizes there are larger but fewer eggs in comparison to those produced by females spawning in streams where the females are large and presumably growth rate was high (Docker & Beamish, 1991). Ova are denser than water and so sink readily, and they possess an adhesive coating which facilitates their retention in the substrate (Okkelberg, 1913; Seversmith, 1953; Yorke & McMillan, 1979).

In male lampreys, early migrants are characterised by the presence of spermatocytes, which are replaced with spermatozoa closer to the spawning period (Ahmadi *et al*., 2011). The development of the reproductive organs is concurrent with the reduction of the gut, which begins to reduce in weight, complexity and diameter (Abou-Seedo & Potter, 1979). Lamprey sperm appears to exhibit particularly long durations of motility, and potentially confers a strong fitness advantage in freshwater by boosting fertilisation rates. In *P. marinus* sperm motility was observed for up to seven minutes (Dabrowski *et al*., 1998), and in *L. camtschaticum* sperm were motile for up to five minutes after activation (Kobayashi, 1993).

1.5.6 *Sex Ratio*

The sex ratios of adult lamprey populations can differ within, as well as among species, and this can result in contrasting descriptions depending on the time at which they are observed. During the upstream migration of *L. fluviatilis* in the Severn Estuary, U.K. a slight excess of males was seen in some years but a preponderance of females in others (Abou-Seedo & Potter, 1979). *Caspiomyzon wagneri* may show either a preponderance of males or an equal sex ratio during the upstream migration (Ahmadi *et al*., 2011). Early in their colonisation of the upper Laurentian Great Lakes freshwater-resident *P. marinus* spawning runs were dominated by males (Applegate, 1950), but *P. marinus* populations in general appear have a male biased sex ratio (Beamish & Potter, 1975) as do populations of *G. australis* (Potter *et al.*, 1983). *Ichthyomyzon unicuspis* has a female biased sex ratio in the Fox River, Michigan (Cochran & Marks, 1995) that appears unusual for a parasitic species. On the spawning grounds males tend to dominate in non-parasitic *L. planeri* populations (Hardisty, 1961), but in the closely related anadromous *L. fluviatilis* the spawning grounds are mainly comprised of females (Jang & Lucas, 2005), sometimes reversing completely during different phases. Both *L. reissneri* and *L. appendix,* non-parasitic lampreys from North America and Japan respectively, also show a general male sex bias on spawning grounds (Seagle & Nagel, 1982; Takayama, 2002).

There is some indication that the sex ratio of adult lampreys may alter according to population density fluctuations. Such periodicity is seen in *C. wagneri*, which exhibits male dominance in two to four year cycles (Ahmadi *et al.*, 2011), or *I. unicuspis* that shows a six to seven year cycle (Cochran & Marks, 1995). However, this trend was not seen in non-parasitic *L. planeri* populations observed almost continuously for *c*. 15 years, instead males always dominated, yet greater proportions of males appeared in those years with the largest total spawning population (Hardisty, 1961). This was attributed to the general preponderance of adult male lampreys coupled with greater female mortality during both metamorphosis, and subsequently their sexual maturation (Hardisty, 1961).

1.5.7 *Spawning Behaviour*

There is much evidence to suggest that pheromones play an important role in the short range attraction of mates and in maintaining cohesion among congregations of spawning petromyzontids. Early work suggested that sex steroids were produced by males in order to attract females to their nests (Adams *et al.*, 1987), but that individuals were only responsive to these pheromones after they had become sexually mature and unresponsive to the migratory pheromones directing their upstream movement (Sorensen & Gallaher, 1996; Bjerselieus *et al.*, 2000). Dense aggregations of spawning adults in restricted patches of available habitat would indicate that once started some cue is responsible for the attraction and retention of later arrivals (Jang & Lucas, 2005). In *P. marinus* a particular component of a pheromone produced by sexually mature males; 7α, 12α, 24-trihydroxy-5α-cholon-3-one

24-sulphate (3kPZS), is highly attractive to sexually mature females, drawing them upstream and onto spawning grounds from hundreds of meters away (Li, 2000; Li *et al*., 2002; Johnson *et al*., 2009; Luehring *et al*., 2011). In addition to this function, 3kPZS and an as yet unknown component of this sex hormone, induce female *P. marinus* to remain in the vicinity of nests for extended periods as well as inducing several spawning behaviours (Johnson *et al*., 2012). The lamprey sex pheromone is secreted from the gills as opposed to the urinary tract in other fishes (Li *et al*., 2002; Siefkes *et al*., 2003; Johnson *et al*., 2009).

All petromyzontids studied to date appear to require remarkably similar environmental and physical conditions in which to spawn successfully. Typically, spawning takes place in the upper reaches of rivers where water flow conditions result in the deposition of suitable substrates, mainly gravels, and water temperatures are suitable for early embryonic development. Lampreys create depressions in the gravel, called redds, into which the gametes will be deposited. Both sexes are usually present during this construction phase, and there appears little compelling evidence to suggest there is a division of labour, although many early authors suggest males initiate nest building (e.g., Young, 1900; Applegate, 1950). Initially, a patch of stones and gravel are swept clean of debris and silt by the vigorous beating of the tail while attached to a larger stone (Seversmith, 1953). This also acts to make the beginning of a depression in the gravel, which is enlarged by the more directed removal of stones using the oral disc and body to lever them downstream and to the side (Hagelin, 1959). During construction lampreys remain oriented in an upstream direction and this ultimately results in an oval or horseshoe-shaped redd, which is deeper on the upstream side and bears a thicker ridge downstream caused by the build-up of stones. Often these redds are created immediately downstream of a larger stone, to which lampreys will attach frequently in order to enlarge the redd, by more tail thrashing, and that will also act as an anchor during the spawning act (Raney, 1939; Hardisty, 1944).

An impressive amount of substrate can be moved by lampreys during nest construction, and there is some indication they petromyzontids have a significant impact on stream ecology (Nika & Virbickas, 2010; Sousa *et al*., 2012). *Petromyzon marinus* pairs, for example, have been estimated to move up to 10.6 kgs of substrate in the course of creating a single redd (Applegate, 1950). In general, the size of the nest varies considerably depending on prevailing conditions and the number of nests being built nearby, as lampreys will construct redds that overlap those of their neighbours, or indeed will nest communally (Dendy & Scott, 1953; Case, 1970; Jang & Lucas, 2005). The diameter of the redd corresponds closely to the body size of the architect, such that in *P. marinus* nest diameter can range 60 – 90 cm, and depth up to 30 cm (Applegate, 1950), while in the non-parasitic *L. aepyptera* and *I. gagei* the nests range 10 – 30 cm in diameter and 3 – 7 cm in depth (Dendy & Scott, 1953; Brigham, 1973). The intermediate body size of *L. fluviatilis* results in redds of 25 to 40 cm in diameter (Gardiner & Stewart, 1997). The depth of water nests are located in also depends largely on the body size of the lamprey species, where larger lampreys such as *P. marinus* will spawn in water up to 2 m deep with a flow rate ranging 1 – 1.5 $m^3 s^{-1}$ (Applegate, 1950). Although found spawning at similar flow rates, the much smaller *L. aepyptera* spawns in water depths of just 7 cm (Brigham, 1973). In general, however, non-parasitic species such as *L. appendix* and *L. reissneri* prefer reduced flows in the region of 0.5 $m^3 s^{-1}$ or less (Takayama, 2002; Mundahl & Sagan, 2007).

The functional significance of these nests is debatable, as it is not clear what the relative importance of substrate type is to the overall survivorship of eggs. At a basic level some form of depression in the gravel will act to retain eggs as they are released nearby, but lampreys do not appear to deposit their fertilised eggs in a directed manner. For example, 85% of *P. marinus* eggs are swept out the nest during repeat spawning events within the same redd, and substrate type has little impact on the hatching success of egg batches (Smith & Marsden, 2009). A second function of the redds could be to reduce the force of water experienced by the lampreys as they spawn, as within the depressions flow rates are much reduced, or even slack (Applegate, 1950). There is some indication that non-parasitic lampreys could utilise the larger and more extensive redds of parasitic species, reducing the energetic demands of building redds and so boost their own reproductive potential (Morman, 1979; Cochran & Lyons, 1994), as seen in the interactions between *L. richardsoni* and the much larger *E. tridentatus* in the Columbia River Basin (Pletcher, 1963; Stone, 2006), *L. camtschaticum* and *L. reissneri* in Arctic Russia (Kucheryavyi *et al*., 2007), or *L. planeri* and *L. fluviatilis* throughout Europe (Huggins & Thompson, 1970; Lasne *et al*., 2010).

The spawning act itself appears highly constrained among petromyzontids, in that there is little variation in the overall pattern of behaviours. It should be noted though that spawning behaviour has not been described for any of the four Southern Hemisphere species, and this demands immediate attention given the deep evolutionary divergence between these and the Petromyzontidae family in the Northern Hemisphere. It begins when the female attaches to a large rock or stone in the anterior section of a nest and orienting her body with the water flow. The male approaches the female from behind and glides along her body with the oral disc open until he reaches the branchial or head region. The approach is typically from either the left or right side of the female's body (Hagelin, 1959). The male then attaches to the female, usually between the first branchial pore and the tip of the oral disc, and throws the lower half of his body across the female, either to the left or right, forming a loose coil around her trunk. This tail-loop is then tightened and both male and female raise their branchial region up from their anchor point at an acute angle and violently vibrate and thrash their tails for several seconds. This results in the expulsion of ova and milt into the redd, which is rapidly covered in sand and small gravel in the downstream ridge. to which the fertilised eggs adhere (Applegate, 1950). Both male and female may then rest for a short period before resuming nest building behaviours such as stone moving.

The action of the male's tail-loop is what causes the eggs to be expressed (Hagelin, 1959), and so there is a strong influence of body size on the ability of a male to successfully complete this function. Petromyzontids tend to mate in a homogomous system i.e., that males and females are of a roughly equal body size, and so strong is the functional requirement for an equal partner size that males appear unable to fertilize the eggs of females > 25% larger than themselves (Beamish & Neville, 1992). As only a small number of eggs are expressed at any one time, for example, 20 to 40 in freshwater-resident *P. marinus* from the Laurentian Great Lakes (Surface, 1899 as reported in Applegate, 1950) and up to 100 for *L. fluviatilis* (Huggins & Thompson, 1970), spawning can last several days for each individual female depending on the stock of eggs available. Spawning may take place every few minutes, with the rest period between each bout increasing as the lampreys near exhaustion.

In an exception to the general rule (i.e., spawning in open gravel beds in relatively shallow water) there have been reports of some lampreys, particularly in the genus *Ichthyomyzon*, spawning beneath cover such as boulders or logs (Morman, 1979; Cochran & Gripentrog, 1992; Mundahl & Sagan, 2007). It is believed these individuals could be taking advantage of suitable microhabitat conditions as a means of spawning in sections of rivers otherwise unsuitable for the typical mode of spawning. There is also a report of *E. mariae* spawning over hard clay without constructing redds, indicating this population is able to utilise atypical substrates (Levin & Holčík, 2006). Alternative male reproductive behaviours have been recorded in several species that may act to negate the effects of body size differences on fertilisation success. For example, small males of *L. fluviatilis* have been seen attaching in a more posterior position, between the first dorsal fin and the last branchial pore, when mating with larger females (Bahr, 1953). In some instances multiple males attach to different positions on a single female (Bahr, 1953; Dendy & Scott, 1953; Heard, 1966; Malmqvist, 1983). However, sneak male mating tactics provide the strongest indication that body size barriers can be overcome in lamprey mating systems, and this has been described in several genera (Hume *et al.*, *in press*). At the point of egg release an unattached male (the "sneaker") rapidly circles the cloaca of a spawning pair, and this has been interpreted as an attempt to achieve fertilisation by the sneaker male (Malmqvist, 1983). Such behaviour has been well documented in *L. appendix* (Cochran *et al.*, 2008), *L. planeri* (Malmqvist, 1983), *L. richardsoni* (Pletcher, 1963) and *L. fluviatilis* (Wüstel *et al.*, 1996 as reported in Cochran *et al.*, 2008).

1.5.8 *Fecundity*

The standing stock of eggs an individual female can express during the reproductive phase limits the overall capacity of the adult population to contribute to the next generation, and gives a basic indication of a species' reproductive potential. In a broad trend the larger parasitic species, and in particular those that forage in marine environments, express many more thousands of eggs than freshwater-resident or non-parasitic species. This would appear to reflect differences in the levels of mortality experienced by these different life history strategies, where high mortality caused by prolonged and demanding migrations is offset by an overabundance of gametes that will contribute to future offspring. In this sense, non-parasitic species trade off a vastly reduced reproductive capacity with a cryptic, and comparatively safe, adult life in natal streams and rivers (Hardisty, 1962). Once ova have been released into the body cavity almost all mature eggs are expressed by females, excepting of course if an individual fails to locate a suitable mate. In one general exception to this Applegate (1950) calculated that a maximum of 5% of mature eggs were not expressed by female *P. marinus* captured following the conclusion of the spawning season, but this was revised to 2.2% on average by Manion & McLain (1971).

Freshwater-resident *P. marinus* from the Laurentian Great lakes may produce 24, 000 to 107, 000 eggs per female (Applegate, 1950), while larger anadromous populations may express up to 210, 000 eggs (Beamish & Potter, 1975). Anadromous populations of *L. camtschaticum* can produce 100, 000 eggs per female (McLory & Gotthardt, 2005) yet freshwater-resident populations express only *c.* 21, 500 (Nursall & Buchwald, 1972). An interesting trend can be seen in *G. australis* where females express *c.* 58, 000 eggs (Hardisty *et al*., 1986) which is surprisingly low given that this species is only marginally smaller than some anadromous *P. marinus* populations, and larger than some *L. camtschaticum*. This could be attributed to the fact that as *G. australis* has an extensive migration into freshwater, the reabsorption of some oocytes is necessary to provide energy, and that the species exhibits a particularly slim body profile, thereby reducing the volume of the body cavity available for retaining mature ova (Potter *et al*., 1982, 1983). The widespread species *C. wagneri* may produce 17, 000 to 51, 000 eggs depending on the population (Nazari & Abdoli, 2010; Ahmadi et al 2011).

In the non-parasitic *I. gagei* fecundity ranges 820 – 2, 500 (Beamish, 1982) while the closely related parasitic *I. castaneus* has a fecundity one order of magnitude greater at 10, 000 to 18, 500 eggs per female (Beamish & Thomas, 1983). This relationship in species pairs is also seen in *L. planeri*, which expresses *c*. 1, 500 eggs (Hardisty, 1964), while *L. fluviatilis* has 7, 500 to 40, 000 eggs (Hardisty, 1944). Other non-parasitic species produce similarly small numbers of eggs, such as: *L. aepyptera* (range 500 – 5, 900, Docker & Beamish, 1991); *L. zanandreai* (*c.* 1, 850, Zanandrea, 1961); *L. appendix* (range 1, 600 – 2, 000, Schuldt *et al*., 1987); *L. kessleri* (range 1, 300 – 2, 100, *L. r.* sp. N range 700 – 2, 500, *L. r.* sp. S range 500 – 3, 000, Yamazaki *et al*., 2001) further illustrating the constraints of body size on actual fecundity.

1.5.9 *Death*

Following their strenuous efforts to migrate upstream, create redds and ultimately reproduce, all petromyzontids die. This period is characterised by the rapid onset of morbidity immediately following the expulsion of their gametes, and lampreys begin to exhibit greatly reduced activity levels and aimless behaviours hours after they have completed their spawning activities. *Lampetra fluviatilis* moves into sheltered areas away from the main river flow, and *L. planeri* buries beneath stones where they wait for death (Hagelin, 1959). *Petromyzon marinus*, however, will sometimes remain attached within the nest, which tends to be much deeper than other species and so they are protected from the strongest currents (Applegate, 1950). *Lampetra planeri* may live for > 60 days following completion of spawning (Sterba, 1962; Korolyev & Reshetnikov, 2008) and *I. gagei* up to 26 days (Dendy & Scott, 1953), but this depends largely on the water temperature and the strenuousness of each individual’s reproductive effort. The breakdown of the body tissue is particularly rapid, and most often it is only the notochord that remains, collecting in deeper pools downstream. Interestingly, *P. marinus* has been shown to actively avoid the odour of dead conspecifics (Wagner *et al*., 2011), ensuring that an individual would negate any potential source of mortality if they were still actively migrating upstream later in the season.

Physical degeneration during the spawning period includes atrophy of the gut, progressive blindness and a breakdown of the epidermis that tends to lead to fungal infection (Applegate, 1950). The corneas of the eye become more opaque as spawning progresses and individuals become unresponsive to visual stimuli, while the skin bears large patches that have sloughed off either in response to attachment by other lampreys, or as a result of abrasion with the substrate. The gut, however, degenerates more slowly, beginning with the cessation of feeding many months prior to spawning. When first re-entering rivers the gut of freshwater-resident *P. marinus* is 7 to 11 mm in diameter, but at the time of spawning itself the intestine resembles a long thread running from the mouth to the anus with a much reduced surface area, and may be less than 1 mm in diameter (Applegate, 1950). Natural death can be postponed if lampreys are delayed in reaching sexual maturation *via*: exposure to cold temperatures (9 months); given a hypophysectomy (13 months); or a gonadectomy (4 months), suggesting that sex hormones, such as corticosteroid, play a role in the maintenance of body tissues (Larsen, 1980). However, death also seems likely to be linked to the exhaustion of energy reserves and a build-up of waste products that are stored in the body tissues, particularly bile products (Larsen, 1980).

In an apparent exception to the rule of death following spawning, an isolated report suggested *E. tridentatus* was capable of spawning for a second time (Michael, 1980). During the spring of 1978 and 1979, and presumably following the initial spawning season, an unspecified number of lamprey kelts (a term typically applied to spent salmonids) were collected in a downstream trap from two rivers and a notch cut in one of the dorsal fins. These lampreys were said to be in good condition and some marked individuals were released downstream of the trap. Several years later (Michael, 1984) a call for additional information on this phenomenon revealed that *"some of the adult downstream migrants* ("kelts") *had eggs which were easily extruded…"*. This would suggest that the author is not clear on what the term kelt refers to, and instead equated lamprey that had not yet spawned, but were dropping back downstream, with spent salmonids (that would have no eggs i.e., "kelts") making their way back to sea to feed.

This is evidenced by the following statement in Michael (1984): *"Marks were applied to some of these fish, and during the following spawning season some marked upstream migrants were captured. These fish were substantially larger than when marked, indicating they found a good food source."* If these downstream migrating lampreys contained eggs that were easily extruded then the gut would already have atrophied and the body cavity filled with ova. There is no physiological or behavioural precedent that suggests these individuals could have resumed feeding in this condition, either in freshwater or at sea, put on subsequent growth and migrated back upstream at a larger body size. In addition, the original paper (Michael, 1980) stated that eight downstream migrants were marked, and two returning marked individuals were recaptured the following year. This either indicates a vanishingly small lamprey population size and very high capture efficiency by the upstream traps, or that the upstream migrants bearing marks on the fins were the result of natural abrasions common to many lampreys on their spawning migration and were not in fact recaptured individuals on a second spawning run. Parsimony would suggest the latter is a more desirable explanation, especially when coupled with the extensive body of literature that indicates repeat spawning in petromyzontids is not possible.

1.6 PAIRED SPECIES

Many lamprey species that share a geographical range are morphologically similar or inseparable up to the point of metamorphosis. Following this process phenotypic differences largely depend on aspects of the adult feeding mode, specifically, whether the individual will subsequently feed or not. These are termed paired species (Zanandrea, 1959) or stem-satellite species (Vladykov & Kott, 1979) and they have evolved in seven of the ten extant lamprey genera. The parallel evolution of non-parasitic lampreys from ancestral parasitic and often migratory types, is both unique to the vertebrate lineage (in that non-trophic adults are not apparent in any other group), and yet the evolution of divergent trophic forms *per se* is common to a range of post-glacial freshwater fish taxa (Taylor, 1999).

That non-parasitic lampreys represent recent evolutionary divergence in response to environmental change and resource availability is no longer in doubt, but the cause and tempo of such extreme adaption remains obscure (Salewski, 2003; Docker, 2009). In some species pairs divergence times range from: tens to hundreds of years (e.g., in response to anthropogenic effects) (Yamazaki *et al.*, 2011); tens of thousands of years (e.g., in response to glacial or other climatic events) (Espanhol *et al*., 2007); and possibly hundreds of thousands of years (e.g., in response to geological change) (Docker *et al*., 1999). This is reflected in the degree to which it is possible to differentiate morphologically or molecularly between such pairs, and particularly where recent divergence times are considered, whether or not both members of the pair continue to exist in sympatry. In many cases genetically indistinguishable lamprey species pairs that have an overlapping geographic range are considered as separate taxonomic entities based on adult trophic strategy (Espanhol *et al*., 2007; Boguski *et al*., 2012; Docker *et al*., 2012).

Variation in lamprey foraging ecology, such as facultative parasitism within a single species, or as seen in lampreys such as *L. fluviatilis* that express large anadromous parasitic forms, praecox anadromous parasitic forms, freshwater-resident parasitic forms and genetically indistinguishable stream-resident non-parasitic forms (i.e., *L. planeri*), confounds the usefulness of rigid taxonomic designations. At least within some paired species it would appear that as a result of ecological divergence in sympatry, and in other pairs possibly following a long subsequent period of geographical isolation, non-parasitic lampreys arose as a means of maximising reproductive output by reducing their adult period and increasing their larval duration. Non-parasitic lampreys have, therefore, undergone a heterochronic shift in their developmental timing i.e., altered the time at which they undergo metamorphosis relative to the onset of sexual maturation (Hardisty, 2006). The extent to which trophic plasticity is necessary in the evolution of non-parasitic lampreys i.e., whether the evolutionary trajectory is step-wise (anadromous – praecox/freshwater-resident – non-parasitic), is obscured by the relative lack of study systems containing all three life history strategies.

1.6.1 *Morphological & Life History Differences*

The theory that non-parasitic forms have evolved from ancestral parasitic populations is now widely accepted. Recent histological evidence has confirmed previous physiological studies that suggested some freshwater-resident lampreys, including *P. marinus* from the Laurentian Great Lakes and non-parasitic *L. planeri*, could still osmoregulate in saline conditions (Morris, 1972; Beamish, 1980). It was also discovered that the non-parasitic *L. appendix*, which diverged from anadromous parasitic *L. camtschaticum c*. 130, 000 years ago, still retains chloride cells (Bartels *et al*., 2011) that enable petromyzontids to osmoregulate in hypertonic environments (Bartels & Potter, 2004). In addition, the complex intestinal structures of several non-parasitic species are considered to represent rudimentary mucosal folds important in the absorption of food within the gut of parasitic species (Hilliard *et al.*, 1983; Yamazaki *et al.*, 2001). Therefore, retention of non-functional, yet well-developed, anatomical structures useful for post-metamorphic feeding still expressed in non-parasitic lampreys indicates their ancestral importance and the relatively recent loss of function.

The potential and actual fecundity of some paired species varies, potentially enabling the separation of each life history type at an early stage in development. For example, the number of mature eggs promoted by *L. fluviatilis* and anadromous *P. marinus* is very close to the number of oocytes the ammocoetes exhibit. While in the closely related non-parasitic *L. planeri* and freshwater-resident *P. marinus* the number of oocytes is far greater than the number of eggs matured by the adults (Hardisty, 1969). This could be seen as the effect of recent divergence, where a large discrepancy between the reproductive potential and an adult's actual fecundity, represents an energetic "waste" that natural selection has not yet winnowed down to the minimum level seen in ancestral populations. However, as the energy from developing oocytes is not in fact "wasted" *per se* (as that energy is retained within the body) it is possible that mass atresia of oocytes is in part necessary for fuelling the post-metamorphic life of recently derived non-parasitic forms (Hardisty, 1963; M. Docker, *pers. com.*). Concurrent with metamorphosis, non-parasitic forms begin to undergo final maturation of the gametes, but then spend the next few months developing secondary sexual characteristics and moving upstream onto the spawning grounds. Here they must construct their nests and take part in the strenuous spawning activities, all fuelled by the lipids assimilated during the ammocoete phase. Therefore, if they could make use of the breakdown of the "untapped" potential stock of eggs, this may act to maintain large numbers of oocytes that would otherwise never be expressed by the mature adult.

This pattern of mass atresia is not evident in all non-parasitic forms though, and *L. planeri* which loses 60 to 90% of its reproductive potential (Hardisty, 1964), may represent an extreme example. Similar levels of atresia were recorded between *I. castaneus* and its non-parasitic derivative *I. gagei*, suggesting an efficient energetic strategy has evolved by establishing actual fecundity early on in the non-parasitic life history strategy (Beamish & Thomas, 1983). In *L. planeri* the situation may be quite the opposite, where although it appears to be an extremely inefficient energetic strategy, the potential fitness benefits may be greater if an individual's life history strategy is not fixed early in life. Presumably both of these strategies represent differences in the divergence times from ancestral species (Hardisty, 1970; Beamish & Thomas, 1983) whereby *L. planeri* has yet to achieve an energetic balance, resulting from the recent adoption of a non-parasitic strategy.

In some systems certain petromyzontids appear highly variable in their trophic ecology, expressing divergent foraging strategies and discrete phenotypic variation within species. This commonly takes the form of anadromy *vs.* freshwater-residence strategies, resulting in two body size modes in a single spawning population, such as seen in *L. fluviatilis* from Loch Lomond, Scotland (Adams *et al*., 2008) or *L. camtschaticum* from Alaska (Heard, 1966) and Japan (Iwata & Hamada, 1986; Yamazaki *et al*., 1998). Other parasitic lamprey populations contain praecox forms that express a small body size, not through freshwater residency, but from a reduced period of feeding at sea. This appears particularly common among *E. tridentatus* populations from the Pacific Coast of North America, where small and large body size lampreys can be found in sympatry (Pletcher, 1963; Beamish, 1980; Kostow, 2002), but is also seen in *L. camtschaticum* from Kamchatka (Kucheryavyi *et al*., 2007; Nazarov *et al*., 2011) and *L. fluviatilis* from Europe (Berg, 1931; Abou-Seedo & Potter, 1970).

There are less common strategies expressed by a few rather enigmatic lamprey species, but which shed some light on the adaptability of lamprey life history strategies and suggest that non-parasitism is not necessarily an evolutionary end-point. The diminutive parasitic *E. minimus*, a freshwater-resident species of a small lake system in Oregon, forages for just a few winter months, but, may be capable of spawning without ever feeding after metamorphosis (Kostow, 2002). Another small freshwater parasitic species *E. danfordi* has also been suggested to exhibit a non-parasitic form, formerly recognised as *E. gracilis* (Renaud *et al*., 2009; Renaud, 2011). Facultative non-parasitism such as this could enable these lamprey populations to survive in the absence of any suitable hosts, the numbers of which may fluctuate year to year given the restricted habitat available to them.

The opposite of this strategy, facultative parasitism by a typically non-parasitic form, has been documented in *L. richardsoni* from Vancouver Island (Beamish, 1985). Here, a small stream population of lampreys exhibits two forms, a typical non-parasitic type and another, that when exposed to fish hosts, will begin feeding parasitically. This is reflected by changes to its morphology, including an anatomical resemblance to its parasitic pair member *L. ayresii*, and a larger size at sexual maturity (Youson & Beamish, 1991). Although the foregut of non-parasitic forms does not typically open such a condition has been documented in both *L. planeri* (Morris, 1972) and *L. appendix* (Gage, 1928), and could represent an example of evolutionary atavism. This is further exemplified by the presence of “giant” *L. appendix* specimens (Manion & Purvis, 1971; Cochran, 2008) that are many times heavier than typical non-parasitic populations and resemble parasitic lampreys in having sharp teeth and a complex intestinal surface.

The strongest process believed to act in maintaining pairs of parasitic and non-parasitic lamprey species is that of size assortative mating; specifically, that homogamy will act to prevent gene flow between small non-parasitic forms and large parasitic forms. This size barrier effect was quantified as resulting in little to no successful spawning where body size differences between mates was 25% or greater (Beamish & Neville, 1992). Although fertilisation success is reduced where size differences are greater (Malmqvist, 1983), this size ratio does however cover many paired species, and differences greater than 25% can still result in some fertilised eggs. Homogamy will not have an effect though where sneak mating behaviours are employed by males. *Lampetra fluviatilis* and *L. planeri* have recently been documented as exhibiting inter-specific sneak tactics, whereby single males attempt to achieve fertilisation of the other species eggs (Hume *et al.*, *in press*). *Lampetra appendix* nests containing three or more lampreys contained a sneaker male on as many as half of all occasions in another study by Cochran *et al.* (2008), and there are unpublished reports of at least three Asian lamprey species exhibiting similar within-species sneak tactics (Iwata & Hamada, 1986).

1.6.2 *Molecular Ecology*

If lamprey species pairs represent “good” species, then differences in body size at sexual maturity between these divergent life history types should act to prevent gene flow between them, caused by assortative mating and reinforced by the subsequent evolution of reproductive isolation. Genetic divergence between life history types that are expressed in a single polymorphic species would suggest that sympatric speciation may be the typical mode that has led to the evolution of non-parasitic forms (Salewski, 2003). Reproductive isolation of fragmented non-parasitic types from migratory parasitic populations could equally be the result of allopatric speciation events as caused by geological, climatic or even contemporary anthropogenic effects (Yamazaki & Goto, 1996; Yamazaki *et al.*, 2011). This is reflected in the differences in tempo of speciation events between lamprey populations, and it may be that anthropogenic effects, such as the creation of barriers to migration, has played an important role in reshaping the direction of gene flow between many localised lamprey species pairs (Yokoyama *et al*., 2009; Yamazaki *et al*., 2011*a*, 2011*b*). In lampreys belonging to the genus *Lampetra* from western North America both shallow and deep divergences were found between cytochrome *b* (cyt *b*) haplotypes that did not reflect current taxonomic nomenclature (Boguski *et al*., 2012). This variation reflects differences in the time at which divergence between non-parasitic and parasitic populations occurred, and indicates the presence of several discrete cryptic non-parasitic species. The highly conserved morphology of non-parasitic forms lends itself to the possibility of yet more cryptic species, as seen between the very highly divergent *Lethenteron* sp. S and other Asian species (Yamazaki & Goto, 1998; Yamazaki *et al*., 2003).

There is evidence that some non-parasitic lampreys evolved from a common parasitic ancestor deeper in the past (range 0.9 – 2.7 MYA) and which are now considered separate species, such as the relationship between the non-parasitic *L. zanandreai* and parasitic *L. fluviatilis* (Tagliavini *et al*., 1994; Docker *et al*., 1999; Caputo *et al*., 2009) and *L. aepyptera* (Martin & White, 2008). With the eventual cessation of gene flow between some isolated populations of parasitic and non-parasitic populations high levels of endemism have resulted, such as seen in: the glacial refugia of the Iberian Peninsula (Pereira *et al*., 2010; Mateus *et al*., 2011, 2012); the relict Mexican species pair *Tetrapleurodon* spp. (Mejía *et al*., 2004); and the lampreys of British Columbia (Taylor *et al*., 2012).

However, in recently diverged petromyzontid paired species that still exist in sympatry, there is no support for genetic differentiation between the life history types belonging to two or more putative species. For example, in *L. fluviatilis* and *L. planeri* using both mitochondrial DNA (mtDNA) and nuclear markers no species specific characters have been found (Schreiber & Engelhorn, 1998; Docker *et al*., 1999; Espanhol *et al*., 2007; Blank *et al*., 2008; Pereira *et al*., 2011), or between *L. camtschaticum* and the non-parasitic *L. reissneri* (Artamonova *et al*., 2011), suggesting that both pairs represent alternative life history strategies of single species. This has been more conclusively demonstrated in the species pairs *I. unicuspis* and *I. fossor* and *L. ayresii* and *L. richardsoni* from North America, where using microsatellite markers no species specific markers could be found where the pair was found in sympatry (Boguski *et al*., Docker *et al*., 2012). This evidence further supports the view that at least some non-parasitic types have evolved independently and repeatedly from parasitic populations, and suggests ongoing gene flow or plasticity in life history strategy.

1.7 THESIS AIMS

The principle focus of this thesis will be the evolutionary ecology of paired lamprey species, utilising a range of approaches, including: ecological, behavioural, taxonomic and molecular genetic studies. This will be achieved by focussing on sympatric populations of European river lamprey *Lampetra fluviatilis* and brook lamprey *L. planeri* from Loch Lomond, Scotland as a case study for explaining the origin, maintenance and significance of lamprey life history diversity in an evolutionary context. This thesis aims to present a greater understanding of this phenomenon based on the following studies:

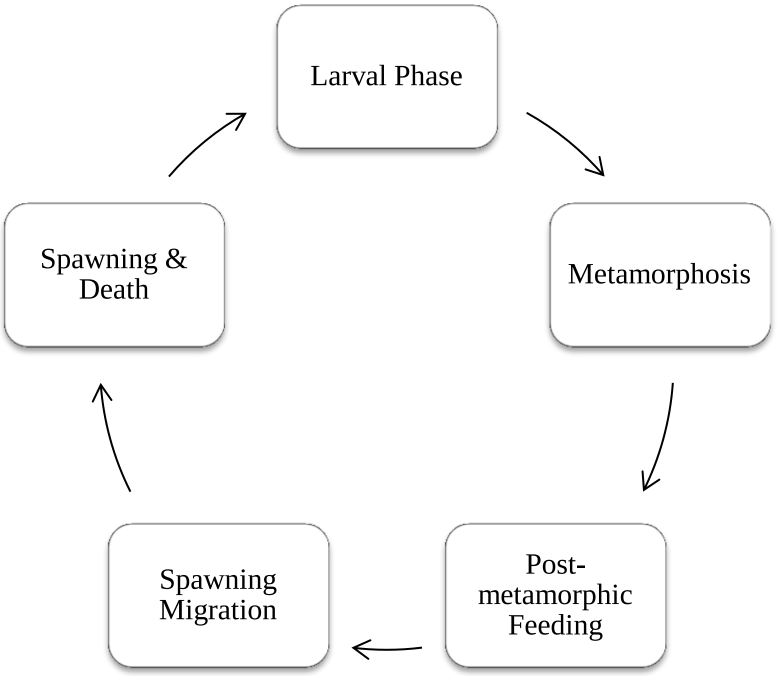
- An extensive review of lamprey literature to evaluate the standing knowledge base and help identify those aspects of lamprey biology likely to yield significant evolutionary insights.
- An evaluation of the foraging strategy of parasitic *Lampetra fluviatilis* within Loch Lomond. This lake contains a freshwater-resident population known to formerly parasitise a host species now thought to be in decline. The response of river lampreys to this decline is examined and dietary plasticity in this species discussed.
- The distribution of lampreys in the Loch Lomond basin was not previously well known. Surveys of adult lampreys undertaking upstream spawning migrations throughout the lake basin were conducted and the timing, extent and duration of this migratory period described. A single river, the Endrick Water, was found to contain the largest populations of lamprey and its significance in regards to the conservation of lampreys exhibiting alternative life history strategies in the lake basin discussed.
- The strength of assortative mating between sympatric anadromous *L. fluviatilis*, freshwater-resident *L. fluviatilis* and non-parasitic *L. planeri* collected from the Endrick Water was tested in an artificial stream environment to test for the presence of pre-zygotic barriers to gene flow. An absence of strong positive mating indicates species specific behavioural cues have not evolved between these paired species. The implications of these findings, and the presence of intermediate phenotypes such as

freshwater-resident forms, are discussed in relation to the potential for gene flow between ecologically divergent lamprey populations.

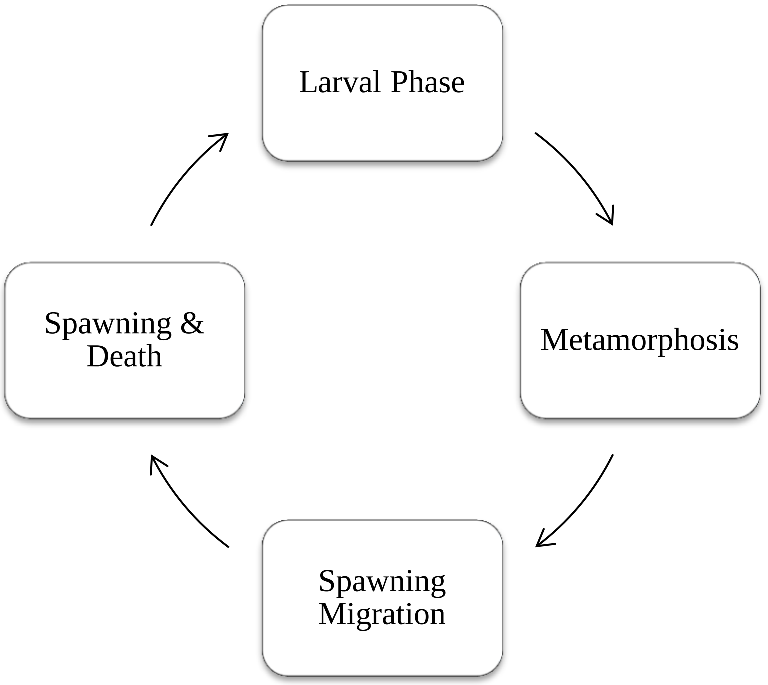
- Observations of spawning among the sympatric populations of the Endrick Water revealed the presence of inter-specific sneak male mating tactics. This behaviour is described for the first time in petromyzontids and its relevance to gene flow patterns between sympatric paired species is discussed.
- If genetic introgression is possible between petromyzontid paired species, due to weak assortative mating or the presence of intermediate phenotypes, then hybrid offspring may be present within the Endrick Water. The viability (survivorship) of hybrid offspring between the sympatric populations from this river was tested *in vitro* to examine the strength of post-zygotic barriers to gene flow.
- Morphological and genetic similarities between petromyzontid paired species have resulted in taxonomic confusion and the uncertainty that non-parasitic forms represent distinct taxa. Traditional morphological taxonomic characters and mtDNA sequences of a range of *L. fluviatilis* foraging strategies, and disjunct populations of non-parasitic *L. planeri*, were examined critically in an attempt to resolve the taxonomy of this lamprey pair. A lack of species-specific characters, either morphologically or genetically, indicates *L. planeri* should be considered a life history variant of *L. fluviatilis*.
- Concluding evidence from all of these studies is discussed in the context of conserving a single phenotypically and ecologically variable lamprey species and recommendations for the conservation and management of *L. fluviatilis* in the U.K. are presented.

Appendix 1.1

Parasitic Lamprey Species



Non-parasitic Lamprey Species



Appendix 1.2

Lamprey Taxonomy

Order PETROMYZONTIFORMES

- Family GEOTRIIDAE
  - Genus *Geotria* Gray 1851
    - **Geotria australis* Gray 1851
- Family MORDACIIDAE
  - Genus *Mordacia* Gray 1851
    - **Mordacia lapicida* (Gray 1851)
    - **Mordacia mordax* (Richardson 1846)
    - †*Mordacia praecox* Potter 1968
- Family PETROMYZONTIDAE
  - Genus *Caspiomyzon* Berg 1906
    - **Caspiomyzon wagneri* (Kessler 1870)
  - Genus *Entosphenus* Gill 1862
    - †*Entosphenus folletti* Vladykov & Kott 1976
    - †*Entosphenus hubbsi* Vladykov & Kott 1976
    - †*Entosphenus lethophagus* (Hubbs 1971)
    - **Entosphenus macrostomus* (Beamish 1982)
    - **Entosphenus minimus* (Bond & Kan 1973)
    - **Entosphenus similis* Vladykov & Kott 1979
    - **Entosphenus tridentatus* Gairdner in Richardson 1836

Genus *Eudontomyzon* Regan 1911

- **Eudontomyzon danfordi* Regan 1911
- †*Eudontomyzon graecus* Renaud & Economidis 2010
- †*Eudontomyzon hellenicus* Vladykov *et al.* 1982
- †*Eudontomyzon mariae* (Berg 1931)
- **Eudontomyzon morii* (Berg 1931)

Genus *Ichthyomyzon* Girard 1858

- **Ichthyomyzon bdellium* (Jordan 1885)
- **Ichthyomyzon castaneus* Girard 1858
- †*Ichthyomyzon fossor* Reighard & Cummins 1916
- †*Ichthyomyzon gagei* Hubbs & Trautman 1937
- †*Ichthyomyzon greeleyi* Hubbs & Trautman 1937
- **Ichthyomyzon unicuspis* Hubbs & Trautman 1937

Genus *Lampetra* Bonnaterre 1788

- †*Lampetra aepyptera* (Abbott 1860)
- **Lampetra ayresii* (Günther 1870)
- **Lampetra fluviatilis* Linnaeus 1758
- †*Lampetra lanceolata* Kux & Steiner 1972
- †*Lampetra pacifica* Vladykov 1973
- †*Lampetra planeri* (Bloch 1784)
- †*Lampetra richardsoni* Vladykov & Follett 1965

Genus *Lethenteron* Creaser & Hubbs 1922

- †*Lethenteron alaskense* Vladykov & Kott 1978

†*Lethenteron appendix* (DeKay 1842)

**Lethenteron camtschaticum* (Tilesius 1811)

†*Lethenteron kessleri* (Anikin 1905)

†*Lethenteron ninae* Naseka, Tuniyev & Renaud 2009

†*Lethenteron reissneri* (Dybowski 1869)

†*Lethenteron zanandreai* (Vladykov 1955)

Genus *Petromyzon* Linnaeus 1758

**Petromyzon marinus* Linnaeus 1758

Genus *Tetrapleurodon* Creaser & Hubbs 1922

†*Tetrapleurodon geminis* Álvarez del Villar 1966

**Tetrapleurodon spadiceus* (Bean 1887)

* Indicates species that typically feed following metamorphosis, either in marine or freshwater environments (i.e., are parasitic).

† Indicates species that do not typically feed following metamorphosis, and remain in natal rivers (i.e., are non-parasitic).

Appendix 1.3

Metamorphic Period

	June	July	August	September	October	November	December	January	February	March	April	May
Geotria australis (P)			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨		
Mordacia lapicida (P)			🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨		
Mordacia mordax (P)									🟨	🟨	🟨	
Mordacia praecox (NP)					🟨	🟨	🟨					
Caspiomyzon wagneri (P)		🟨	🟨	🟨	🟨	🟨	🟨					
Entosphenus folletti (NP)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Entosphenus hubbsi (NP)			🟨	🟨	🟨							
Entosphenus lethophagus (NP)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Entosphenus macrostomus (P)		🟨	🟨	🟨								
Entosphenus minimus (P)				🟨	🟨	🟨	🟨					
Entosphenus similis (P)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Entosphenus tridentatus (P)		🟨	🟨	🟨	🟨	🟨						
Eudontomyzon danfordi (P)		🟨	🟨	🟨	🟨							
Eudontomyzon graecus (NP)					🟨	🟨	🟨	🟨	🟨	🟨		
Eudontomyzon hellenicus (NP)					🟨	🟨	🟨	🟨				
Eudontomyzon mariae (NP)		🟨	🟨	🟨	🟨	🟨						
Eudontomyzon morii (P)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Ichthyomyzon bdellium (P)	🟨	🟨	🟨	🟨								
Ichthyomyzon castaneus (P)					🟨	🟨	🟨					
Ichthyomyzon fossor (NP)			🟨	🟨	🟨							
Ichthyomyzon gagei (NP)			🟨	🟨	🟨	🟨	🟨	🟨				
Ichthyomyzon greeleyi (NP)			🟨	🟨	🟨							

Ichthyomyzon unicuspis (P)						🟨	🟨	🟨				
Lampetra aepyptera (NP)			🟨	🟨	🟨				🟨			
Lampetra ayresii (P)		🟨	🟨	🟨								
Lampetra fluviatilis (P)	🟨	🟨	🟨									
Lampetra lanceolata (NP)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Lampetra pacifica (NP)					🟨	🟨	🟨					
Lampetra planeri (NP)	🟨	🟨	🟨	🟨	🟨	🟨						
Lampetra richardsoni (NP)				🟨	🟨	🟨						
Lethenteron alaskense (NP)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Lethenteron appendix (NP)			🟨	🟨	🟨							
Lethenteron camtschaticum (P)			🟨	🟨	🟨	🟨	🟨	🟨				
Lethenteron kessleri (NP)		🟨	🟨	🟨	🟨	🟨						
Lethenteron ninae (NP)		🟨	🟨	🟨								
Lethenteron reissneri (NP)		🟨	🟨	🟨								
Lethenteron zanandreai (NP)	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛	⬛
Petromyzon marinus (P)	🟨	🟨	🟨	🟨	🟨						🟨	🟨
Tetrapleurodon geminis (NP)	🟨	🟨	🟨								🟨	🟨
Tetrapleurodon spadiceus (P)	🟨	🟨	🟨								🟨	🟨

Key

(P)	Parasitic
(NP)	Non-parasitic
🟨	Metamorphosis known to occur during this month
⬛	No data available

"Salar could not shake off Petromyzon. The lamprey's mouth was stuck firmly his left ventral side below the medial lie of nerves, forward of the ventral fins. Indifferent to the salmon's slipping and turning rushes, to his rolling staggers as he changed from one tide pressure to another, Petromyzon sucked the scales closer to his teeth and began to rasp away and swallow skin and curd and flesh. He drew blood, and fed contentedly."

Henry Williamson (1935), *Salar the Salmon*

"Vedius Pollio kept in ponds huge lampreys that had been trained to eat men, and he was accustomed to throw to them such of his slaves that he desired to put to death. Once, when he was entertaining Augustus, his cup-bearer broke a crystal goblet, and without regard for his guest, Pollio ordered the fellow to be thrown to the lampreys."

Pliny the Elder (77), *Natural History*

Chapter Two

Evidence of a recent decline in lamprey parasitism of a nationally rare whitefish *Coregonus lavaretus* in Loch Lomond, Scotland: is there a diamond in the ruffe?

2.1 ABSTRACT

Lamprey-induced scarring of the nationally rare *Coregonus lavaretus*, a known host of a freshwater-resident population of European river lamprey *Lampetra fluviatilis*, has declined precipitously since the establishment of several non-native fish in Loch Lomond. Recent evidence points to the possibility that *L. fluviatilis* in this lake may have altered its trophic ecology in response to the negative impact non-native species have had on their favoured host.

2.2 INTRODUCTION

Lampreys (Petromyzontiformes) comprise a relatively enigmatic group of fish that express a range of specialised foraging strategies prior to sexual maturation. These include parasitic or predacious modes of feeding on actinopterygian fishes in marine or freshwater environments, where the blood and/or body tissues of hosts are removed (Renaud *et al.*, 2009); carrion feeding and scavenging (Holčík, 1986; Kan & Bond, 1981); or in many species the complete absence of a post-larval feeding phase (Hardisty & Potter, 1971). Some large European lake systems contain populations of lampreys known to feed exclusively within the lake itself; including lakes Onega and Ladoga in the Russian Federation (Berg, 1948), several lakes in Finland (Valovirta, 1950; Tuunainen *et al.*, 1980), Lough Neagh, Northern Ireland (Goodwin *et al.*, 2006) and Loch Lomond, Scotland (Maitland, 1980).

However, these populations remain mostly uncharacterised, to a large extent due to inherent difficulties in observing adult lampreys foraging under natural conditions and a reliance on the observation of prey that have survived being fed on by lampreys. This methodology does not lend itself to clearly defined descriptions of petromyzontid foraging ecology as it reveals little information about either host mortality or lamprey behaviour, and there must necessarily be some interpolation between sparse or incidental data. Yet scarring data from surviving hosts remain a critical source of information, particularly in a conservation context (DFO, 2010). Many freshwater-resident lamprey species are also endemic (Taylor *et al.*, 2012) and with the notable exception of sea lamprey *Petromyzon marinus* L. 1758 in the Laurentian Great Lakes most of these populations are drastically understudied.

Loch Lomond is both the largest area of freshwater in the U.K. (71 km^2) and contains the greatest number of fish species of any lake in Scotland (Winfield *et al.*, 2010). Fifteen species are native, including populations of the nationally rare whitefish *Coregonus lavaretus* (L. 1758) (known locally as powan). This study refers to the more usual *C. lavaretus*, in preference to *C. clupeoides* Lacepède 1803 for Loch Lomond coregonids, as suggested by Kottelat & Freyhof (2007). Recent work has shown these fish to be indistinguishable from other putatively identified “species” of *C. lavaretus* in the U.K. (Etheridge *et al*., 2012). Loch Lomond also supports a freshwater-resident European river lamprey *Lampetra fluviatilis* (L. 1758) population, and both *L. fluviatilis* and *C. lavaretus* currently experience substantial national and international conservation protection. Since 1970 a further six non-native species have been recorded (Adams, 1994; Etheridge & Adams, 2008), the most pernicious being the deliberate introduction of ruffe *Gymnocephalus cernuus* (L. 1758) to the lake prior to 1982 (Maitland *et al.*, 1983). This event marked a clear watershed moment in the ecology of the Loch Lomond system, none more so than its effect on the trophic interactions between piscivorous species and *C. lavaretus*.

Gymnocephalus cernuus are thought to have a deleterious effect on the *C. lavaretus* population, as they are a major predator of their ova (Adams & Tippett, 1991; Etheridge *et al*., 2011). However, prior to the introduction of *G. cernuus*, *C. lavaretus* were themselves a favoured prey item for several predatory species, including: otters *Lutra lutra* (L. 1758) (McCafferty, 2005), grey herons *Ardea cinerea* (L. 1758) (Adams & Mitchell, 1995), cormorants *Phalacrocorax carbo* (L. 1758) (Adams *et al*., 1994), and Northern pike *Esox lucius* L. 1758 (Adams, 1991), all of which subsequently altered their trophic ecology to feed heavily on *G. cernuus* as the population increased exponentially through the 1980s (Maitland & East, 1989). One additional species that was known to utilise *C. lavaretus* as a key prey item in the past is *L. fluviatilis* (Maitland, 1980). This parasitic lamprey is typically anadromous, feeding within estuaries before returning to rivers in order to spawn (Maitland *et al*., 1984), but in Loch Lomond the population comprises two components, one of which retains anadromous tendencies while the other remains within the lake itself to feed (Maitland *et al*., 1994; Adams *et al*., 2008).

Casual observation had suggested that the number of *C. lavaretus* exhibiting evidence of lamprey feeding, collected from the lake during routine monitoring and other scientific studies in recent years, was remarkably low compared with past experience. Given the significant increase in scientific and conservation interest of both *C. lavaretus* (Etheridge *et al*., 2010*a*, *b*; Etheridge *et al.*, 2011; Etheridge *et al*., 2012*a*, *b*) and *L. fluviatilis* populations of Loch Lomond (Adams *et al*., 2008; Hume, 2011; Hume *et al*., 2012; Hume *et al*., *in press*) it was deemed an appropriate time to re-examine the *C. lavaretus* population for lamprey-induced feeding scars (*sensu* Maitland, 1980). Here, evidence is presented that suggests a change in trophic interactions between *L. fluviatilis* and *C. lavaretus* that has occurred since the introduction of non-native fish species to Loch Lomond, by comparing the proportion of *C. lavaretus* that were historically parasitised (pre-1980) to the proportion parasitised in 2010. Such data are discussed in regard to how it could be used to infer *L. fluviatilis* foraging behaviour within Loch Lomond, in light of several competing hypotheses, and some ways in which important knowledge gaps can be filled are suggested.

2.3 MATERIALS & METHODS

Coregonus lavaretus were collected in gillnets set overnight in December 2009 through to February 2010 as part of a separate study, and examined for lamprey-induced scars; that is Type A and B, Stage IV marks *sensu* Ebener *et al*. (2006). Scars appear as roughly circular patches of scale-less skin, sometimes with a shallow depression, and are easily recognised and distinguished from damage caused by nets or birds. Depth and location of nets varied widely throughout the study period, and knot-to-knot mesh of nets deployed ranged from 25 to 38 mm. The presence/absence of lamprey-induced scars, and their frequency on individuals where present, was recorded for each *C. lavaretus* specimen along with fork length (FL) (± 1 mm). A mixed collection of several other fish species during the study period was similarly examined for lamprey-induced scars. The 2010 results on the frequency of lamprey-induced scars on *C. lavaretus* in the lake were compared to historic data for the period 1951-79 (Maitland, 1980). In the historic data set, only *C. lavaretus* known to be collected between December and February were included and data from these years combined (1951-79) so that comparisons could be made with the single 2010 data set.

2.4 RESULTS

The proportion of *C. lavaretus* in Loch Lomond bearing scars in 2010 was significantly lower than during the period 1951-79 (χ^2, d.f. = 1, $P < 0.001$) (Table 2.1). The frequency of scarred *C. lavaretus* in the period 1951-79 ranged from 26 to 43% (mean 36.6%), while in 2010 only 6% of *C. lavaretus* exhibited lamprey-induced scars. The incidence of multiple scars was also lower in 2010, where two scars were the maximum observed on an individual, compared with up to eight scars in 1951-79. The mean lengths of *C. lavaretus* collected in 2010 were, however, significantly greater than that of 1951-79, both for unscarred (T-test, d.f. = 341, $P < 0.05$) and scarred individuals (d.f. = 21, $P < 0.05$) (Fig. 2.1). Scarring was not randomly distributed amongst *C. lavaretus* size classes, but was most frequently recorded from individuals of 250 – 350 mm fork length (FL) (Fig. 2.2). Across both sampling periods, 90.9% (n = 379) of *C. lavaretus* within this size range were scarred at least once, and no specimens outside this size range were scarred three or more times. Individuals < 250 mm FL were scarred at low frequencies (7%, n = 30), and no specimen < 210 mm FL bore scars although fish as small as 150 mm were examined.

Five other fish species were collected during sampling in 2010: roach *Rutilus rutilus* (L. 1758) (n = 443), *E. lucius* (n = 4), perch *Perca fluviatilis* L. 1758 (n = 39), *G. cernuus* (n = 1) and brown trout *Salmo trutta* L. 1758 (n = 24). Only *R. rutilus* exhibited lamprey-induced scars (2.3%, n = 10), and those individuals had a similar mean FL (224, range 202 – 248 mm), compared with unscarred specimens (n = 129, mean 228, range 183 – 290 mm).

2.5 DISCUSSION

It is apparent that *C. lavaretus* were historically an important constituent of the diet of *L. fluviatilis* in Loch Lomond (Maitland, 1980 and references therein), and that presently the evidence for continued parasitism of the *C. lavaretus* population has declined drastically. A single major event (i.e., the introduction of *G. cernuus* by 1982) has occurred in Loch Lomond since the collection of the historic data and four hypotheses, not necessarily mutually exclusive, could be used to explain the apparent differences between the historic and recent data sets. An explanation of their relevance to interpreting the foraging ecology of *L. fluviatilis*, and ways in which to address any remaining knowledge gaps follows:

1) *A decline in the L. fluviatilis population size* – If the number of *L. fluviatilis* entering the lake to feed each year has declined since 1979 this might explain why fewer *C. lavaretus* were parasitised and subsequently collected in 2010, compared with the historic data. No quantitative data exist, however, on the numbers of adult *L. fluviatilis* between 1951 and 1979 making comparisons impossible. Yet, what sporadic data are available suggests adult *L. fluviatilis* numbers have at least remained stable since 1983 (summarised in Hume, 2011).
2) *An increase in C. lavaretus population size* – Similarly, if the number of *C. lavaretus* available to *L. fluviatilis* each year has increased since 1979 this could result in the reduced probability of capturing *C. lavaretus* specimens in 2010 that had been parasitised. Recent evidence from hydroacoustic surveys actually suggests that the *C. lavaretus* population is in decline (Winfield *et al*., 2008), likely the result of elevated ova predation by *G. cernuus* (Etheridge *et al*., 2011). Such a situation might, however, be expected to result in similar or greater proportions of scarred *C. lavaretus* in recent years, if the *L. fluviatilis* population size itself has remained stable. This is difficult to separate from the possibility that *L. fluviatilis* may find it more difficult to detect a less abundant host. An experimental design featuring choice chambers containing diffuse *vs.* concentrated *C. lavaretus* holding water could be used as means of testing the relative importance of olfaction in the ability of petromyzontids to detect potential hosts and orient towards them (Kleerekoper & Mogensen, 1963).
3) *Higher C. lavaretus mortality rate* – If, as has been suggested by Winfield *et al.* (2008), the population of *C. lavaretus* is now comprised mainly of individuals 40 – 99 mm FL, then it is possible *L. fluviatilis* continue to parasitise *C. lavaretus* yet they are not surviving to be counted as scarred individuals in the population. However, Maitland (1980) suggested *C. lavaretus* < 250 mm were rarely parasitised, possibly as the result of a pelagic life history strategy, and certainly very few individuals < 250 mm were recorded with scars either historically or recently. Examining the frequency of scars on individuals cannot inform estimations of mortality in the population though, only suggest that a substantial proportion of *C. lavaretus* have survived to be counted (Schneider *et al*., 1996). Therefore, an experimental approach utilising *C. lavaretus* of different size classes (e.g., < 250 mm, 250 – 350 mm, > 350 mm) and exposing them to lamprey parasitism in a controlled environment (Farmer & Beamish, 1973) could provide data necessary in forming any robust conclusions about the effect of *L. fluviatilis* parasitism on its host.

4) *Host switching* – *Lampetra fluviatilis* could have altered its feeding preferences to parasitise alternative hosts, and certainly native *S. trutta* and *R. rutilus* were historically parasitised in Loch Lomond (Maitland, 1980). However, the mixed species collection of 2010 found that the proportion of *R. rutilus* scarred by *L. fluviatilis* has also declined in comparison to the 1951-79 period (5% to 2.3%) and *S. trutta* were not found to be scarred at all. Elsewhere, freshwater-resident *L. fluviatilis* in Lough Neagh switch from feeding on native to non-native hosts throughout the year, and the latter now contribute a significant proportion of their diet (Inger *et al*., 2010). In Loch Lomond, *G. cernuus* is numerically the most abundant non-native species (Adams, 1994), yet there is no evidence *L. fluviatilis* have begun to parasitise them since their colonisation was first detected in 1982. It is possible, however, that *G. cernuus* experiences greater mortality due to their smaller body size (Kitchell, 1990) compared to *C. lavaretus* [100 – 150 mm *vs*. 150 – 400 mm; Maitland (2000)], and are therefore unlikely to be collected while bearing a lamprey-induced scar. Certainly other predatory species altered their trophic ecology in Loch Lomond to exploit the establishment of *G. cernuus* (see above), and some lamprey species are known to utilise very small hosts (Cochran & Jenkins, 1994). Although a large-scale survey of all fish species bearing fresh wounds in Loch Lomond during the summer feeding period of *L. fluviatilis* would be most likely to yield critical data, ethically and logistically this would be very difficult to justify. Alternatively, either a targeted examination of the *G. cernuus* population during the summer trophic period of *L. fluviatilis*, or a captive feeding experiment utilising both *C. lavaretus* and *G. cernuus* hosts within aquaria could establish the relative rates of mortality in these species as a result of *L. fluviatilis* feeding.

Drawing these four hypotheses together and teasing apart the relative effects each may have on the other is not simple, and the results of future studies outlined above will be likely to shed more light on a complex situation. Yet, even though it is not possible to conclusively explain these data with any one theory, a combination of these may be used to infer some key points about the trophic ecology of *L. fluviatilis* in Loch Lomond, and petromyzontids elsewhere, and how it may have changed in response to the introduction of non-native species.

Prior to the introduction of *G. cernuus* to the lake, *C. lavaretus* was exceptionally abundant in Loch Lomond and exploited by a variety of species including *L. fluviatilis*.

Coregonus lavaretus of a size 250 – 350 mm FL were, in particular, heavily parasitised by the *L. fluviatilis* population, and many exhibited multiple scars within this size range. As *C. lavaretus* appear to undergo a change in habit from pelagic to at least partly benthic foraging at *c.* 250 mm FL (Maitland, 1980; Etheridge *et al*., 2010*b*), it seems likely that at this point *C. lavaretus* become vulnerable to *L. fluviatilis* searching for suitable hosts. *Coregonus lavaretus* < 250 mm are probably not exposed to foraging *L. fluviatilis* and so are rarely scarred. *Coregonus lavaretus* > 350 mm that have already "passed through" the stage of lamprey-vulnerability, probably suffer increased rates of mortality due to repeated parasitic attacks, and do not survive to be counted as scarred, as explained by the lack of evidence for increasing scar numbers on larger individuals.

As *G. cernuus* also forage benthically there exists the possibility that *L. fluviatilis* searching for a suitable host (i.e., *C. lavaretus* 250 – 350 mm FL) will encounter *G. cernuus* in high numbers, yet parasitised *G. cernuus* are not surviving to be counted as scarred given their small body size in comparison to *C. lavaretus*. This could also explain the reduced incidence of multiple scars on *C. lavaretus* of suitable size in 2010; as *L. fluviatilis* in the lake are no longer prioritising formerly abundant *C. lavaretus* hosts but feeding heavily on the now abundant *G. cernuus*. At present this appears to be the most parsimonious explanation for evidence of a change in *L. fluviatilis*-*C. lavaretus* trophic interactions, and suggests that *L. fluviatilis* populations may be adaptable in their foraging ecology (Inger *et al*., 2010). Although undoubtedly of conservation concern, curiously the establishment of *G. cernuus* in Loch Lomond may act to secure the long term stability of two of its most endemic populations.

2.6 FIGURES & TABLES

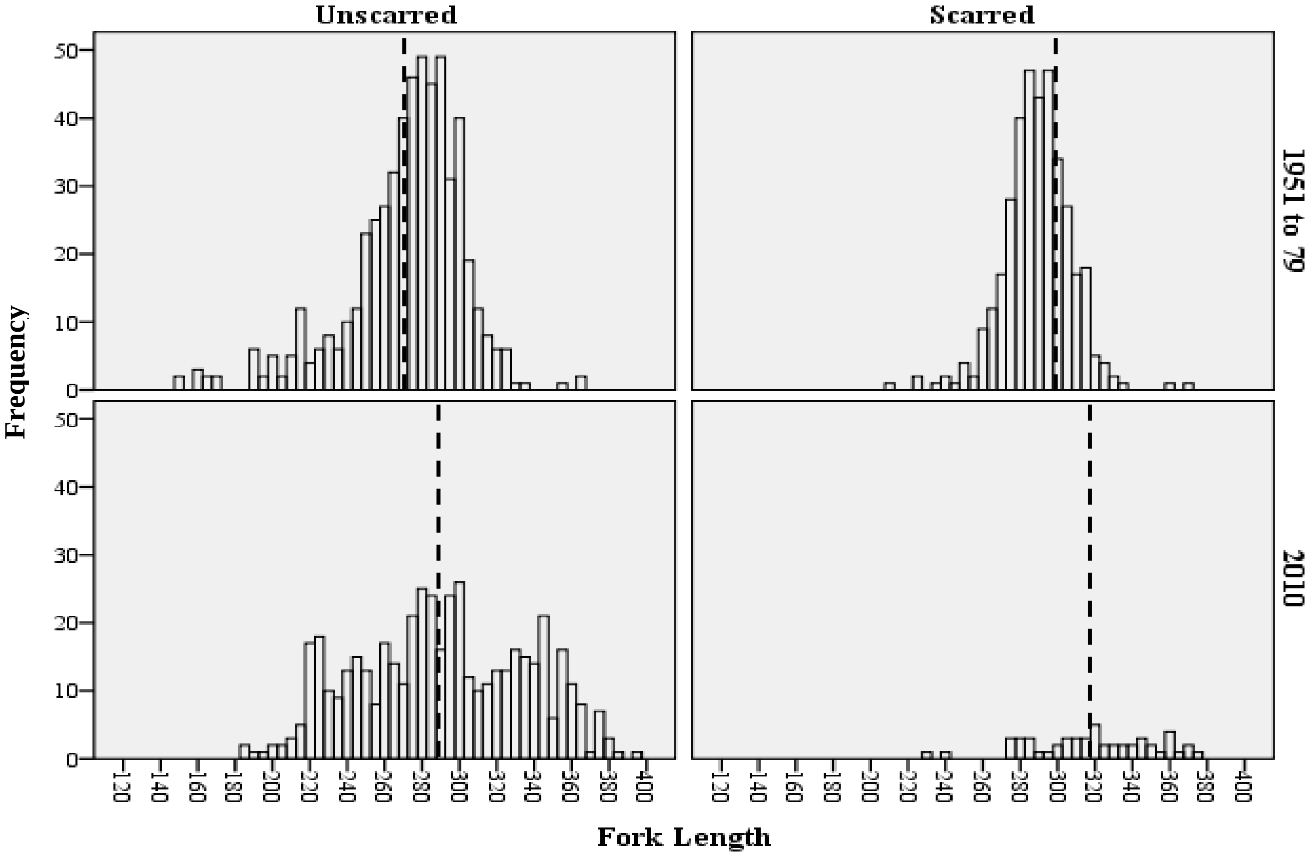


Fig.2.1 Length frequency distribution of all *Coregonus lavaretus* collected from Loch Lomond during both historic (1951-79) and recent (2010) periods, with and without the presence of lamprey-induced scars. Fish were measured to fork length (FL ± 1 mm) and the vertical dashed line indicates the mean FL for each of the four categories (historic *vs.* recent, scarred *vs.* unscarred).

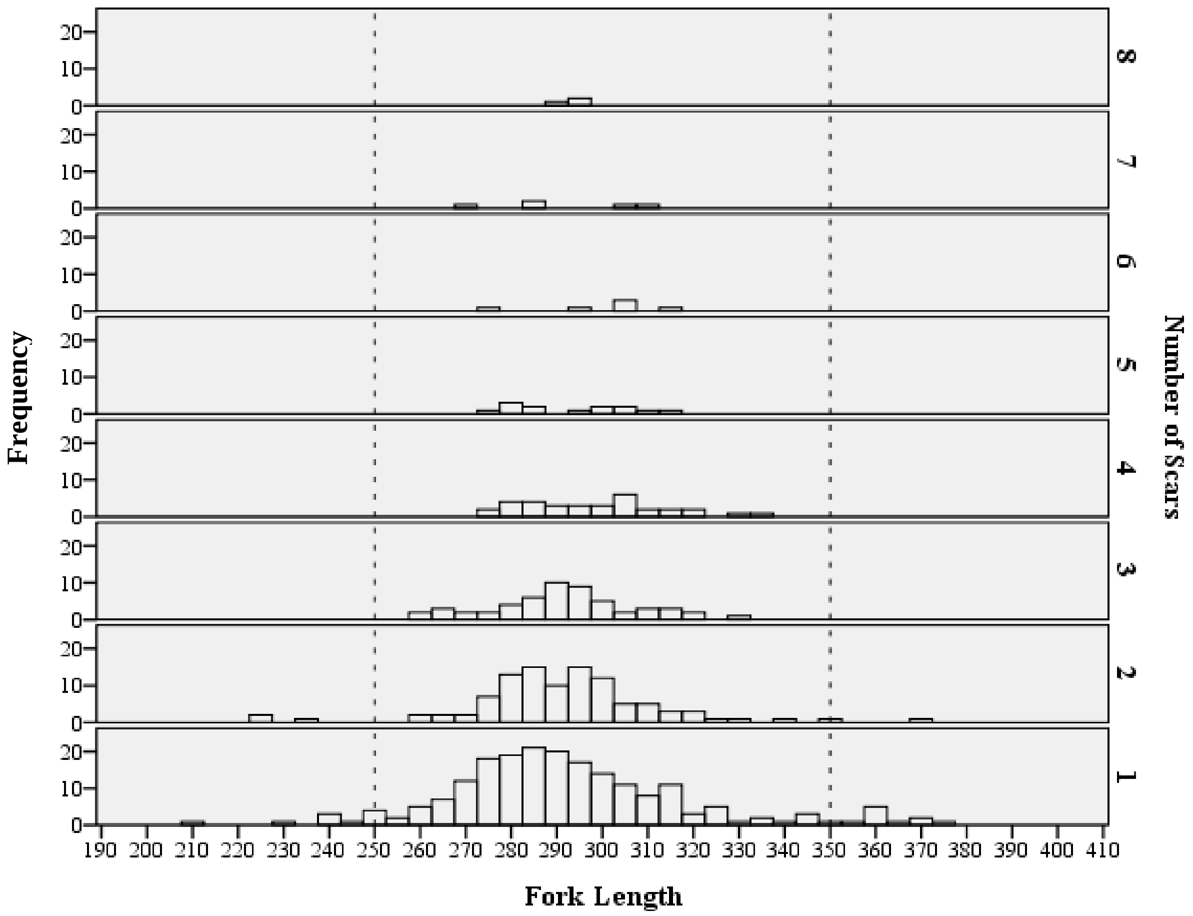


Fig.2.2 Length frequency distribution of all *Coregonus lavaretus* collected from Loch Lomond exhibiting one or more lamprey-induced scar, during both historic (1951-79) and recent (2010) periods combined (*n* = 417). Fish were measured to fork length (FL ± 1 mm) and the vertical dashed line indicates the delineation of size classes < 250 mm, 250 - 350 mm, and > 350 mm FL.

Table 2.1 Frequency of historic (1951-79) and recent (2010) lamprey-induced scars recorded from *Coregonus lavaretus* collected in Loch Lomond. The proportion of fish scarred during each time period, as well as the number of scars recorded from individual fish, is indicated.

Sample Period	**Number of *C. lavaretus* Examined**	**% Scarred**	**Number of Scars**							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1951-79	1079	36.6	183	98	54	33	13	6	5	3
2010	364	6	18	4	0	0	0	0	0	0

“The first marvel is Loch Lomond (stagnum Lumonoy). In it are sixty islands and men dwell there, and it is surrounded by sixty rocks and an eagle’s nest is on every one, and sixty rivers flow into it, and there issues not therefrom to the sea save one river, which is called Leven.”

Nennius (*c.* 800)

“The gourmets in the cities of Elbing and Memel wait with great anticipation for the appearance of the first lamprey fisherman in early autumn. I remember the happy feeling in Memel when the rifle shot was heard, or the red flag was flown over a beach snack bar proclaiming that fresh, roasted lampreys were available…Who has in recent years eaten or seen lampreys? Certainly they appear now and again in the fish markets as delicacies either marinated or roasted but their purchase depends on a well-filled purse.”

Gunther Sterba (1963), *Die Neunaugen*

Chapter Three

Pre-spawning migration of lampreys, *Lampetra* spp., in the Loch Lomond basin, Scotland

3.1 ABSTRACT

Sexually maturing lampreys must undertake a period of upstream migration prior to spawning. In European river and brook lampreys this occurs at different spatial scales as a result of differences in life history strategy. Within Loch Lomond, Scotland a single population of river lampreys exhibits partial migration, where one component is anadromous and migrates to marine waters to feed, while another component is potamodromous and migrates within freshwater. This situation is unique to the U.K. and the population is, therefore, of high conservation and scientific value. Maturing lampreys were trapped within several tributaries surrounding the Loch Lomond basin as they moved upstream between 2009 and 2012. River lampreys more commonly migrated in the autumn while brook lampreys moved upstream mostly in spring. The catch rate differed significantly between the three lamprey groups ($P < 0.05$) but not across years ($P > 0.05$) as a result of large variation in sample size and climatic conditions. Catch rate was weakly negatively correlated with warming water temperatures ($F = 0.562$, $P > 0.05$) and weakly positively correlated with increasing river flow ($F = 0.248$, $P > 0.05$). The Endrick Water was found to contain the largest adult lamprey population in the Loch Lomond basin and is the only tributary currently supporting sexually mature potamodromous river lampreys. The protection of this river system is paramount to the continued presence of a partially migrating river lamprey population in Loch Lomond.

3.2 INTRODUCTION

The seasonal migrations of animal populations are often spectacular, and have long occupied the collective human conscience, whose ancient calendars were punctuated by the arrival or departure of economically and culturally important species (Palmer, 1978; Jackson *et al*., 2004). Migration acts to redistribute populations in time and space, influencing eco-evolutionary processes by impacting both individuals as well as entire ecosystems (Chapman *et al*., 2011*a*). Such intra-annual movement impacts on applied goals such as the conservation and management of migratory species, as well as providing a powerful force for evolutionary processes to act on, and so a clear understanding of the underlying causes and consequences of migration are necessary for explaining many of the patterns in biodiversity we see today. Migration has been documented in a wide variety of taxa, and is especially well known from avian and mammalian species whose movements are relatively conspicuous and easy to track.

Fish though are comparatively difficult to track given their propensity for travelling beneath the surface of water and across large distances (Chapman *et al*., 2012*a*). Fishes do, however, express a range of migratory behaviours; encompassing short distances between freshwater lakes and rivers (Skov *et al*., 2008), as well as expansive oceanic journeys (Block *et al*., 2004). Even between populations and within species there is a diversity of migratory patterns, expressed by individuals in response to a suite of environmental factors (Chapman *et al*., 2012*b*). Many fish species exhibit partial migrations, where a single population may comprise migratory and resident components, neither of which may be a fixed strategy for individuals (Chapman *et al*., 2011*b*). Such infra-specific diversity in migratory behaviour is often just one part of a more complex life-history polymorphism expressed by a phenotypically plastic species (Chapman *et al*., 2012*a*). Alternative life history strategies are particularly common to fishes inhabiting post-glacial lakes, often resulting from adaptation to different foraging strategies or environments (Robinson & Parsons, 2002; Boulet *et al*., 2012). Such divergent ecological adaptation can, in some systems, result in the long-term stability of a polymorphic population exhibiting partial migration (McPhee *et al*., 2007).

Of all the fish taxa where partial migration has been suggested to occur, perhaps the least well understood are the lampreys (Petromyzontiformes) (Chapman *et al*., 2012*b*). This order comprises ten genera, seven of which contain pairs of species of migratory parasitic and non-parasitic stream-resident lampreys occurring in sympatry (Renaud, 2011). A lack of genetic differentiation between these putative species is evidence that many species pairs in fact represent alternative life history strategies within partially migrating populations (Schreiber & Engelhorn, 1998; Docker *et al*., 2012; Boguski *et al*., 2012). The European river lamprey *Lampetra fluviatilis* and European brook lamprey *L. planeri* are one such species pair, with a wide geographic range covering most of western Europe, that exhibit no species-specific molecular genetic differences (Espanhol *et al*., 2007), and are frequently found in sympatry (Hardisty & Potter, 1971). Following the completion of larval development within riverine sediments *L. fluviatilis* typically migrates downstream to estuaries to feed, while *L.*

planeri does not feed as an adult and instead matures and remains resident in natal rivers. In some areas *L. fluviatilis* populations contain potamodromous individuals (hereafter freshwater-residents) that migrate downstream to large bodies of freshwater to feed (Goodwin *et al*., 2006; Inger *et al*., 2010) and which, at least for those populations examined to date, are genetically indistinguishable from the anadromous component using mitochondrial DNA (Hume, *unpub. data*). Petromyzontids begin sexual maturation as they travel towards spawning grounds (Dziewulska & Domagala, 2009), which are typically located in gravel patches in the middle to upper reaches of rivers (Applegate, 1950).

Lamprey populations are generally declining throughout their range, and two major drivers of this trend are barriers to migration and the degradation of riverine habitat (Renaud, 1997; Freyhof & Brooks, 2011). Due to its anadromous tendencies and relative abundance *L. fluviatilis* has received the greatest attention of three lamprey species found in the U.K., both from a research perspective as well its commercial value to fisheries (Masters *et al*., 2006). Despite a notable reduction in some population sizes from formerly significant rivers [e.g., the tidal River Ouse, England, U.K. (Jang & Lucas, 2005)] *L. fluviatilis*, as well as *L. planeri*, are listed as of Least Concern on the IUCN's Red List (Freyhof & Brooks, 2011). They are both, however, protected by the Bern Convention and the European Habitats Directive 92/42/EEC, as species whose conservation requires the designation of Special Areas of Conservation (SACs) (Kelly & King, 2001). The Endrick Water, Loch Lomond, Scotland, U.K. is one such SAC that is listed for the stream-resident *L. planeri,* as well as a population of *L. fluviatilis* containing both an anadromous and a freshwater-resident component (i.e., is a partially migrating *L. fluviatilis* population) (Bond, 2003).

The ecology of lampreys in Loch Lomond has been of interest for several decades (Maitland, 1980; Morris, 1989; Maitland *et al*., 1994; Adams *et al*., 2008), although notes on their pre-spawning migration are limited to observations from the Endrick Water alone. Very little is known of the behaviour or spawning locations of upstream migrants in the Endrick Water. The aim of this study was to describe the characteristics of the pre-spawning migration of both the anadromous and freshwater-resident components of *L. fluviatilis*, as well as *L. planeri* populations, from within the Loch Lomond basin. The aim was to record the extent, timing and duration of upstream movement of lampreys present in the system, and to elucidate the environmental factors important in inducing and maintaining their upstream migration. Additionally, this study aims to provide baseline data that could be used to a) inform conservation managers of crucial periods in the lamprey life-cycle by describing aspects of lamprey ecology in an SAC critical to their continued protection, and b) provide a springboard for more focussed research on important sites for lampreys within the Loch Lomond basin.

3.3 MATERIALS & METHODS

Loch Lomond is the largest lake in Scotland by surface area (71 km^2), comprising three sub-basins and draining a combined catchment area (CA) of 769 km^2. Tributary rivers and streams are extensive, the largest of which is the Endrick Water, itself draining a CA of 220 km^2. The Endrick Water enters the south basin of the lake and has a mean annual discharge of 7.54 m^3 s^{-1}. Other significant tributaries include the River Falloch that drains to the north basin, and the Finlas, Fruin (CA 161 km^2) and Luss Waters (CA 35.3 km^2), all of which drain into the western portion of the lake. The River Leven is the lake's major outflow and is located in the south basin, entering the Clyde Estuary in west-central Scotland. The River Leven is regulated for domestic water supply by a barrage constructed in 1971 acting to maintain high water levels in summer months. All other rivers mentioned in the text are unregulated.

Adult lampreys are not apparent in river systems between May and September in Loch Lomond, and so actively migrating adult lampreys (i.e., sexually maturing individuals) were collected as they undertook their annual spawning migration, using a highly selective static double-funnel trap (Morris & Maitland, 1987). Traps were installed during September 2009 and fished continuously until May 2012. Damaged traps were replaced as soon as possible. The total number of traps in operation remained constant throughout the study period (n = 26). Eight rivers distributed around the lake's three sub-basins were sampled using varying numbers of traps (Fig. 3.1): south basin (River Leven n = 2, Fruin Water n = 3, Finlas Water n = 2, Endrick Water = 5); mid-basin (Luss Water n = 2, Ross Burn n = 1, Wood Burn n = 2) and north basin (River Falloch n = 3). Two tributary streams were also selected: Blane Water (n = 3), which drains into the Endrick Water and Ben Glas Burn (n = 3), which drains into the River Falloch.

Traps were examined weekly except during periods of high flow and/or extreme weather which prevented normal operations. All fish removed from traps were identified to species and their abundance recorded (Table 3.1). Captured lampreys were transported alive to holding facilities at the Scottish Centre for Ecology and the Natural Environment, University of Glasgow. All lampreys were measured (± 1 mm) and weighed (± 0.01 g) within 24 hours of being removed from traps. Lamprey catches from the Endrick Water were adjusted for trapping effort to a catch-per-unit-effort (CPUE) measure representing the number of lampreys per trap per day. CPUE was calculated for this site alone due to the relatively larger sample sizes available. An attempt was made at establishing an estimated population size for lampreys in the Endrick Water, as well as estimating trap efficacy at this site, using a mark-recapture technique. Three hundred *L. planeri* (selected because of their relative abundance) were collected from traps in the Endrick Water mainstem between January and March 2011, fin-clipped and released approximately 500 m downstream of the traps on March 27th 2011.

The Endrick Water mainstem was the only site in this study monitored by a velocity-area gauging station. It was located 2 km upstream of the chosen trapping site, and mean daily flow rates (m^3 s^{-1}) were made available for the duration of the trapping period. In addition, mean daily water temperatures for the Endrick Water were recorded throughout the trapping period by DS1921g-F5 thermochron units (HomeChip) mounted within traps. Differences in abundance between *Lampetra* spp. trapped across all years was tested with Chi-Squared analysis, and differences in CPUE tested by way of Analysis of Variance and a post-hoc Tukey's Honestly Significant Different test. Correlations between CPUE and both water temperatures and flow rate from the Endrick Water are also shown.

3.4 RESULTS

Throughout the three year study period, adult lampreys first appeared in traps in rivers draining into Loch Lomond during October (n_{total} = 62) (Fig. 3.2). A small peak in the frequency of captured lampreys indicates that during November a larger number of individuals were migrating upstream (n_{total} = 89), although low frequencies of lampreys continued to be captured until February (range 39 – 46). The month of March produced the greatest catches across the entire study period (n_{total} = 343) and indicates the peak of lamprey pre-spawning migration in Loch Lomond tributaries. High frequencies of adults were also trapped in April (n_{total} = 176), although these numbers rapidly dropped during May when few adults were collected (n_{total} = 10). This overall migration pattern was largely driven by *L.*

planeri being most abundant in traps during the month of March (Fig. 3.4). In contrast, both anadromous and freshwater-resident components of *L. fluviatilis* were trapped more often in October and November than any other month (Fig. 3.3).

The Endrick Water accounted for 95.8% (n_{total} = 838) of all lampreys trapped during the three year period. Freshwater-resident *L. fluviatilis* were captured in both the Endrick Water mainstem (n_{total} = 36) as well as its major tributary the Blane Water (n_{total} = 9), and were collected from no other river system during this study. *Lampetra planeri* were collected only from the Endrick Water (n_{total} = 767) and Ben Glas Burn, a minor tributary of the River Falloch (n_{total} = 18). Anadromous *L. fluviatilis* were the most widely distributed adult lamprey in the Loch Lomond basin, being recovered from traps located in four separate tributaries (Endrick Water, including Blane Water n_{total} = 26; Fruin Water n_{total} = 4; Finlas Water n_{total} = 1; Luss Water n_{total} = 1). Across the three year study period and during all months when lampreys were trapped (October – May) *L. planeri* were significantly more abundant than either anadromous or freshwater-resident *L. fluviatilis* (Chi-squared test, d.f. = 2, χ = 1277, $P < 0.01$) within the Endrick Water. Although in the Endrick Water CPUE differed widely across the three years (Table 3.2) there were significant differences in catch rate between the three groups (ANOVA, d.f. = 2, 90, $F = 5.38$, $\eta^2 = 0.11$, $P < 0.01$). *Lampetra planeri* was significantly more abundant and therefore had a statistically higher CPUE than either *L. fluviatilis* migratory component (Tukey HSD, $P < 0.05$ for both). No individuals from the mark-recapture experiment were however recovered from any traps in the Endrick Water.

Lampreys undergoing the pre-spawning migration varied in body length and weight. Anadromous *L. fluviatilis* were typically the largest individuals ascending rivers in Loch Lomond (mean length 323 mm, range 257 – 374 mm; mean weight 59.12 g, range 27.96 – 80.98 g), while *L. planeri* were usually the smallest (mean length 145 mm, range 103 – 195 mm; mean weight 4.73 g, range 1.56 – 12.69 g). These discrete modes were overlapped by the presence of freshwater-resident *L. fluviatilis* from the Endrick Water catchment. These individuals averaged 217 mm in length (range 145 – 269 mm) and 15.97 g in weight (mean; range 4.45 – 30.71 g). Lamprey populations in Loch Lomond exhibited a notable reduction in total body length throughout the duration of the pre-spawning migration (Fig. 3.5). Anadromous *L. fluviatilis* trapped between October and January averaged 332 mm (range 323 – 343 mm), but in March and April individuals measured 287 mm (mean; range 282 - 293 mm). This represents a reduction in body length of 13.6% between migrating lamprey collected in autumn and spring. Freshwater-resident *L. fluviatilis* expressed greater reductions in body length between early and late migrants, where autumn individuals averaged 234 mm (range 226 – 242 mm) and spring individuals averaged 179 mm (range 173 – 185 mm). Thus, a reduction of 23.5% took place during the 6 – 7 month migratory period. *Lampetra planeri* exhibited a similar extent of reduction during the migratory period as anadromous *L. fluviatilis*, averaging 159 mm in October (range 129 – 181 mm) and 138 mm in April (range 103 – 170 mm). They were therefore 13.2% smaller in spring compared with autumn.

During the pre-spawning migration in the Endrick Water catchment water and air temperatures ranged 1 – 12.3°C (mean ± S.D. 7.14 ± 4.08) (Fig. 3.6*a*), and -1.2 – 14.3°C (mean ± S.D. 8.03 ± 4.26) (Fig. 3.6*b*) respectively. A linear relationship between water temperature and CPUE for all lampreys trapped in the Endrick Water was not observed, although this non-significant trend was negative ($R^2 = 0.019$, $P > 0.05$) (Fig. 3.7). This suggests that pre-spawning migration could be negatively correlated with higher water temperatures. Mean daily flow rates in the Endrick Water during the pre-spawning migration were 0.47 – 34.07 $m^3\ s^{-1}$ (mean ± S.D. 12.34 ± 7.6 $m^3\ s^{-1}$) (Fig. 3.8). There was a positive yet non-significant trend between CPUE for all lampreys trapped in the Endrick Water and mean daily flow rate, although again this linear relationship was exceedingly weak ($R^2 = 0.008$, $P > 0.05$ (Fig. 3.9). This suggests a positive relationship between increasing flow and the number of lampreys moving upstream may exist, but was not supported in this study.

3.5 DISCUSSION

Maturing populations of migratory lampreys initiate re-entry into rivers and begin upstream movement within the Loch Lomond basin during the month of September. Individuals belonging to the anadromous and freshwater-resident component of *L. fluviatilis* have commonly travelled 14 km upstream in the Endrick Water mainstem by October and November, and anadromous individuals can penetrate rivers located in more northerly positions along the lake’s western shore, as far north as Luss Water, by mid-October. The estimated minimum migration distance of the anadromous component of *L. fluviatilis* (*c*. 45 km) is well within the range of that observed in other populations (Gaudron & Lucas, 2006). Upstream movement is sporadic throughout the winter months and it would seem most *L.*

fluviatilis within the lake basin migrate during autumn months. In contrast, the peak migratory period for *L. planeri* is March, although some individuals begin upstream movement during October. Tight coordination of maturing *L. planeri* in spring has been recorded in other populations (Hardisty, 1944; Malmqvist, 1980).

Lampreys collected during autumn and winter months were larger than those migrating immediately before the spring spawning period. This trend is apparent in all maturing petromyzontids (Larsen, 1980) and is not representative of two discrete stocks within each population as seen elsewhere i.e., large autumn migrants and small spring migrants (Abou-Seedo & Potter, 1979). *Lampetra fluviatilis* populations elsewhere are largely autumn migrants (Hardisty, 1973; Maitland *et al*., 1984) or spring migrants only (Berg, 1948), but others will exhibit more protracted migrations such as that noted in Loch Lomond (Sjoberg, 1980). Particularly for the migratory *L. fluviatilis*, upstream movement in either autumn or spring may be due to differential exposure to environmental and pheromonal cues initiating pre-spawning physiological changes and behaviour, resulting from the spatial segregation of individuals during the juvenile feeding period (Sorensen, 2003; Vrieze & Sorensen, 2001; Vrieze, 2011). Alternatively, some maturing individuals may choose to overwinter (Beamish, 1980; Ahmadi *et al.,* 2011) either in the lake itself or the estuary if conditions within the chosen river are unsuitable, such as exhibited by freshwater-resident *Petromyzon marinus* from the Laurentian Great Lakes (Applegate, 1950).

Petromyzontids typically migrate upstream during the hours of darkness (Hardisty, 2006). The negative phototactic response declines as sexual maturation progresses however (Applegate, 1950), as was noted within the Endrick Water, where on May 09th 2011 approximately 200 *L. planeri* were observed moving upstream at 1300 hrs. at a water temperature of 10.5°C. Traps positioned parallel to a low-height in-stream barrier (bridge apron) resulted in large catches of lampreys, suggesting that lampreys were migrating close to the river substrate and exhibited searching behaviour when faced with an obstruction.

The distance covered by individual lampreys in Loch Lomond during the pre-spawning migration is not known. However, for those that spawn within the Endrick Water mainstem some estimate of the minimum distance can be made. Anadromous *L. fluviatilis* that have fed within the Clyde Estuary must first enter and ascend the River Leven, a distance of *c.* 20 km from potential feeding grounds near the mouth of the River Clyde. These individuals must then travel a minimum of 9 km across the south basin of the lake to the mouth of the Endrick Water. The most suitable spawning grounds accessible to lampreys within this river system are located *c*. 16 km from the mouth of the river, and so anadromous *L. fluviatilis* spawning here will have travelled a minimum of 45 km from their estuarine feeding grounds. Foraging freshwater-resident *L. fluviatilis* potentially utilise the entire length of the Loch Lomond basin (36.4 km) and therefore individuals of this population may undertake pre-spawning migrations of up to 50 km to spawn in the Endrick Water. *Lampetra planeri* is not known to travel great distances towards spawning grounds, although a limited upstream movement must necessarily be made from downstream larval habitats. In the Endrick Water productive larval habitats are located along most of the lower reaches of the river, and so individuals of this population likely migrate anywhere from < 1 – 5 km depending on the vicinity of suitable spawning habitat.

Adult lampreys were distributed throughout tributaries entering the lake's three sub-basins; however the Endrick Water, located in the south basin, is evidently the most important river for spawning lampreys in Loch Lomond. No freshwater-resident *L. fluviatilis* were recorded from any other site during this study, and those data presented here are strongly suggestive that this one river is a stronghold for lampreys in the lake basin. Numbers of maturing adults of the freshwater-resident component of *L. fluviatilis* have remained stable here since at least the 1980s, but the anadromous component appears to fluctuate more frequently (Maitland *et al*., 1994; Gardiner *et al*., 1997; Adams *et al*., 2008). This may be a result of the more stochastic processes inherent to an anadromous lifestyle, such as greater mortality at sea (Fukuwaka & Suzuki, 2002). Anadromous *L. fluviatilis* individuals were collected in the Fruin, Finlas and Luss Waters, and this represents the first recorded incidence of this species within those tributaries (Maitland *et al*., 1994) perhaps suggesting a recent range expansion within the basin. *Lampetra planeri* is abundant in the Endrick Water, yet was trapped in only one other river during this study. Ben Glas Burn is a minor tributary of the River Falloch located to the north of the lake, and is likely to contain a highly localised population of *L. planeri* attracted from the River Falloch itself, as this stream frequently dries out during periods of low rainfall and contains no larval habitat. Surveys using electrofishing equipment have, however, revealed the presence of *L. planeri* in all rivers examined in the present study (Maitland *et al*., 1994; Hume, *unpub. data*).

Catch-per-unit-effort varied widely between years as well as between the three lamprey populations in the Endrick Water and this is almost certainly partly the result of extreme climatic variation during the study period. The efficacy of the trap design employed in this study was severely hampered by river flow conditions. During periods of high flow the traps were frequently lifted from the substrate and became suspended in mid-water, rendering them unsuitable for capturing lampreys which travel upstream close to the river bottom (Nazari & Abdoli, 2010). In periods of low flow the river became too shallow for the traps to operate efficiently as they became exposed and lampreys were unable to enter. The three years of the study period encompassed some of the most extreme weather events on record for this region. The autumn of 2009 and 2010 was characterised by prolonged and heavy rainfall causing severe flooding, and was followed in both years by heavy snowfall and extended periods of freezing temperatures throughout winter. An exceptionally dry and warm spring occurred in 2011, resulting in unusually low river levels for this period.

Such variation no doubt weakened the linear relationship between water temperature, flow rate and CPUE within the Endrick Water, although non-significant trends were observed. Lamprey numbers were weakly but positively correlated with increasing flow rates, and indicate that lampreys were either stimulated to continue upstream migration immediately following periods of increased flow, or were actively migrating during them. As this was outside statistical significance however this trend is difficult to interpret. Lampreys are relatively poor swimmers in relation to other fishes, given their lack of paired fins and gas bladder (Kemp *et al*., 2011), and it seems most probable that lampreys were stimulated by a reduction in flows following greater discharge to resume moving against the current (McLaughlin *et al*., 2003; Quintella *et al*., 2004). Such a strategy would enable them to overcome in-stream barriers as a result of raised water levels (Andrade *et al*., 2007; Kemp *et al*., 2011; Russon *et al*., 2011), and the presence of fresh oxygenated water could supply the aerobic capacity required to continue migrating (Claridge *et al.*, 1973). A non-significant yet negative correlation between CPUE and water temperature indicates that the duration of the pre-spawning migration could be constrained by warming spring temperatures (Clemens *et al*., 2009). The instinct to attain the spawning grounds prior to warmer temperatures would ensure that lampreys begin spawning before low oxygen tension in the water reduces their capacity for upstream movement or the exertion required to spawn (Patton *et al*., 2011), and safeguards any subsequently developing embryos against lethally high temperatures (Macey & Potter, 1978).

In the spring of 2011 an attempt was made at estimating the relative population size of lampreys in the Endrick Water using a mark-recapture technique. Three hundred *L. planeri* had a small triangle of tissue removed from the second dorsal fin and were released 500 m downstream of traps located in the mainstem during a period of low flow. No marked individuals were subsequently recovered from upstream traps or located in the vicinity of the release site. No lampreys were trapped in the Endrick Water following the release date of the marked *L. planeri* individuals, and it is likely that these individuals spawned downstream of the release site shortly after this time as a result of rapidly warming water temperatures. A similar study by Maitland *et al.* (1994) at the same site employed 426 marked lampreys released between December 1983 and February 1984 where nine individuals were subsequently recovered 7 – 56 days later, up to 5 km upstream, suggesting the more recent attempt occurred too late in the migration period to induce any further upstream movement.

The Endrick Water is designated as a Special Area of Conservation (SAC), within which the populations of *L. planeri*, and both migratory components of *L. fluviatilis*, are designated as features of significant conservation concern on the basis that this site is considered to be one of the best in the U.K. for these species (Bond, 2003). One major focus of this conservation effort aims to ensure that the distribution and extent of habitats supporting these species are maintained over the long term. However, of the 40.5 km currently under protection in the Endrick Water, spawning *L. fluviatilis* utilise < 10%, significantly increasing the impact of any detrimental effects in the vicinity of these sensitive areas. The Endrick Water SAC is both the single most important river for petromyzontids within the Loch Lomond basin and the most densely populated by humans (Doughty & Maitland, 1994). Anthropogenic pressures such as water abstraction for domestic supply, gravel extraction and diffuse pollution from extensive areas of arable land are all likely to negatively impact the adult lamprey populations within the Endrick Water (Maitland *et al*., 1994).

Mitigation measures, such as the addition of gravel beds in river stretches suitable for spawning lampreys, have the potential to contribute significantly to increasing lamprey population sizes in Loch Lomond (*sensu* McManamay *et al*., 2010), as it would appear this is a limiting factor, particularly in the Endrick Water (McEwen & Gardiner, 2001). The presence of extensive sections of river downstream of potential spawning sites suitable for the rearing of larval lampreys within the Endrick Water (Gardiner *et al*., 1995; Forth

Fisheries Foundation, 2004; Watt *et al*., 2011), and a lack of any major obstacles to adult upstream migration, suggests that the relatively low numbers of spawning adults in this system are the result of low recruitment. As larval pheromones are now known to play a key role in the attraction of maturing adults and their retention in rivers (Vrieze *et al*., 2010), any increase in the larval population of the River Endrick could result in greater recruitment to future generations within the Loch Lomond basin as a whole, given that this tributary is the largest in the region and its proximate location to the lake's outflow. Adult attraction to larval pheromones has also been shown to lack species specificity (Sorensen, 1998; Gaudron & Lucas, 2006; Yun *et al*., 2011; Stewart & Baker, 2012), and therefore there is a potential benefit to populations of the locally and nationally rare sea lamprey *P. marinus* (Maitland *et al*., 1994; Hume & Adams, 2012).

It is now apparent that the Endrick Water is a major, if not the sole stronghold for lampreys in Loch Lomond. The presence of a partially migrating population of *L. fluviatilis* within the lake basin is unique to the U.K. and has become the focus of intense scientific interest in recent years (Maitland, 1980; Morris, 1989; Maitland *et al*., 1994; Hardisty, 2006; Adams *et al*., 2008; Hume *et al*., 2012). Yet, conservation-oriented goals are not generally focussed on common species expressing significant within-species diversity (de Guia & Saitoh, 2007). Therefore, the inclusion of freshwater-resident *L. fluviatilis* from Loch Lomond as qualifying features in the Endrick Water SAC, that expresses a phenotype, trophic ecology and migratory strategy atypical for the species, represents a progressive movement likely to contribute to its continued presence within the lake. However, knowledge gaps such as the robust identification of spawning sites and a clear estimation of population size within the Endrick Water must be filled to ensure future management decisions relate specifically to the requirements of the freshwater-resident component. In addition, the identification of foraging grounds for the anadromous component of *L. fluviatilis*, and its migratory route back into freshwater, are of high priority in order that this species remains protected in all supporting habitats throughout the greater Loch Lomond basin.

3.6 FIGURES & TABLES

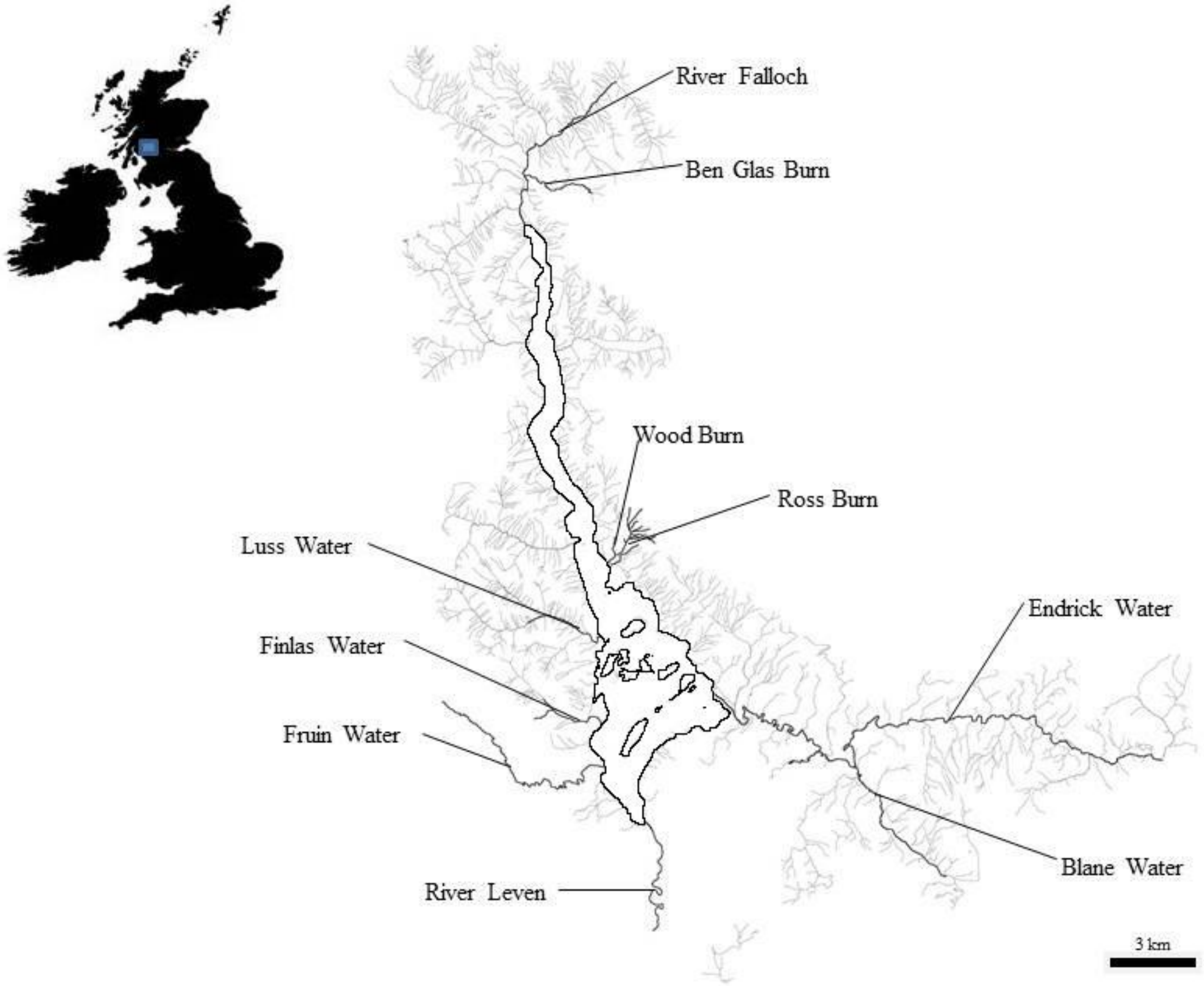


Fig.3.1 Map of the Loch Lomond basin. The location of major watercourses and tributaries fitted with static lamprey traps between autumn 2009 and spring 2012 are indicated.

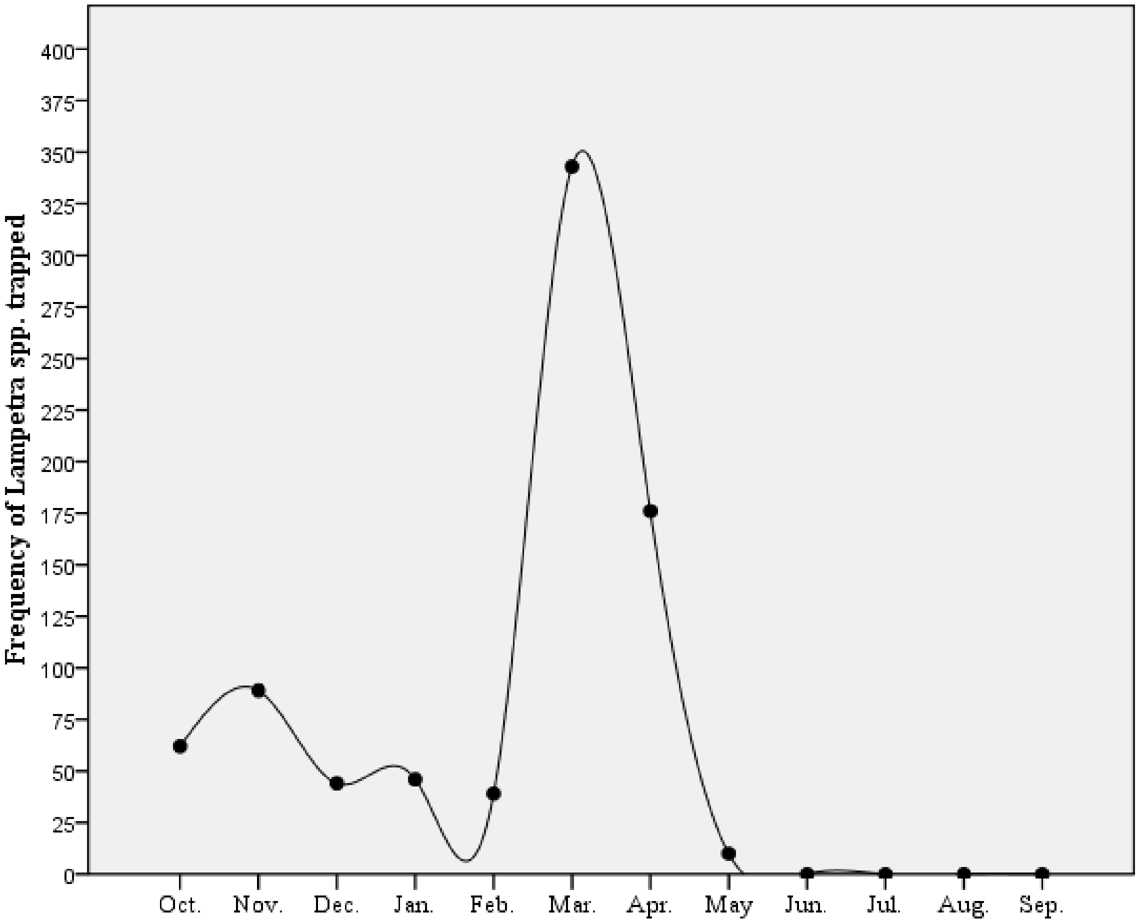


Fig.3.2 Number (monthly totals with fitted cubic spline interpolation) of adult lampreys belonging to anadromous and freshwater-resident *Lampetra fluviatilis*, and *L. planeri*, trapped at all study sites between 2009 and 2012 ($n_{traps} = 26$).

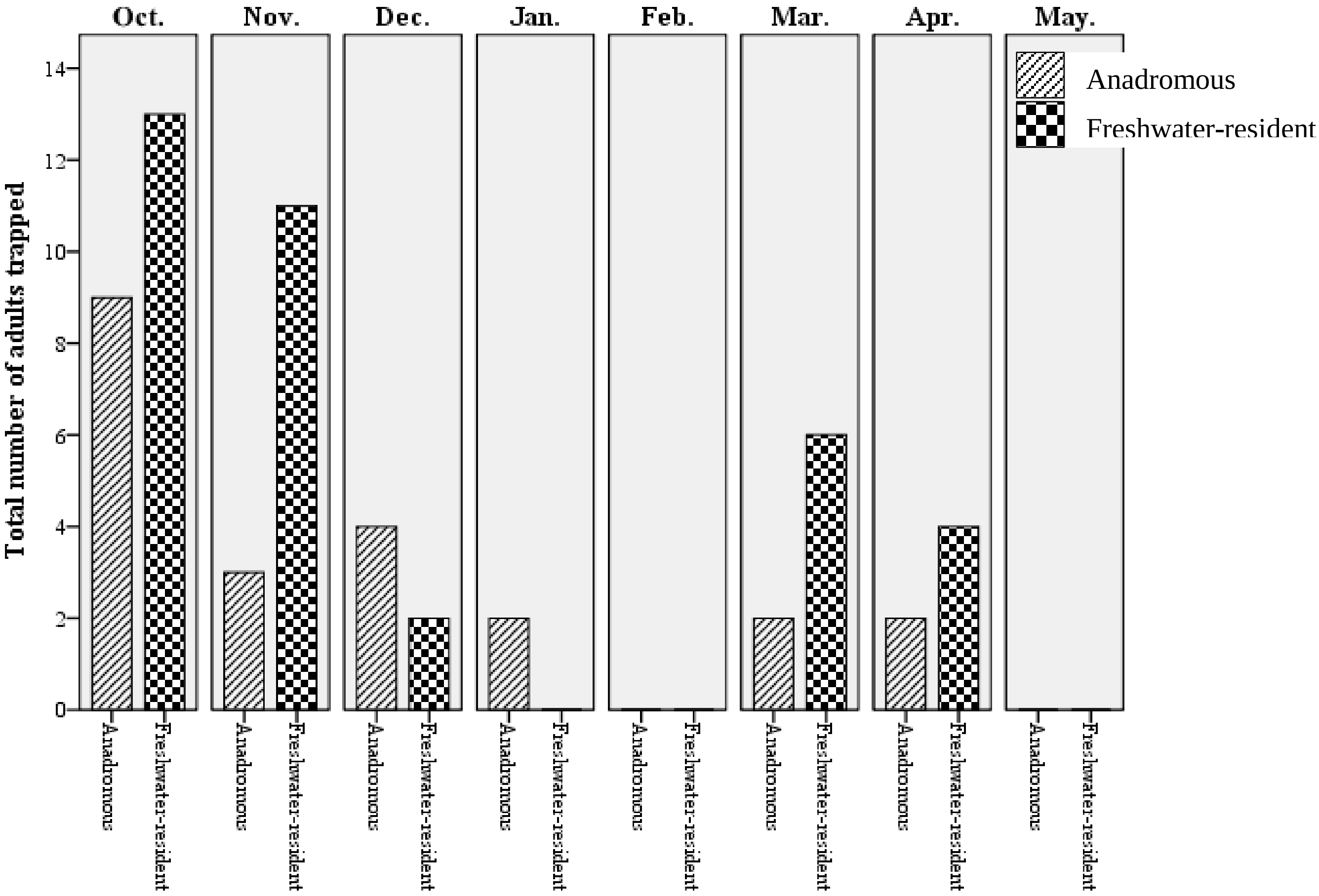


Fig.3.3 Number (monthly totals) of adult *Lampetra fluviatilis* belonging to both migratory components trapped at all study sites ($n_{traps} = 26$) during the pre-spawning migration between 2009 and 2012.

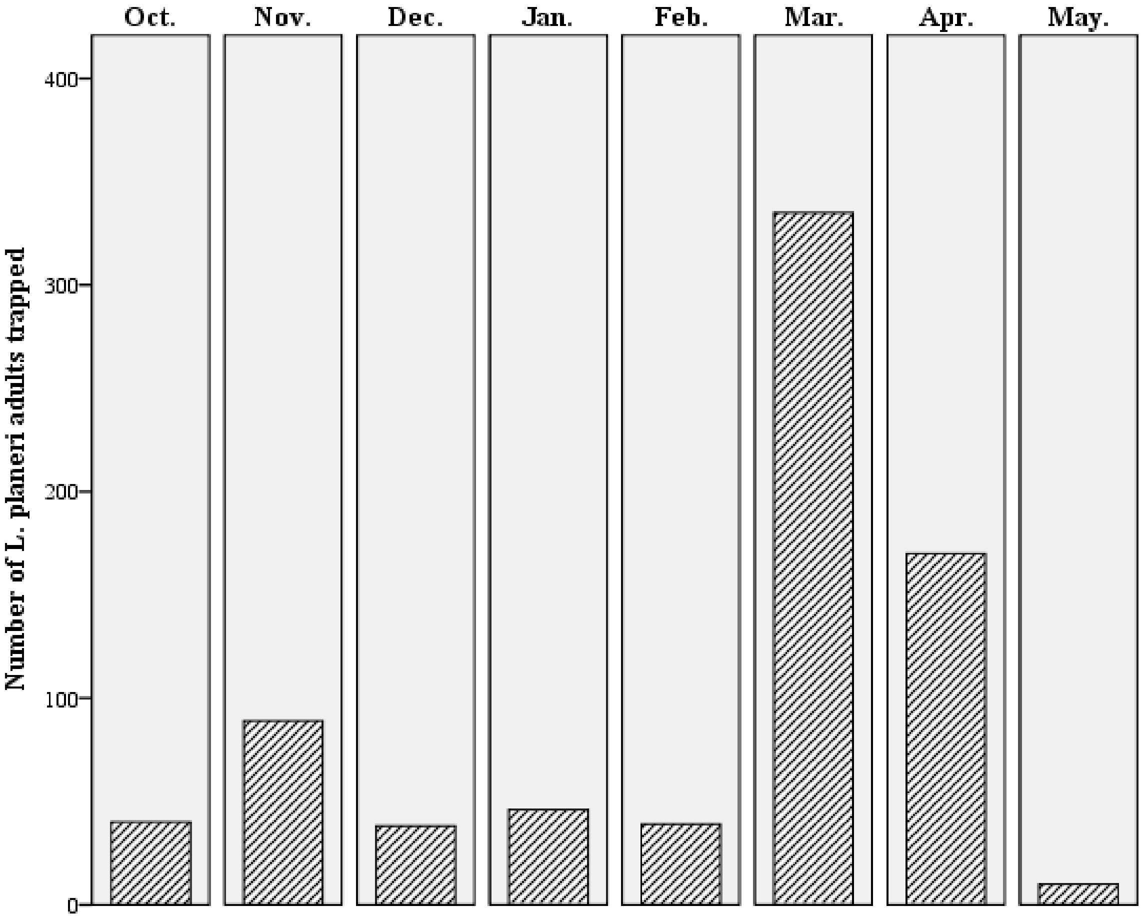


Fig.3.4 Number (monthly totals) of adult *Lampetra planeri* trapped at all study sites (n_{traps} = 26) during the pre-spawning migration between 2009 and 2012.

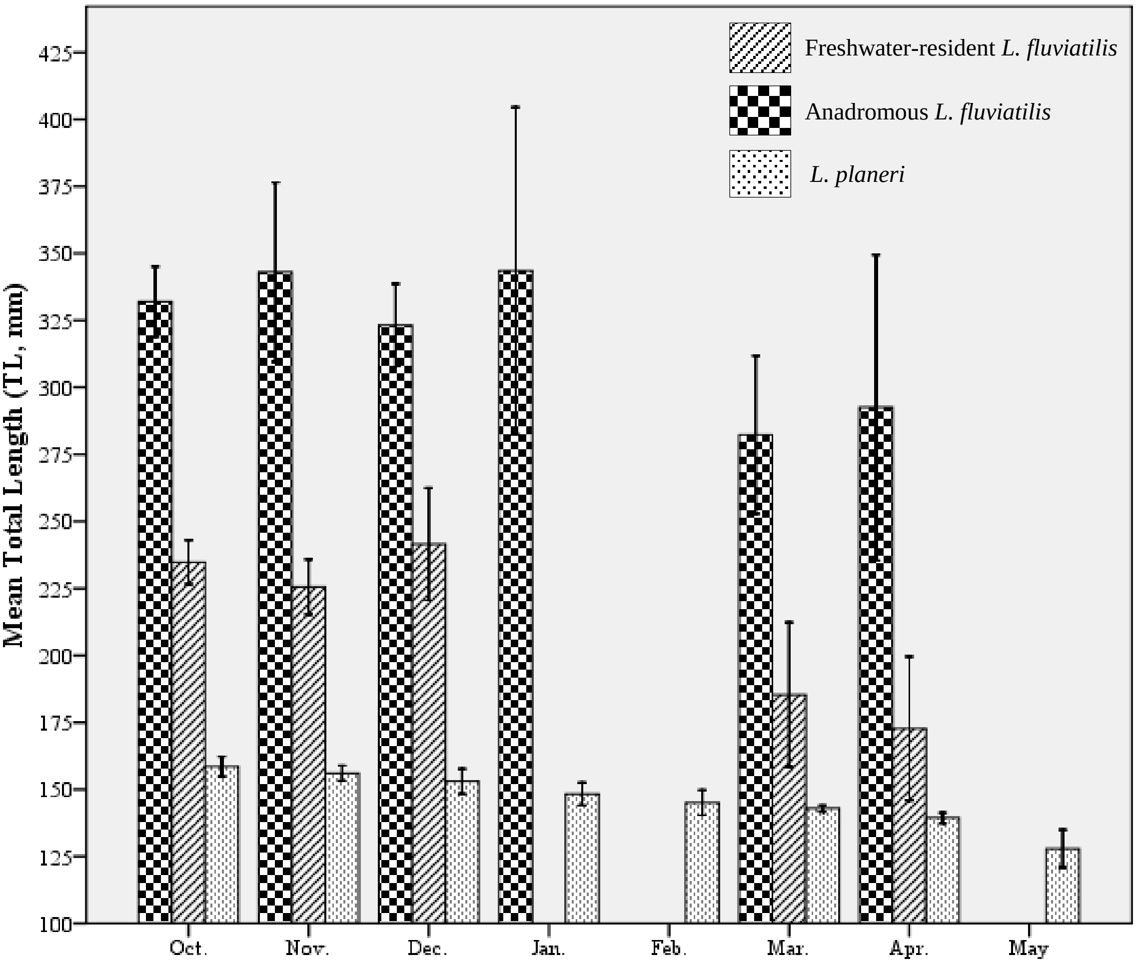


Fig.3.5 Total length (mean ± S.D.) during all months of the pre-spawning migration for both migratory components of *Lampetra fluviatilis*, and *L. planeri*, trapped from all study sites (n_{traps} = 26) between 2009 and 2012.

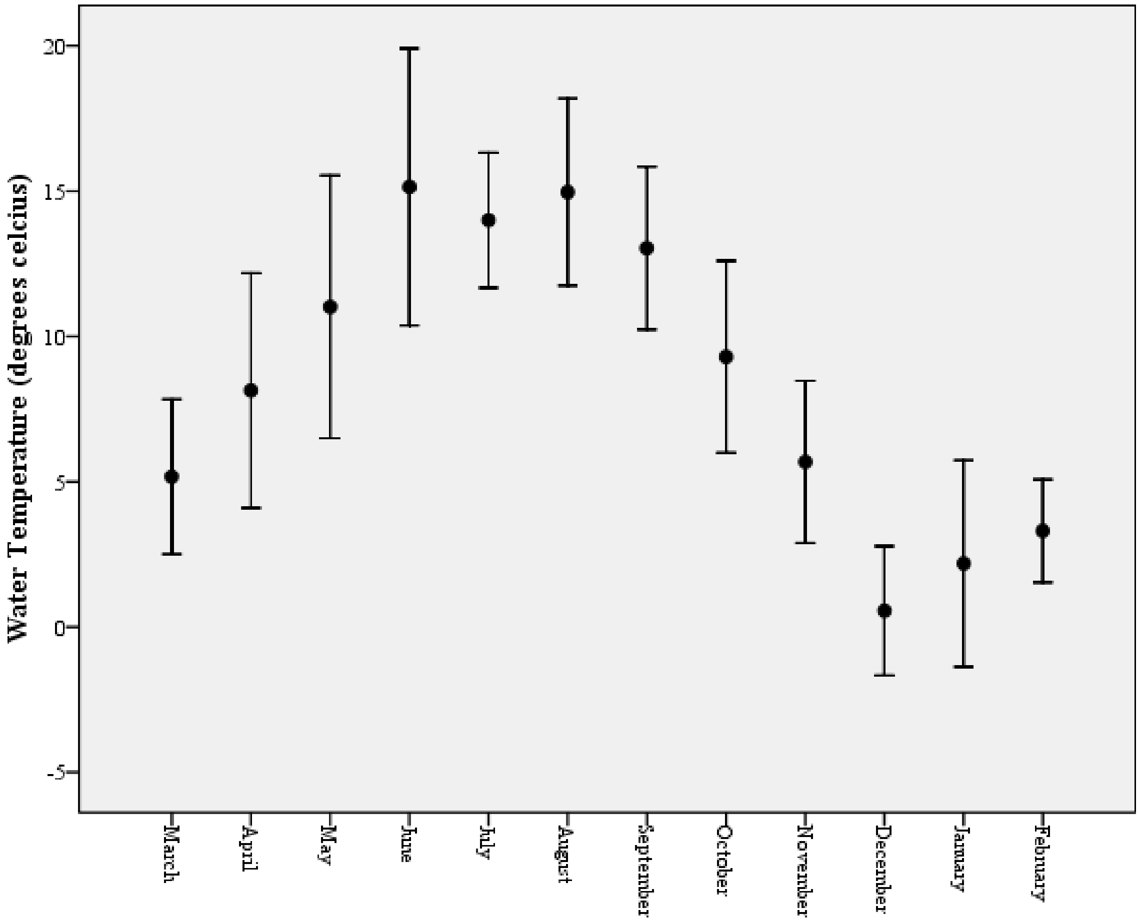


Fig.3.6*a* Mean (± S.D.) monthly water temperatures (°C) for the Endrick Water during the three year study period.

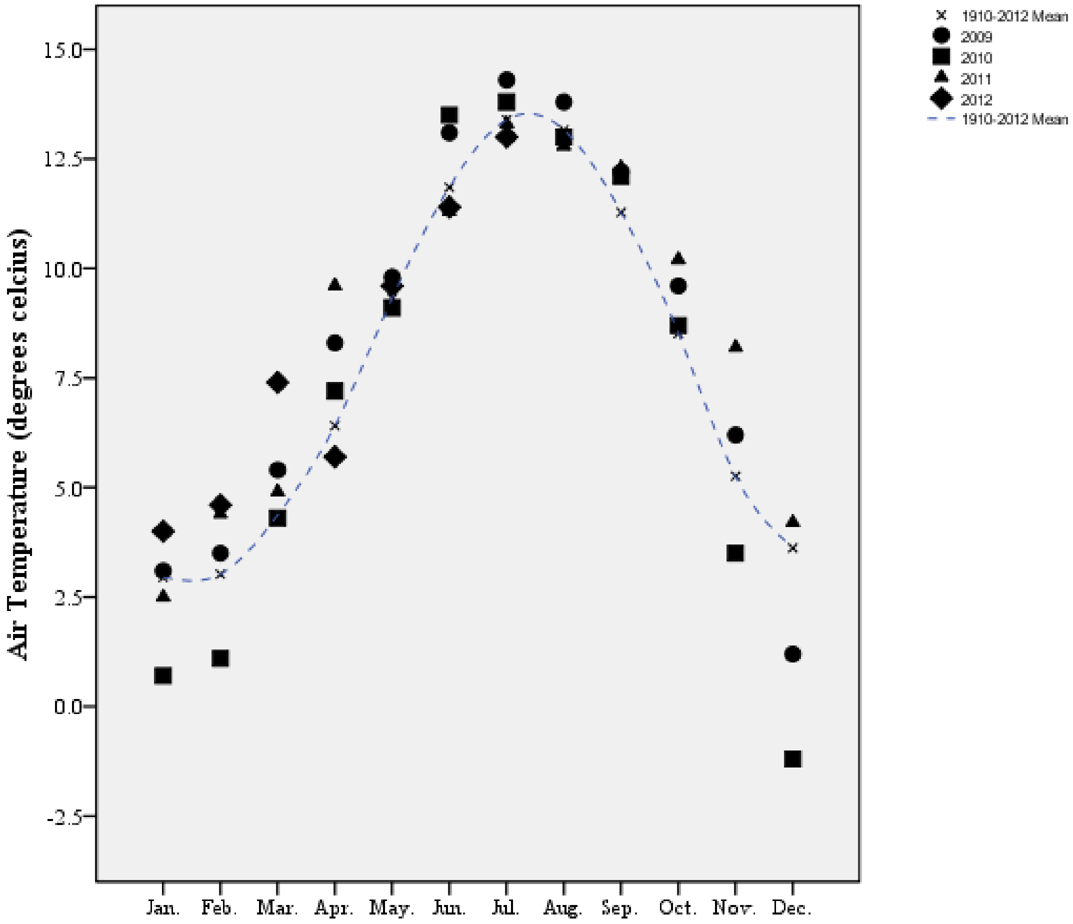


Fig.3.6*b* Mean monthly air temperatures (°C) between January 2009 and July 2012 recorded from west-central Scotland, and compared with the 100 year average for this region.

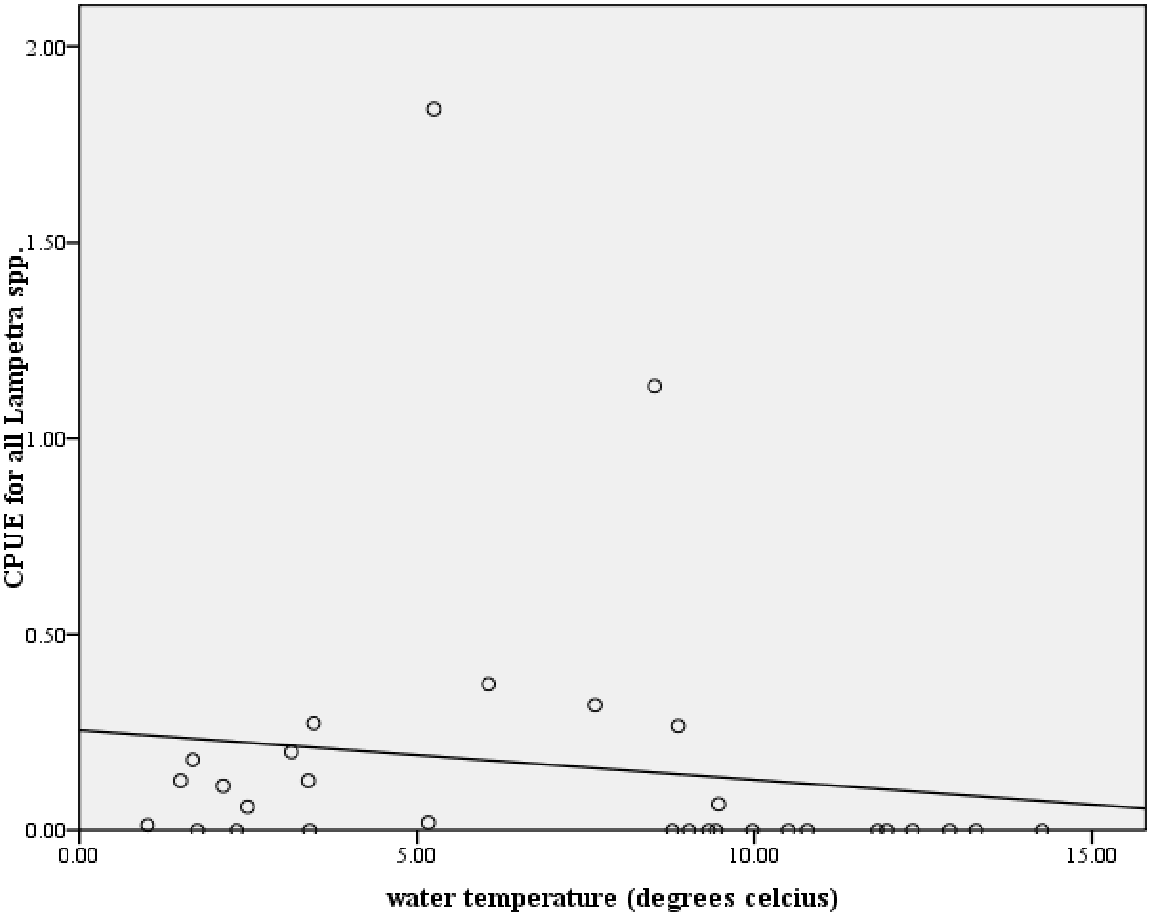


Fig.3.7 Catch-per-unit-effort (CPUE) for all adult lampreys collected from the Endrick Water (n_{traps} = 5) between October and May 2009-2012. The correlation between mean water temperature (°C) and CPUE was not statistically significant ($F = 0.562$, $R^2 = 0.019$, $P > 0.05$).

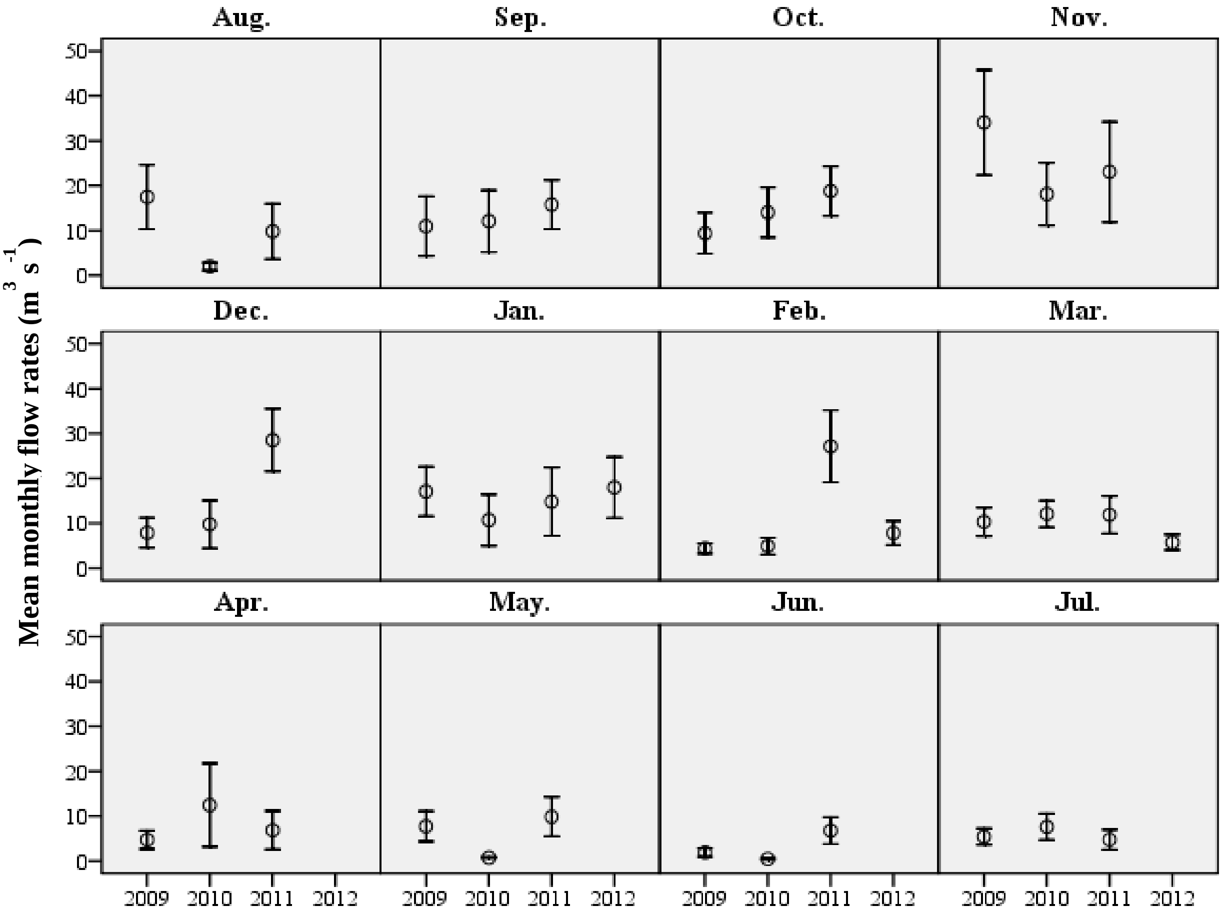


Fig.3.8 Mean (± S.D.) monthly flow rates ($m^3 s^{-1}$) for the Endrick Water between August 2009 and March 2012.

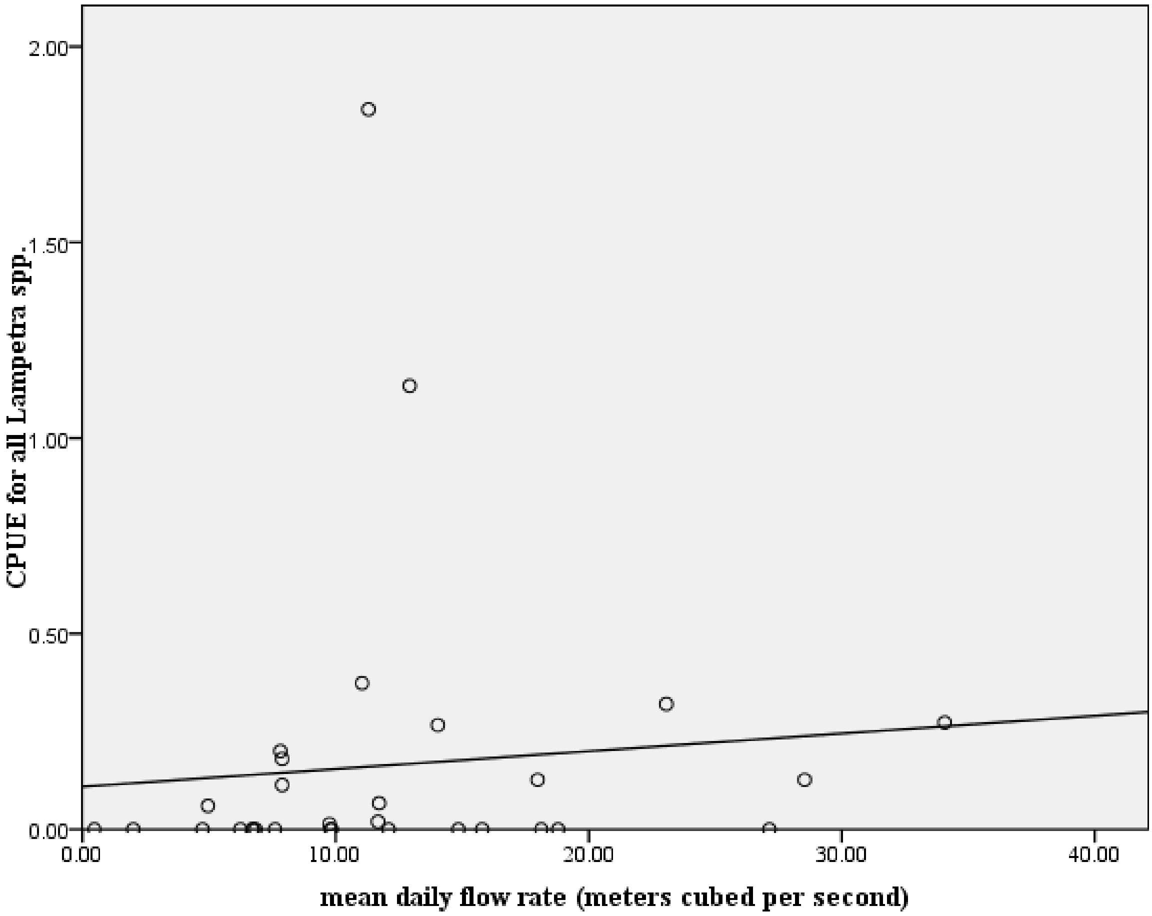


Fig.3.9 Catch-per-unit-effort (CPUE) for all adult lampreys collected from the Endrick Water (n_{traps} = 5) between October and May 2009-2012. The correlation between mean daily flow rates (m^3 s^{-1}) and CPUE was not statistically significant (F = 0.248, R^2 = 0.008, P > 0.05).

Table 3.1 Record of all fish species removed from static traps ($n_{traps} = 26$) located in ten tributaries surrounding the Loch Lomond basin between September 2009 and May 2012.

Species	**Endrick Water**	**Blane Water**	**River Leven**	**Fruin Water**	**Finlas Water**	**Luss Water**	**Ben Glas Burn**	**Ross Burn**	**Wood Burn**	**Total**
anadromous *L. fluviatilis*	23	3	0	4	1	1	0	0	0	**32**
freshwater-resident *L. fluviatilis*	36	9	0	0	0	0	0	0	0	**45**
L. planeri	767	0	0	0	0	0	18	0	0	**785**
***Lampetra* spp. ammocoete**	10	0	1	0	0	0	0	0	0	**10**
***Lampetra* spp. transformer**	1	0	0	0	0	0	0	0	0	**1**
***Petromyzon marinus* ammocoete**	1	0	0	0	0	0	0	0	0	**1**
brown trout, *Salmo trutta*	55	0	0	2	8	4	6	10	2	**87**
Atlantic salmon smolt, *Salmo salar*	9	1	0	0	3	1	0	0	0	**14**
gudgeon, *Gobio gobio*	6	1	0	0	0	0	0	0	0	**7**
stone loach, *Barbatula barbatula*	106	1	0	1	13	1	0	0	0	**122**
European eel, *Anguilla anguilla*	8	0	0	0	0	1	0	2	0	**11**
3-spine stickleback, *Gasterosteus aculeatus*	7	0	3	0	0	0	0	0	2	**12**
dace, *Leuciscus leuciscus*	1	0	0	0	0	0	0	0	0	**1**
ruffe, *Gymnocephalus cernuus*	3	0	0	0	0	0	0	0	0	**3**
minnow, *Phoxinus phoxinus*	1	0	0	0	0	0	1	0	0	**2**
perch, *Perca fluviatilis*	1	0	0	0	0	0	0	0	0	**1**

Table 3.2 Comparison of mean (± S.D.) catch-per-unit-effort (CPUE) for both migratory components of *Lampetra fluviatilis* and *L. planeri* collected from the Endrick Water (n_{traps} = 5) between October and May 2009-2012. CPUE for all lampreys collected in the Endrick Water combined is indicated separately. CPUE represents the mean of monthly totals for each year. Statistical differences between these catch rates (ANOVA) are also indicated.

	2009-10	**2010-11**	**2011-12**	**ANOVA**	***P***
All lampreys				$F_{2, 90}$ = 5.381	0.006
anadromous *L. fluviatilis*	0.005 ± 0.006	0.009 ± 0.019	0.004 ± 0.006	$F_{3, 27}$ = 0.600	0.621
freshwater-resident *L. fluviatilis*	0.012 ± 0.019	0.013 ± 0.027	0.004 ± 0.008	$F_{3, 27}$ = 1.334	0.284
non-parasitic *L. planeri*	0.264 ± 0.363	0.236 ± 0.573	0.127 ± 0.137	$F_{3, 27}$ = 3.91	0.991

“One summer day I was amused by watching the singular proceedings of two lampreys in a small ditch of clear running water near my house…The two little creatures were most busily and anxiously employed in making little triangular heaps of stones, using for the purpose irregularly-shaped bits of gravel about the size of large peas. When they wished to move a larger stone, they helped each other in endeavouring to roll it into the desired situation. Occasionally they both left off their labours and appeared to rest for a short time, and then to return to the work with renewed vigour. The object of their building I am not sufficiently learned in the natural history of the lamprey to divine; but I conclude that their work had something to do with the placing of their spawn. It seems so singular a manœuver on their part to build up regular little pyramids of gravel, bringing some of the stones from the distance of two feet against the current and rolling them to the place with evident difficultly, that the lampreys must have some good reason which induces them to take this trouble.”

Charles St. John (1893), *Short Sketches of the Wild Sport & Natural History of the Highlands*

Chapter Four

No evidence of behavioural barriers to gene flow between sympatric lamprey populations exhibiting alternative life history strategies

4.1 ABSTRACT

The process of speciation, particularly those events typified by ecological divergence in sympatry, is surprisingly poorly understood. This is particularly true of the process resulting in gene pool segregation. One mechanism through which this may occur is *via* the evolution of assortative mating between diverging populations, which may, or may not, lead to full speciation. In lampreys (Petromyzontiformes) pairs of closely related species frequently co-occur that appear reproductively isolated due to body size constraints in mating and differences in adult body size resulting from alternative life history strategies. Selection against heterotypic mates, therefore, is believed to act as a barrier to gene flow between sympatric lampreys. Such barriers were not found to be evident among sympatric populations of lampreys from Loch Lomond, Scotland expressing three alternative life history strategies, two of which comprise putative species. Heterotypic mate selection was in fact a common occurrence, demonstrating that life history strategy and subsequent adult body size does not alter the spawning behaviour between potential mates. Positive assortative mating was evident in an intermediate body size life history type (Isolation Index, $If > 0$), yet females of this type were themselves favoured by heterotypic males expressing more extreme strategies. This indicates that pre-zygotic barriers may not be acting to prevent gene flow between some sympatric lamprey species pairs.

4.2 INTRODUCTION

Speciation, the mechanism that results in reproductive isolation and subsequent evolutionary divergence between two sister populations, is a surprisingly poorly understood process. Once thought only to be possible in allopatry for sexually reproducing organisms, it is now apparent that reproductive isolation between populations diverging where their distributions overlap, wholly or in part, is more common than previously thought (Bush, 2001; Coyne & Orr, 2004). Among sexually reproducing organisms three non-allopatric models of speciation are frequently proposed: parapatric speciation, where sister species evolve while adapting to contiguous, but spatially segregated habitats, across a narrow contact zone (Bush, 1994; Berner *et al*., 2009); sympatric speciation, where sister species are not spatially segregated but diverge in response to disruptive selection caused by resource specialisation and maladapted hybrids (Coyne, 2007); and hybrid speciation, where new species evolve rapidly from matings between individuals of two closely related species often as a result of changes in chromosome number (Bush, 2001; Mable *et al*., 2011). However, classifying modes of speciation is unsatisfactory, as it divides a continuum into discrete categories by concentrating on the extremes and ignoring the rate of speciation, which varies in spatial contexts (Butlin *et al*., 2008; Hendry *et al*., 2009).

The most acute difficulty in our understanding of speciation is how reproductive isolation between diverging species might develop (Conde-Padín *et al*., 2008). In events broadly categorised as "ecological speciation", that is where adaptive divergence within a population leads to complete reproductive isolation, this can be driven by natural selection and does not always require that divergent populations come into secondary contact following a period of isolation. Mating preferences evolve separately among both populations that reduce the likelihood of maladapted between-type matings, and may even occur over contemporary timescales (Dieckmann *et al*., 2004; Hendry *et al*., 2007). Theoretical and empirical work suggests that reproductive isolation between such diverging groups of individuals can evolve both rapidly, and despite initially high levels of gene flow (Doebeli & Dieckmann, 2003; Bolnick & Fitzpatrick, 2007; Berner *et al*., 2009; Via, 2009; Smadja & Butlin, 2011). Therefore, ecological speciation will occur following ecological divergence and the presence, or subsequent development, of assortative mating (Rolán-Alvarez, 2012; Servedio & Kopp, 2012). A simple preference for phenotypically similar mates will, for example, reduce the probability of mating between individuals from populations diverging through ecological adaptive processes if that process involves any phenotypic change that is favoured under sexual selection, and therefore drive reproductive isolation in sympatry (Bolnick & Kirkpatrick, 2012).

Polymorphism, the occurrence of more than one discrete phenotype within a population (Ford, 1945), is common in a wide range of organisms including: invertebrates (Conde-Padín, 2007; Merrill *et al*., 2010; Pérez-Barros *et al*., 2011); amphibians (Takahashi *et al*., 2010) and birds (Cooke *et al*., 1988; Bearhop *et al*., 2005; Pryke & Griffith, 2008). However, polymorphisms appear frequently among fishes, particularly in north temperate lake systems, where alternative divergent forms can result from phenotypic divergence associated with: adoption of resident *vs.* anadromous strategies (Hendry & Stearns, 2004; Radhakrishnan *et al.*, 2012), or phenotypic change resulting from adaptation to alternative foraging environments within a system (Robinson & Parsons, 2002; Berner *et al.*, 2008, 2009; Boulet *et al.*, 2012). The expression of multiple phenotypes can be further accentuated by polychromatisms (Herder *et al.*, 2000; Elmer *et al.*, 2010) and differential growth driven by resource availability (Bernatchez & Dodson, 1990; Moles *et al.*, 2011).

These processes can lead to difficulties in asserting the validity of the status of some species, particularly where variation among populations is high (e.g., Adams & Maitland, 2007). The phylogenetic relationships, inter-relatedness and specific status of lampreys (Petromyzontiformes), for example, has been debated for decades (Enequist, 1937; Potter & Hilliard, 1987; Docker *et al.*, 1999; Gill, 2003). Contention arises when one considers the parallel evolution of non-parasitic, stream-resident lampreys, commonly termed brook lampreys, from a parasitically-feeding and often migratory ancestor (Docker, 2009). Some parasitic and non-parasitic forms continue to share an overlapping geographic range (Renaud, 1997), and the term “paired species” was coined by Zanandrea (1959) to describe such a situation. Species pairs have been described in seven of ten petromyzontid genera, where the filter-feeding larvae (ammocoetes) are morphologically and ecologically similar (Goodwin *et al.*, 2008) but where the adults can be readily distinguished (Renaud, 2011).

Brook lampreys do not exhibit a post-metamorphic feeding phase, and so the conventional view is that differences in body size between adults of lamprey species pairs is a result of somatic growth during the parasitic feeding phase, which does not occur in brook lampreys. The mating system of petromyzontids is widely believed to require homogomous mates (i.e., of a similar body size) as no successful fertilisation of eggs is thought to occur where body size differences between mates is 25% or greater (Malmqvist, 1983; Beamish & Neville, 1992). This results from the inefficiency of external fertilisation, where sperm is released too far from the site of ova expression should the size difference between males and females be too great (Pletcher, 1963). Thus, body size is believed to be a robust barrier to gene flow between divergent lamprey life history types. Consequently, specific status is often bestowed on lamprey populations based solely on life history strategy and particularly body size (Beamish & Withler, 1986; Potter & Hilliard, 1987).

However, some lamprey species with a wide geographic distribution appear highly variable in their life history strategy, producing several divergent adult forms (Abou-Seedo & Potter, 1979; Nazarov *et al*., 2011; Taylor *et al*., 2012) and the conventional view of lamprey taxonomy (i.e., frequent co-occurring, yet distinct parasitic and non-parasitic species pairs) has always had its opponents (reviewed in Docker, 2009). The lack of fixed genetic differences between sympatric species pairs (Schreiber & Engelhorn, 1998; Blank *et al*., 2008; Boguski *et al*., 2012; Docker *et al*., 2012), evidence of communal spawning on shared nesting grounds (Huggins & Thompson, 1970; Cochran & Gripentrog, 1992; Lasne *et al*., 2010), as well as inter- and intra-specific sneak mating tactics exhibited by males (Pletcher, 1963; Malmqvist, 1983; Cochran *et al*., 2008; Hume *et al*., *in press*), suggest that in certain cases, species specific mating cues may not have evolved.

The European river lamprey *Lampetra fluviatilis* and European brook lamprey *L. planeri* are a sympatric species pair with a wide geographic range covering most of western Europe (Hardisty & Potter, 1971; Renaud, 2011). *Lampetra fluviatilis* is an anadromous species and following a larval period of three to four years in freshwater it metamorphoses and migrates downstream where it forages by removing muscle tissue from various estuarine and inshore teleosts for a period of 12 - 18 months (Hardisty & Potter, 1971; Maitland *et al*., 1984; Renaud *et al*., 2009). *Lampetra planeri* is a non-parasitic species; it has a larval growth period of at least five years, and following metamorphosis it remains within natal streams where it does not feed (Hardisty, 1961; Hardisty, 2006). Both species spawn in spring, and where found in sympatry, are known to utilise similar spawning habitat (Huggins & Thompson, 1970; Jang & Lucas, 2005). In a few European lake systems this species pair can be found sympatrically with a freshwater-resident population of *L. fluviatilis* (Berg, 1948; Valovirta, 1950; Tuunainen *et al*., 1980; Goodwin *et al*., 2006; Inger *et al*., 2010). In Loch Lomond, Scotland, a freshwater-resident *L. fluviatilis* population is known to forage within the lake for a period of 6 months (Maitland, 1980; Adams *et al*., 2008). Individuals expressing this life history strategy spawn in only one afferent river of the lake and there is no apparent temporal or spatial spawning segregation from sympatric *L. fluviatilis* expressing an anadromous life history or from the non-parasitic *L. planeri* (Maitland *et al*., 1994; Hume, 2011).

Possibly as a result of a reduced parasitic feeding phase, freshwater-resident *L. fluviatilis* in Loch Lomond mature at a smaller size compared with anadromous *L. fluviatilis*, but they are larger than the non-parasitic *L. planeri*. Given that sexually mature body size is believed to play a key role in lamprey speciation (*sensu* Beamish & Neville, 1992), and that body size appears correlated with life history strategy, the lampreys of Loch Lomond offer a unique system for testing this theory. Therefore, complete reproductive isolation between the extreme life history strategies of non-parasitic, stream-resident and anadromous parasitic individuals is expected to result from behavioural isolation acting between heterotypic mates. Individuals expressing an intermediate life history strategy (i.e., freshwater-resident parasitic) are expected to exhibit less polarised responses to heterotypic mates, given the wider choice of potential spawning partners. Here the strength of assortative mating between three such sympatric lamprey populations, exhibiting divergent life history strategies and expressing discrete adult body sizes is examined in a comprehensive multiple-mate choice trial within an artificial stream environment.

4.3 MATERIALS & METHODS

4.3.1 *Collection and maintenance of experimental animals*

Between October 2010 and April 2011 adult lampreys were collected in static traps (Morris & Maitland, 1987) as they migrated upstream to spawning grounds in the Endrick Water (56°3’17·3” N, 4°27’16·2” W), which drains into the south basin of Loch Lomond. Adult anadromous and freshwater-resident *L. fluviatilis,* as well as non-parasitic *L. planeri* from the Loch Lomond catchment, can be separated using standard lamprey taxonomic characteristics (Morris, 1989; see Renaud, 2011 for criteria). Anadromous *L. fluviatilis* mature at 323 mm (mean; range 257 – 374 mm); freshwater-resident *L. fluviatilis* at 217 mm (mean; range 145 – 269 mm); and *L. planeri* mature at a mean length of 145 mm (range 103 – 195). Hereafter, a “population” refers to only one life history strategy i.e., the anadromous *L. fluviatilis,* freshwater-resident *L. fluviatilis* or *L. planeri* “population”.

Each population was held in a separate 175 litre tank using Loch Lomond water at ambient temperature on a flow-through system, and exposed to artificial light that tracked natural photoperiod. Lampreys were examined periodically to assess the progress of sexual maturation. Ripe lamprey females become swollen with eggs that are usually visible through a patch of translucent skin near the cloaca, and also develop a post-cloacal finfold. Sexually mature male lampreys can be identified by an obvious genital papilla that extends several millimetres from the cloaca. By April 1st 2011 the majority of captive lampreys were ready to spawn.

Twelve sexually mature individuals from each of the three populations (six males and six females) were selected for inclusion in mate choice trials. These were anaesthetised using a benzocaine solution and measured to the nearest 1 mm total length (L_T) (Fig. 4.1). To prevent any size-bias effects individuals from within each population were size matched from among the total length-range available from captured lampreys (Fig. 4.2). Prior to inclusion in any mate choice trial (see below), lamprey were held in 10 L tanks as same-sex, same-type pairs to prevent spawning. These tanks were maintained under the same conditions as the population holding tanks.

4.3.2 *Mate choice trials*

An artificial stream measuring 5.72 m in length was used to simulate natural conditions as closely as possible during mate choice trials. The base of the stream was covered to a depth of approximately 4 cm by gravel (0.5 – 2 cm diameter) collected locally. The stream was partitioned by fine mesh (1 mm) screens, creating six discrete sections measuring 91 cm long by 58 cm wide. Water from Loch Lomond was pumped through the stream at velocities of 5 – 20 cm s^{-1}, and temperatures ranged 8.5 – 11.5°C throughout the study period. Artificial, low-light levels on a natural photoperiod were maintained throughout.

Mate choice trial groups consisted of a single female and three males (one from each population). Each female was exposed to all 18 males over all trials, creating 108 mate choice trials in total (= 36 trials with each female type). Groups were placed in a stream section and allowed to acclimate for *c*. 5 minutes before observations began. Direct observation of each mate choice trial lasted six hours, during which time all spawning activity was recorded. Following a trial, each female was removed and allowed to rest for at least six hours. This prevented the exhaustion of the female's egg stock during any single trial. Male groups were rested after every second trial. Only females of the same type were tested in succession to reduce any possible residual effects from pheromones, or other stimuli. Following each trial, the stream section was examined for eggs that were collected using a siphon. The gravel was then scoured to remove any traces of nests, and that section remained empty for at least 12 hours. After all six females of a population were tested with all 18 males, the stream was drained for a period of 24 hours, and the gravel scoured before refilling with water. The experimental period lasted 15 days, during which no individuals died.

Petromyzontid spawning comprises several discrete behaviours (Table 4.1). Typical spawning occurs within nests created in shallow gravel beds, and begins when females attach to an object immediately upstream of the nest. A male then attaches to the female's head and wraps his tail around her trunk, forming a tight loop which acts to express the ova from her body. Both partners vibrate rapidly as gametes are released and fertilisation takes place externally within the nest. Spawning in this study is here defined as those behaviours beginning with a male attaching to a female, and which may or may not culminate in the release of gametes. Therefore, spawning behaviour in this study constituted the following: male attachment; male attachment + unsuccessful gamete release; male attachment + successful gamete release. This definition is considered to be an expression of mate preference for both sexes, given that females are capable of rejecting a male, and so can be used as a measure of behavioural isolation between populations.

An isolation index was calculated for each mate choice trial based on the frequency of spawning (*If*):

$$If = \frac{number\ of\ homotypic\ spawnings - number\ of\ heterotypic\ spawnings}{total\ number\ of\ spawnings}$$

The isolation index ranges between -1 and 1, where: *If* = 0 indicates random mating; *If* > 0 indicates positive assortative mating; and *If* < 0 indicates negative assortative mating. In this study positive assortative mating is defined as a preference for phenotypically similar mates (i.e., expressing the same life history strategy). Student's t-tests were used to test for significant deviations from 0 in *If*, the null hypothesis being that there was no preference for mating between individuals belonging to the same population. Analysis of variance was used to test for differences in the frequencies of spawning behaviour exhibited by different populations.

4.4 RESULTS

Overall, spawning was recorded on 963 occasions from 66 mate choice trials. Forty-two trials resulted in no spawning (six with anadromous *L. fluviatilis* females; six with freshwater-resident *L. fluviatilis* females; 30 with *L. planeri* females). There were significant differences in the total frequency of observed spawning behaviour between trials containing females from different populations (ANOVA, d.f. = 2, $P < 0.001$). Trials containing anadromous *L. fluviatilis* females accounted for 30.7% of recorded spawning (n = 296, N = 30 trials), freshwater-resident *L. fluviatilis* females 67% (n = 645, N = 30 trials) and *L. planeri* females 2.3% (n = 22, N = 6 trials).

Of all spawning pairs recorded (n = 963) 33.6% were homotypic (n = 324) and 66.4% were heterotypic (n = 659) (Fig. 4.3). In trials containing anadromous *L. fluviatilis* females, 50.3% of recorded spawning were homotypic (n = 149; N = 24 trials), while 49.7% were heterotypic (with freshwater-resident *L. fluviatilis* males n = 121, 40.9%, N = 24 trials; with *L. planeri* males n = 26, 8.8%, N = 12 trials). In trials containing freshwater-resident *L. fluviatilis* females 24.8% of recorded spawning were homotypic (n = 160, N = 23 trials), and 75.2% were heterotypic (with anadromous *L. fluviatilis* males n = 212, 32.9%, N = 24 trials; with *L. planeri* males (n = 273, 42.3%, N = 24 trials). In trials containing *L. planeri* females 68.2% of recorded spawning behaviour were homotypic (n = 15, N = 6 trials) and 31.8% were heterotypic (with freshwater-resident *L. fluviatilis* males n = 7, N = 1 trial; with anadromous *L. fluviatilis* males n = 0).

There were significant differences in the frequency with which different male life-history types spawned with females across all trials (ANOVA, d.f. = 2, 107, F = 13.6, $P < 0.01$). When anadromous *L. fluviatilis* females were available: anadromous and freshwater-resident *L. fluviatilis* males engaged in spawning with equal frequency (Tukey HSD, P = 0.430 for anadromous; P = 0.529), but anadromous and freshwater-resident *L. fluviatilis* males did so more often than male *L. planeri* individuals ($P < 0.001$). In trials with freshwater-resident *L. fluviatilis* females, there were no significant differences in the spawning frequency of different male life-history types ($P > 0.05$ for all). When female *L. planeri* individuals were available in trials *L. planeri* males spawned more often with them compared with both anadromous ($P < 0.001$) and freshwater-resident *L. fluviatilis* males ($P < 0.001$). It should be noted that only a small number of trials containing *L. planeri* females resulted in spawning (N = 6, see above).

For anadromous *L. fluviatilis* and *L. planeri* populations, the value of *If* was variable, and average Index values were not significantly different from zero, indicating random mating (Student's t-test, t = -1.311, d.f. = 29, P = 0.20 for anadromous *L. fluviatilis*; t = 2.429, d.f. = 5, P = 0.059 for *L. planeri*). However, average Index values for the freshwater-resident *L. fluviatilis* population differed significantly from zero ($t = 5.176$, d.f. = 29, $P < 0.001$), indicating that positive assortative mating was occurring within this life history type (Fig. 4.4).

4.5 DISCUSSION

The mechanisms through which reproductive isolation between emerging species develops following ecological divergence in sympatry are complex and not widely agreed upon (Baldauf *et al*., 2009; Berner *et al*., 2009; Bolnick & Kirkpatrick, 2012). As phenotypic and behavioural divergence increases, or following reinforcement from secondary contact, assortative mating caused by a preference for similar mates may, or may not, lead to complete reproductive isolation and full speciation, either in parapatry or sympatry (Merrill *et al*., 2010). In this study, there was no evidence of reproductive isolation *via* strong assortative mating between three sympatric lamprey populations exhibiting very different life history strategies, and which currently belong to two putative species. In contrast, heterotypic spawning was most frequently observed, with both males and females reacting in the same manner to both homo- and heterotypic mates. Therefore, behavioural isolation between these life history types does not appear to have resulted in a barrier to gene flow.

Trials containing females of both anadromous and freshwater-resident *L. fluviatilis* resulted in similar frequencies of spawning with males, particularly in trials containing freshwater-resident *L. fluviatilis* females. However, there were significant differences in the total frequency of observed spawning in trials containing females of *L. planeri*, providing some evidence of uni-directional mate preference acting between this species pair. It should be noted that both male and female *L. planeri* appeared unwilling to participate in spawning, although nest building behaviours were evident throughout the study (Appendix 4.1). In general, non-parasitic lamprey species spawn gregariously, possibly as a way of increasing available mates and allowing for the creation of larger and more suitable nests for spawning (Mundahl & Sagan, 2005). One explanation for observations of inter-specific nesting between anadromous and non-parasitic lamprey species pairs is that the non-parasitic types were taking advantage of a larger nest created by the parasitic species, thereby increasing the spawning habitat available for the non-parasitic population to utilise.

Lamprey spawning is believed to be linked closely to adult body size, where homogamy is the general rule (Beamish & Neville, 1992). Deviation from a 1:1 body length ratio appears to reduce fertilisation success at *c.* 25% size differences, both within and between species (Malmqvist, 1983). Such differences between males and females result in low fertilisation rates due to poorly aligned genital papillae, and the inaccurate expression of milt from males onto the ova (Hagelin, 1959; Malmqvist, 1983; Beamish & Neville, 1992). Although fertilisation success is reduced where size differences are greater, this ratio does, however, cover many species pairs (Docker, 2009), and differences marginally less than 25% can still result in some fertilised eggs. In this study there was no strong evidence for size-assortative mating between the three populations, as both large anadromous *L. fluviatilis* and small *L. planeri* males spawned frequently with freshwater-resident *L. fluviatilis* females of an intermediate size. This also indicates that there is only a weak preference to spawn with individuals sharing their own life history strategy. As expected, little spawning behaviour between anadromous parasitic *L. fluviatilis* and stream-resident, non-parasitic *L. planeri* was observed and this likely represents at least some modest ability to detect body size in prospective mates.

If mate selection was random among multi-modal lamprey populations such as these, it might be expected that intermediate body sizes (i.e., freshwater-resident *L. fluviatilis*) would be the focus of most spawning effort, given that they represent a potential mate to both larger and smaller individuals. Indeed, there was no difference in the frequencies with which males of all three life history types attempted to spawn with freshwater-resident *L. fluviatilis* females in this study. The freshwater-resident *L. fluviatilis* population, however, expressed positive assortative mating, indicating that they themselves preferred to mate with similar individuals. Given their position as intermediate between two otherwise extreme body sizes, it remains unclear whether or not such individuals regard larger (i.e., anadromous *L. fluviatilis*) and smaller (i.e., stream-resident *L. planeri*) lampreys as potential conspecifics in this system.

Certainly, species specific mating cues between *L. fluviatilis* and *L. planeri* appear to be lacking. Alternative reproductive behaviours were exhibited by males of all three life-history types, although with the exception of sneaker males (Hume *et al.*, *in press*) this was not fully quantified and their function remains unclear. These behaviours are believed to be natural and not the result of context-dependent sexual selection by males influenced by the presence of potential competitors (Callander *et al.*, 2011). Neither was female mate choice expected to have been an artefact of access to low-quality males (Robertson & Butler, 2013), given that both larger and smaller males were available in this study as opposed to the more common no-choice assays employed elsewhere (Williams & Mendelson, 2010; Berden & Fuller, 2012).

This study has shown that pre-zygotic barriers to gene flow in the form of strong assortative mating do not occur between sympatric populations of *L. fluviatilis* and *L. planeri* in the Endrick Water. Additionally, populations exhibiting alternative life history strategies, such as freshwater-resident *L. fluviatilis,* do not appear to be behaviourally isolated from both members of this species pair. As life history strategy and adult body size in lampreys are intimately linked, preference for either anadromous parasitic or stream-resident, non-parasitic strategies should result in a strong selection bias against individuals expressing intermediate phenotypes. Freshwater-resident parasitic females of *L. fluviatilis* do, however, appear highly favoured by both larger and smaller males expressing a more extreme life history, suggesting either a limited ability to detect type-specific cues, or a lack thereof. Compatibility between the gametes of *L. fluviatilis* and *L. planeri,* including freshwater-resident *L. fluviatilis* from Loch Lomond (Hume *et al*., 2012, *in press*), suggests that hybrid offspring resulting from either direct spawning, incidental gene flow caused by nest-sharing (Lasne *et al*., 2012), or sneak mating tactics between petromyzontid species or life history types, are not necessarily selected against.

Ultimately, differences in life history strategy between members of lamprey paired species should result in genetic isolation as a result of size-assortative mating, but only if life history type has a genetic component (Adams & Huntingford, 2002). In recently derived pairs that still exist in sympatry there is no support for genetic differentiation between life history types. For example, in *L. fluviatilis* and *L. planeri* using both mitochondrial DNA (mtDNA) and nuclear genes no species specific markers have been found (Schreiber & Engelhorn, 1998; Docker *et al*., 1999; Espanhol *et al*., 2007; Blank *et al*., 2008; Pereira *et al*., 2011) suggesting both represent alternative life history strategies of a single species, ongoing gene flow and/or recent divergence times. A similar pattern is seen in the Asian species pair *Lethenteron camtschaticum/L. reissneri* (Artamonova *et al*., 2011). Most definitively, the North American species pairs *I. unicuspis* and *I. fossor* (Docker *et al*., 2012) and *Lampetra ayresii*/*L. richardsoni* (Boguski *et al*., 2012) do not exhibit even subtle mtDNA or microsatellite allele frequency differences where they co-occur.

The evolution of non-parasitic lampreys has been suggested to require intermediate phenotypes or life history strategies (i.e., freshwater parasitic forms) prior to the abandonment of post-larval feeding and the subsequent evolution of non-parasitic, stream-resident individuals (Salewski, 2003). Coupled with molecular evidence, those data presented here instead point to plasticity in life history strategy among wide-ranging parasitic lamprey species such as *L. fluviatilis* (Hindar & Johnsson, 1993). Patterns in evolution such as this are common to post-glacial fishes, where ancestral anadromous species have given rise to multiple freshwater-resident or fluvial populations expressing discrete phenotypes (Taylor, 1999). However, phenotypic divergence in response ecological adaptive processes and evolutionary constraints, and the subsequent evolution of reproductive isolation resulting from assortative mating between divergent forms, is not inevitable (Raeymaekers *et al.*, 2010). Rates of speciation resulting from the reproductive isolation of these diverging populations are highly variable between systems, and many species designations are therefore very contentious (e.g., Etheridge *et al.*, 2012).

It seems most congruent with the available data that the lampreys of Loch Lomond, and indeed most paired lamprey species, in fact represent alternative life history strategies arising from a single gene pool. This study indicates that despite the propensity for gene flow, resulting from a lack of behavioural isolation, lampreys expressing alternative life history strategies could have arisen sympatrically *via* weak assortative mating. Although not strictly necessary for the maintenance of non-parasitic populations after they have evolved (if those individuals prefer to mate with similarly small mates), it remains to be seen what role phenotypically variable species such as *L. fluviatilis* have on the mediation of gene flow between parasitic and non-parasitic forms, and whether the anadromous parasitic life history can appear again from isolated non-parasitic populations.

4.6 FIGURES & TABLES

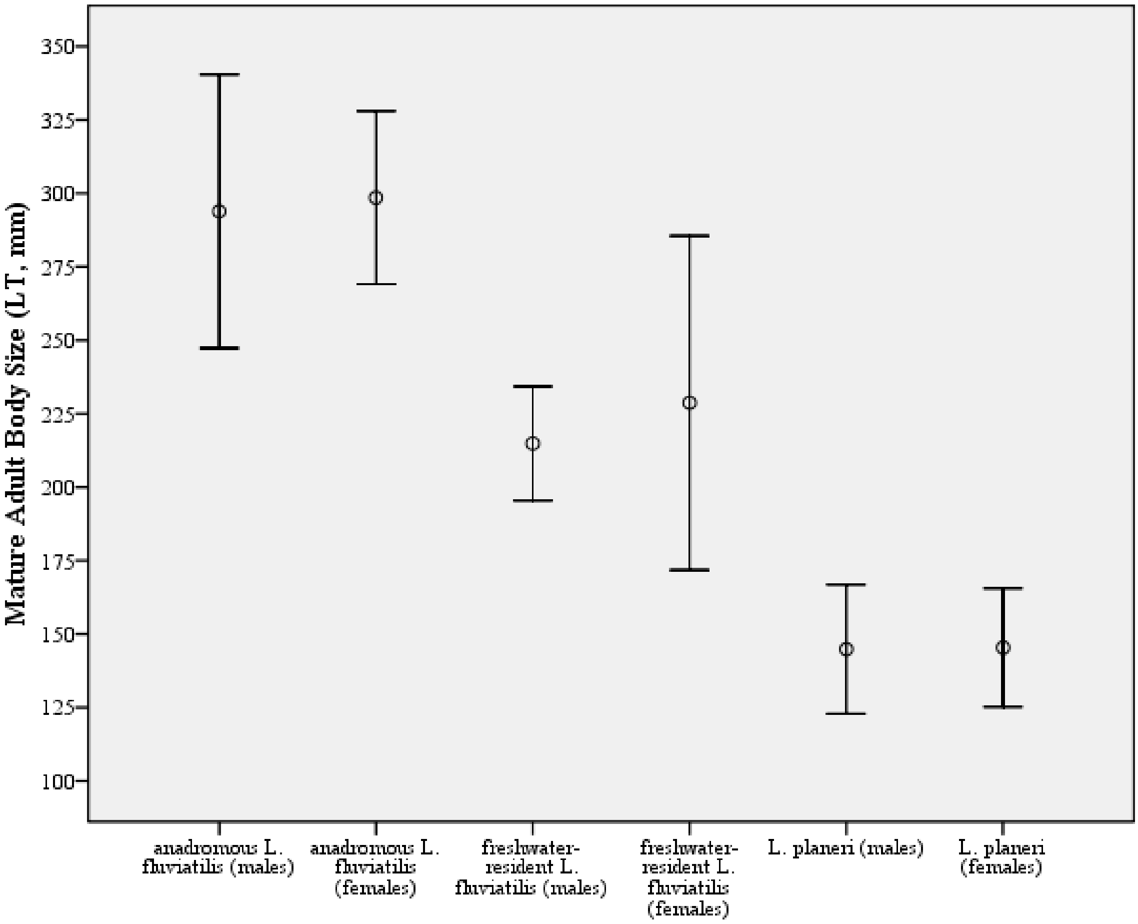


Fig.4.1 Length range (total length L_T, mean ± S.D.) of both male and female anadromous *Lampetra fluviatilis* (*n* = 12), freshwater-resident *L. fluviatilis* (*n* = 12) and *L. planeri* (*n* = 12) from the Endrick Water, Loch Lomond selected for mate choice trials.

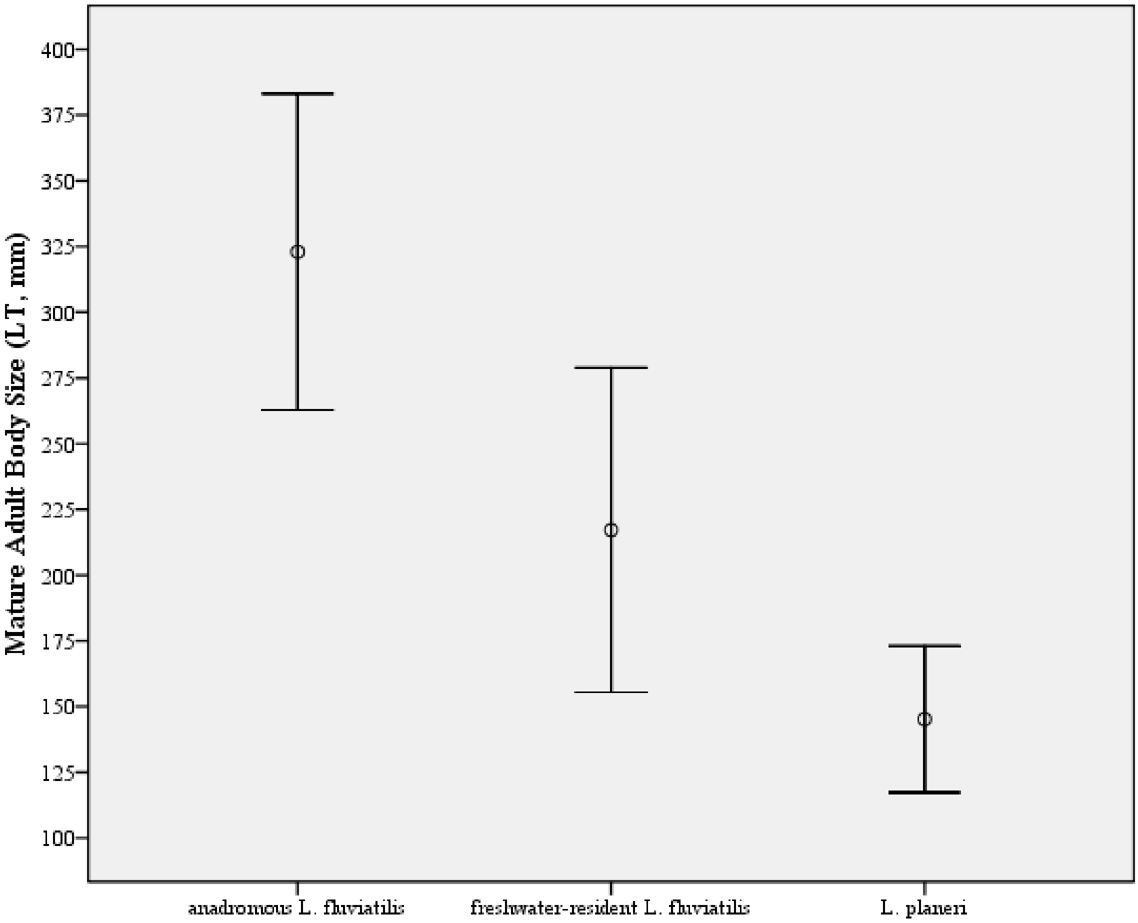


Fig.4.2 Length range (total length L_T, mean ± S.D.) of sexually mature anadromous *Lampetra fluviatilis* (*n* = 23), freshwater-resident *L. fluviatilis* (*n* = 35) and *L. planeri* (*n* = 318) collected from the Endrick Water, Loch Lomond.

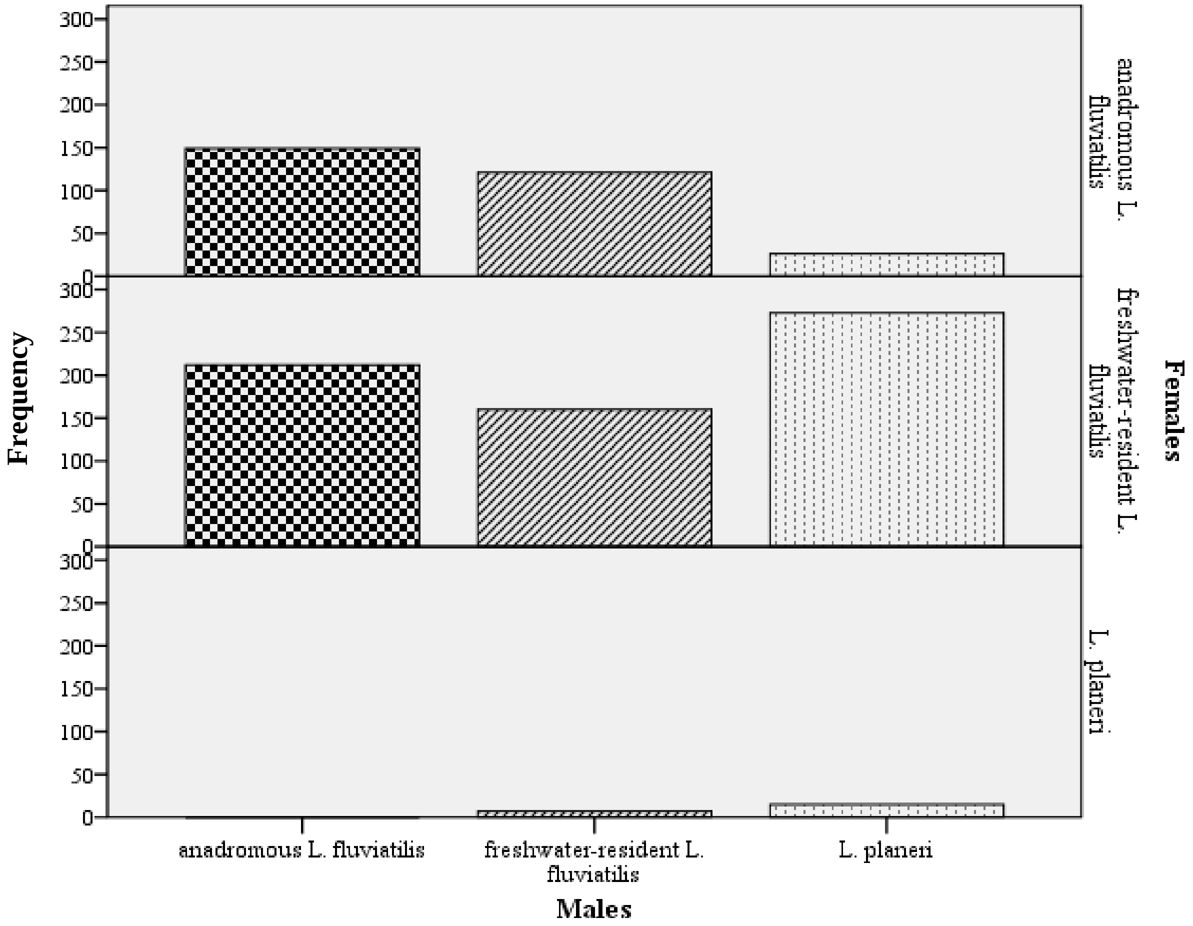


Fig.4.3 Frequency of spawning interactions (total) between anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* from the Endrick Water, Loch Lomond during mate choice trials in an artificial stream (n = 963, N = 62 trials).

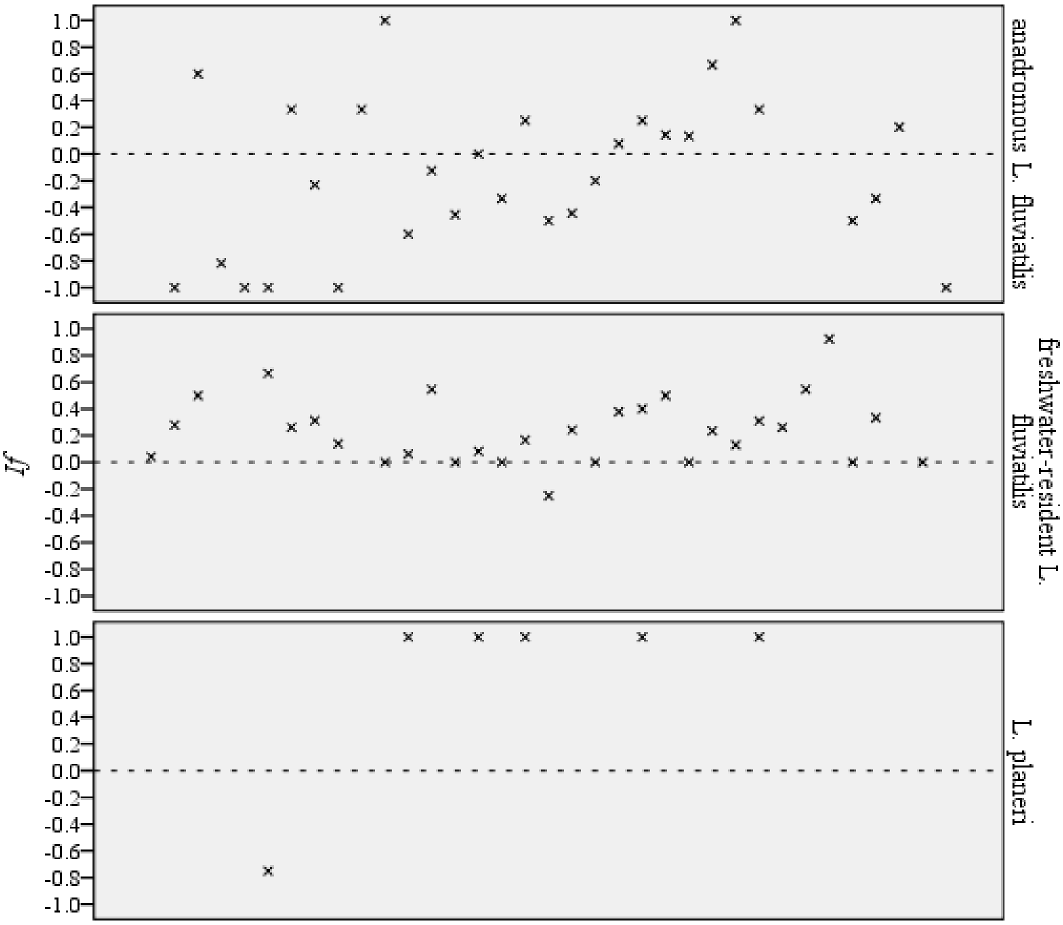


Fig.4.4 Isolation Index (*If*) testing the relative strength of assortative mating between anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* from the Endrick Water, Loch Lomond during mate choice trials in an artificial stream (*N* = 62 trials). Each cross represents a trial containing a single female lamprey of the indicated life history strategy, where spawning took place. The position of the cross in the panel represents the mating preference of the female involved in that trial. I_f = (*number of homotypic encounters – number of heterotypic encounters) x total number of encounters*$^{-1}$. The Y-intercept indicates the perceived position of random mating on the Isolation Index ($I_f = 0$), where $I_f > 0$ positive mating occurs (see text for definition).

Table 4.1 Description of *Lampetra* spp. spawning behaviours as documented during field and laboratory observations. A brief explanation of the significance of each is included.

Behaviour	**Description**
Female tail waving	Female attaches to rock out-with a nest, upstream of gravel depression. Tail is waved in a slow and deliberate manner (distinct from tail-thrashing behaviour employed during nest cleaning). May act to attract males either through dispersal of pheromones excreted from branchial region[1], or mechanically through changes in water pressure.
Male gliding	Male moves the oral disc along female's body, both in anterior-posterior and posterior-anterior directions. May be employed to detect female body size[2] or to discriminate sexes[3], as male-male interactions were observed (Appendix 4.1).
Male attachment	Male attaches oral disc to the head of female[4], usually anterior of the eye. Other positions were noted though, including: on branchial and trunk regions, as well as to rocks outside the nest.
Female rejection	Female releases from attachment in the nest and shakes the male loose.
Unsuccessful gamete release	Male attempts to form a tail-loop around the female but cannot complete it. Males may be larger/smaller than females and so unable to express eggs from her body[5]. May also be an indication of lateralisation as some individuals appeared to preferentially form tail-loops to the left or right.[4, 6]
Successful gamete release	Following the completion of the tail-loop both male and female raise their branchial regions at an acute angle and the male slides the tail-loop along the female in a posterior direction. Both then shake violently, expelling gametes into the nest[2].
Sneak male tactics	At the point of gamete release an additional male that is not attached to either the female or nest, circles tightly around the urogenital area of the spawning pair[7]. Or, a male resting near a nest is stimulated by a pair spawning within the nest and also begins to quiver[8]. This latter behaviour may not result in gamete release.
Multiple male attachment	More than one male attaches to a single female, either simultaneously, or following an attachment by the initial male. These males may form multiple tail-loops or just one male succeeds. Up to three males were seen attached to a single female.

[1] (Pickering & Morris, 1977); [2] (Hagelin & Steffner, 1958); [3] (Reighard, 1903); [4] (Hardisty, 2006); [5] (Beamish & Neville, 1992); [6] (Hagelin, 1959); [7] (Cochran *et al*., 2008); [8] (Pletcher, 1963)

Appendix 4.1

Table 4.2 Total frequency of all spawning pairs and their outcomes from mate choice trials containing anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* collected from the Endrick Water, Loch Lomond. Trials took place in an artificial stream environment. Spawning pairs can elicit four outcomes as follows: *I* = female rejects male attachment and detaches from the nest; *II* = female does not respond to male attachment; *III* = typical spawning takes places yet no ova are expressed; *IV* = typical spawning takes place and ova are expressed.

		Females											
		anadromous *L. fluviatilis*				freshwater-resident *L. fluviatilis*				*L. planeri*			
		I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Males	anadromous *L. fluviatilis*	15	46	17	27	12	67	60	36	0	0	0	0
	freshwater-resident *L. fluviatilis*	11	47	8	0	5	39	35	32	0	0	0	0
	L. planeri	4	13	0	0	9	179	31	11	5	2	3	1
	anadromous + freshwater-resident *L. fluviatilis*	4	3	11	13	0	4	5	1	0	0	0	0
	anadromous *L. fluviatilis* + *L. planeri*	1	0	0	0	2	15	2	2	0	0	0	0
	freshwater-resident *L. fluviatilis* + *L. planeri*	1	0	0	0	2	10	1	0	0	0	0	0
	anadromous + freshwater-resident *L. fluviatilis* + *L. planeri*	0	0	0	0	0	0	1	1	0	0	0	0

Table 4.3 Total frequency of male-male attachments during mate choice trials containing anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* collected from the Endrick Water, Loch Lomond. Trials took place in an artificial stream environment. All males rapidly detached from their nest if another male attached. Abbreviations: *n/a* = not applicable. Only a single male of each life history type was present in any one trial.

		Males		
		anadromous *L. fluviatilis*	freshwater-resident *L. fluviatilis*	*L. planeri*
Males	anadromous *L. fluviatilis*	n/a	3	0
	freshwater-resident *L. fluviatilis*	19	n/a	0
	L. planeri	2	1	n/a

Table 4.4 Total frequency of nest building behaviours exhibited during mate choice trials by anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* collected from the Endrick Water, Loch Lomond. Trials took place in an artificial stream environment.

		anadromous *L. fluviatilis*		freshwater-resident *L. fluviatilis*		*L. planeri*	
		♂	♀	♂	♀	♂	♀
Nest building behaviour	*Gravel loosening*	3	2	7	0	2	5
	Tail thrashing	162	51	69	7	68	14
	Stone moving	173	35	185	90	356	115

Table 4.5 Total frequency of antagonistic behaviour recorded during mate choice trials containing anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* collected from the Endrick Water, Loch Lomond. Trials took place in an artificial stream environment. Antagonistic behaviour was said to have occurred when a nest building male attached to the trunk region of another individual and swam a short distance away from the nest before releasing them. Abbreviations: *n/a* = not applicable. Only a single male of each life history type was present in any one trial.

		Females			***Males***		
		anadromous *L. fluviatilis*	freshwater-resident *L. fluviatilis*	*L. planeri*	anadromous *L. fluviatilis*	freshwater-resident *L. fluviatilis*	*L. planeri*
Aggressive Males	anadromous *L. fluviatilis*	1	0	0	n/a	17	12
	freshwater-resident *L. fluviatilis*	0	0	2	18	n/a	7
	L. planeri	0	0	0	10	3	n/a

“The lamprey…is found principally in the Severn, the Thames and in Scotch waters. Formerly but little use was made of it, except to be dried and burnt as a candle. The flesh is sweet and good, and of much nourishment: it increases lust, and by reason of its richness causes surfeits if much eaten.”

W. T. Fernie (1905), *Meals Medicinal*

Chapter Five

Sneak male mating tactics between lampreys exhibiting alternative life history strategies

5.1 ABSTRACT

Pairs of closely related sympatric lamprey species expressing different life history strategies are believed to be reproductively isolated as a consequence of size-assortative mating reducing gene flow between them. Previous studies, however, suggest that some male lampreys are capable of exhibiting alternative reproductive strategies in the form of sneaker males. This study reports sneak mating tactics among European river and brook lampreys, but most dramatically, it documents for the first time inter-specific sneak mating between members of a petromyzontid species pair, and suggests its commonality in some systems may result in high levels of gene flow between putative species.

5.2 INTRODUCTION

Lampreys (Petromyzontiformes) exhibit an evolutionary tendency to produce small, non-parasitic stream-resident species from larger, parasitic and often migratory species. These so-called paired species (Zanandrea, 1959) are believed to have arisen rapidly in sympatry through the development of barriers to gene flow as a result of size-assortative mating (Beamish & Neville, 1992; Salewski, 2003). Petromyzontids are generally homogomous spawners (i.e., mating at approximately 1:1 size ratios), resulting in reduced fertilisation rates where the sexes begin to diverge more widely in size, with zero fertilisation observed when differences are greater than or equal to 25% (Hagelin, 1959; Malmqvist, 1983; Beamish & Neville, 1992). This trend results from the spawning mechanics of the lamprey mating pair; where a male attaches to the female's head and wraps his tail around her trunk, forming a tight loop that acts to express the ova from her body (Hagelin & Steffner, 1958). Both partners vibrate rapidly as gametes are released and fertilisation takes place externally within the nest.

Ultimately, differences in life history strategy between members of lamprey species pairs should result in genetic isolation as a result of size-assortative mating, if life history type has a genetic component (e.g., Adams & Huntingford, 2002). However, in recently derived pairs that still exist in sympatry there is no support for genetic differentiation between life history types. For example, in the European river lamprey *Lampetra fluviatilis* and its non-parasitic derivative *L. planeri* using both mitochondrial DNA (mtDNA) and nuclear genes no species specific markers have been found (Schreiber & Engelhorn, 1998; Docker *et al.*, 1999; Espanhol *et al.*, 2007; Blank *et al.*, 2008; Pereira *et al.*, 2011) suggesting both represent alternative life history strategies of a single species, or recent divergence times. The same pattern is seen in the Asian species pair *Lethenteron camtschaticum/L. reissneri* (Artamonova *et al.*, 2011). Most definitively, the North American species pair *Ichthyomyzon unicuspis/I. fossor* do not exhibit even subtle mtDNA or microsatellite allele frequency differences where they co-occur (Docker *et al.*, 2012). This evidence supports the view that at least some non-parasitic lampreys have evolved independently and repeatedly from parasitic populations, and suggests ongoing gene flow and the possibility of plasticity in life history strategy (e.g., Hindar & Johnsson, 1993).

Mechanisms explaining this pattern of gene flow focus on the potential ability of paired species to circumvent the effects of homogamy. Although fertilisation success is reduced where size differences are greater, the perceived 25% limit does, however, overlap many paired species (Docker, 2009), and differences greater than this can still result in some fertilised eggs (Malmqvist, 1983; Beamish & Neville, 1992). Homogamy will not have any effect though where alternative mating behaviours are employed by males (Taborsky, 2008). Alternative mating behaviours have been described in two petromyzontid genera (*Lampetra* and *Lethenteron*), where males are often referred to as “satellites” (e.g., Cochran *et al.*, 2008). During typical spawning a satellite male will rapidly circle the urogenital area of a spawning pair at the point of gamete release, attempting to gain fertilisation of the female’s eggs. Sneaker males, that achieve fertilisation success not through direct competition with other males or by successful attraction of females, but through swift release of their gametes during the spawning of another pair, are common to many teleost groups (Gross, 1984). Therefore, the term “sneaker male” should be applied to male lampreys exhibiting “satellite” spawning behaviour to indicate the similarity between petromyzontids and other fish groups. In studies to date, this behaviour has been observed only in intra-specific pairings (Cochran *et al.*, 2008).

In Loch Lomond, Scotland the Endrick Water is known to contain three phenotypically discrete lamprey populations at spawning time, each of which exhibits very different life history strategies (Maitland *et al*., 1994). A population of anadromous *L. fluviatilis* and non-parasitic *L. planeri* can be found alongside a freshwater-resident population of *L. fluviatilis* (Morris, 1989). Anadromous *L. fluviatilis* feed parasitically within estuarine environments for 12 – 18 months (Maitland *et al.*, 1984) while freshwater-resident *L. fluviatilis* feed exclusively in the freshwater lake for not more than six months (Maitland, 1980; Adams *et al*., 2008). *Lampetra planeri* does not feed following the completion of its larval development. As a result of this *L. planeri* matures at a mean length of 145 mm (range 103 – 195 mm), freshwater-resident *L. fluviatilis* at 217 mm (range 145 – 269 mm), and anadromous *L. fluviatilis* at 323 mm (range 257 – 374 mm) (Hume, this study). There is no temporal segregation between the populations and they become sexually mature and migrate to the spawning grounds simultaneously (Hume, 2011).

During the course of a wide-ranging investigation of these lamprey populations a detailed study was made of their spawning preferences (Hume *et al*., *in prep.*). The purpose of this report is to document and present new information on the presence of sneak mating tactics within this life history complex. Although reported previously within species, this record provides the first conclusive evidence for inter-specific sneak mating behaviour between any petromyzontid species pair.

5.3 MATERIAL & METHODS

Between October 2010 and April 2011 adult lampreys were collected in static traps (Morris & Maitland, 1987) as they migrated upstream to spawning grounds. Adult anadromous *L. fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* from the Loch Lomond catchment can be separated using standard lamprey taxonomic characteristics (Morris, 1989; *sensu* Renaud, 2011). Individuals from each of the three populations were held in a separate 175 litre tank using Loch Lomond water at ambient temperature on a flow-through system, and exposed to artificial light that tracked natural photoperiod. Lampreys were examined periodically to assess the progress of sexual maturation. Ripe females become swollen with eggs, which are usually visible through a patch of translucent skin near the cloaca, and also develop a post-cloacal finfold (Hagelin & Steffner, 1958). Sexually mature male lampreys can be identified by an obvious genital papilla that extends several millimetres from the cloaca. By April 1st 2011 the majority of lampreys were ready to spawn.

An artificial stream measuring 5.72 m in length was used to simulate natural conditions as closely as possible. The base of the stream was covered to a depth of approximately 4 cm by gravel (0.5 – 2 cm diameter) collected locally. Water from Loch Lomond was pumped through the stream at velocities of 5 to 20 cm s^{-1}, and temperatures ranged from 8.5 to 11.5°C throughout the study. Artificial, low-light levels were maintained throughout. Twelve individuals from each of the three populations (6 male, 6 female) were selected for spawning. Spawning groups comprised a single female and three males (one from each population) and each group was separated into individual sections of the stream partitioned by fine (1 mm) mesh screens. A spawning group was placed in a stream section and allowed to acclimate for five minutes before observations began. Groups were observed for six hours before males were rotated between different sections. Direct observation of spawning groups lasted 15 days, during which all spawning activity was recorded. Successful gamete release was confirmed visually by the appearance of small (*c.* 1 mm) yellow ova in a nest. The study was terminated on 16th April 2011, and no individuals died during the observation period.

5.4 RESULTS

Overall, spawning was observed on 963 occasions (i.e., where a male attached to a female, formed a tail-loop, and vibrated rapidly). Of the total number of times gametes could be confidently said to have been expressed in a nest during such spawning (n = 125), sneak mating tactics (Fig. 5.1) where noted in 13.6% (n = 17). Freshwater-resident *L. fluviatilis* males were seen to exhibit sneaking on seven occasions, *L. planeri* on six, and anadromous *L. fluviatilis* on four (Table 5.1). Inter-specific sneak mating tactics between *L. planeri* and anadromous *L. fluviatilis* females were witnessed twice, and with freshwater-resident *L. fluviatilis* females on four occasions. In addition, both life history strategies of *L. fluviatilis* employed sneak mating tactics on one another, where anadromous (n = 2) and freshwater-resident (n = 5) males were observed sneaking on their smaller and larger conspecifics respectively.

5.5 DISCUSSION

These observations indicate that sneak mating is relatively common in this system, even when considering the small sample size (n = 36 individuals) and being conservative in the identification of sneak behaviour (i.e., in considering only those instances when gametes were expressed with a high degree of certainty). Other alternative reproductive behaviours were exhibited by males of all three populations. In many cases (*c*. 25% of observed spawning) males attached to a rock in the vicinity of a nest containing a spawning pair would vibrate rapidly at the same moment the pair were attempting to release eggs. It may be that these males were responding to the mechanical stimuli of nearby spawners, as opposed to exhibiting any attempt to achieve sneak fertilisation (Pletcher, 1963). In far fewer occasions (< 5% of observed spawning) smaller males would attach to the trunk region of larger spawning females, belonging to the same or different population, and when the pair began to vibrate the smaller male would curl his tail beneath them. Again, rather than representing any attempt to achieve sneak fertilisation it may be that these males were stimulated to attempt to curl their tail around the female as they would during typical spawning, but due to their lower position on her body this could not be achieved. These alternative behaviours were not fully quantified in this study, and their precise function remains unclear.

Inter-specific sneak mating between petromyzontid paired species has not been described previously, although several species pairs are known to nest communally, such as: *I. unicuspis/I. fossor, I. castaneus/I. gagei* (Cochran *et al*., 2008), and *I. bdellium/I. greeleyi* (Cooper, 1983 as reported in Cochran *et al*., 2008). Cochran *et al*. (2008) noted that on one occasion a male *I. gagei* attached to a female *I. castaneus* within a nest, but no eggs were released and the female showed no response to the male attachments. Non-parasitic *Lethenteron appendix* males exhibit intra-specific sneak mating, but it does not occur sympatrically with its parasitic pair member *L. camtschaticum* (Cochran *et al*., 2008). However, "giant" individuals of *L. appendix*, which likely follow a parasitic life history strategy, could be maintained in the gene pool by sneak matings with typical *L. appendix* (Cochran, 2008).

To date *L. fluviatilis/L. planeri* is the only species pair for which both sneaker males (Malmqvist, 1983; Wüstel *et al*., 1996) and communal nesting has been documented (Huggins & Thompson, 1970; Lasne *et al*., 2010). Additionally, it was recently confirmed that post-zygotic barriers to hybridisation, at least in the form of gamete incompatibility between this pair, including freshwater-resident *L. fluviatilis* from Loch Lomond, do not exist (Hume *et al*., 2012). Alternative male reproductive strategies, such as those described here, are mechanisms that could negate the effects of reproductive isolation, created by size assortative mating, acting between petromyzontid species pairs mating in the typical manner (Beamish & Neville, 1992). The findings of this report suggest such behaviours are a plausible explanation for the patterns of gene flow exhibited by some sympatric lamprey species pairs exhibiting divergent life history strategies.

5.6 FIGURES & TABLES

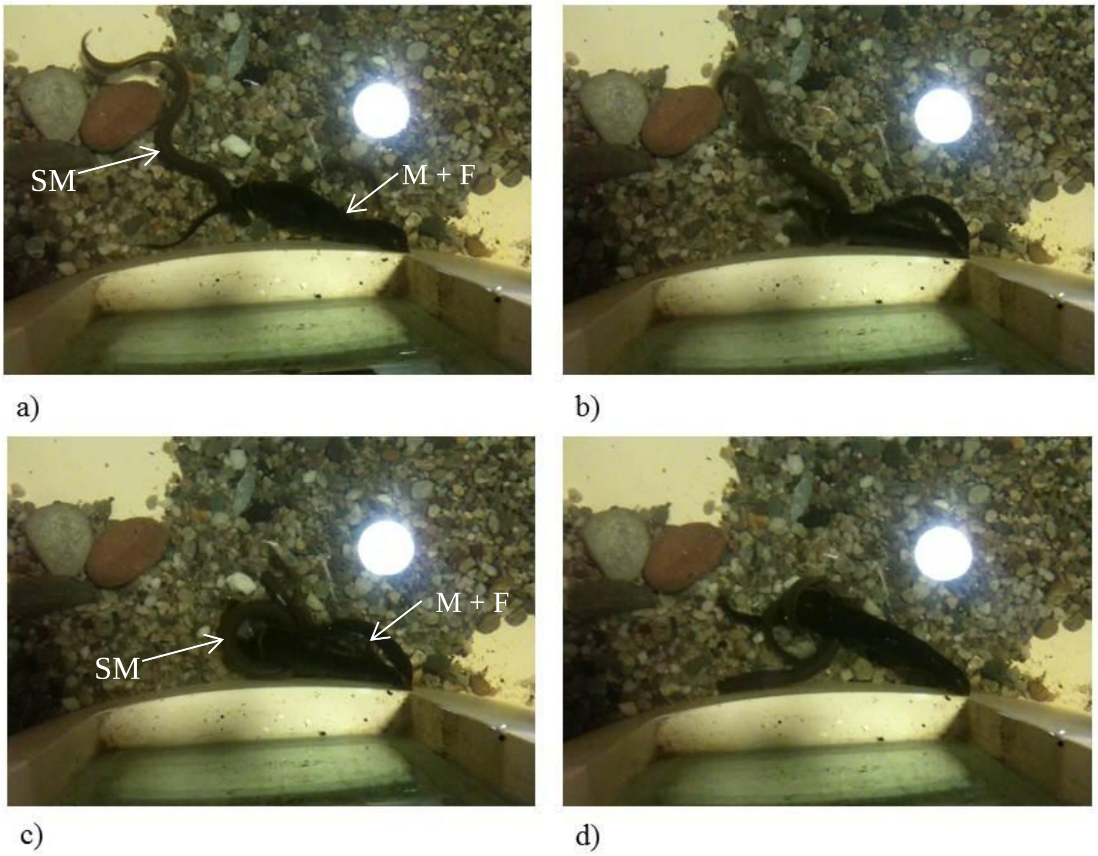


Fig.5.1 Example of sneak mating tactics as photographed between anadromous and freshwater-resident *Lampetra fluviatilis*, showing (*a*) a freshwater-resident *L. fluviatilis* pair forming with a larger anadromous *L. fluviatilis* (sneaker male) nearby. (*b*) The sneaker male approaches the urogenital area of the typically spawning pair, tightly circling clockwise around their bodies (*c*) at the point of gamete release. (*d*) The sneaker male then unwinds and travels away from the pair. Abbreviations: M = male, F = female, SM = sneaker male.

Table 5.1 Instances of sneak mating recorded between anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* in an artificial stream environment. Gamete release was confirmed in all cases. Percentage size differences between sneaker males and spawning females are included. Abbreviations: *fw* – freshwater.

Sneaker Male	**Spawning Pair**	**Length of Sneaker Male (mm)**	**Length of Spawners (mm) ♂ + ♀**	**% Size Difference Between Sneak and ♀**
anadromous *L. fluviatilis*	fw-resident ♂ + anadromous ♀	298	220 + 315	5.4
anadromous *L. fluviatilis*	fw-resident ♂ + anadromous ♀	298	220 + 272	9.6
anadromous *L. fluviatilis*	fw-resident ♂ + fw-resident ♀	327	200 + 217	50.7*
anadromous *L. fluviatilis*	fw-resident ♂ + fw-resident ♀	327	200 + 241	35.7*
L. planeri	anadromous ♂ + anadromous ♀	141	298 + 272	48.2*
L. planeri	anadromous ♂ + anadromous ♀	129	304 + 315	59*
L. planeri	anadromous ♂ + fw-resident ♀	129	304 + 283	54.4*
L. planeri	anadromous ♂ + fw-resident ♀	164	327 + 283	42*
L. planeri	fw-resident ♂ + fw-resident ♀	164	200 + 241	32*
L. planeri	fw-resident ♂ + fw-resident ♀	129	205 + 193	33.2*
freshwater-resident *L. fluviatilis*	anadromous ♂ + anadromous ♀	220	298 + 315	30.2*
freshwater-resident *L. fluviatilis*	anadromous ♂ + anadromous ♀	226	304 + 295	23.4*
freshwater-resident *L. fluviatilis*	anadromous ♂ + anadromous ♀	248	327 + 297	16.5*
freshwater-resident *L. fluviatilis*	anadromous ♂ + anadromous ♀	220	298 + 297	25.9*
freshwater-resident *L.*	anadromous ♂ + anadromous ♀	217	306 +315	31.1*

fluviatilis				
freshwater-resident *L. fluviatilis*	anadromous ♂ + fw-resident ♀	200	327 + 241	17
freshwater-resident *L. fluviatilis*	anadromous ♂ + fw-resident ♀	229	270 + 222	3.2

* Denotes instances where the sneaker male belongs to a different life history strategy to the spawning female.

“Lampreys, it is said, are of the female sex only and conceive from intercourse with snakes; as a result, fishermen catch it with a snake’s hiss.”

Anonymous (*c.* 1200), *Aberdeen Bestiary*

Chapter Six

Post-zygotic hybrid viability in sympatric species pairs: a case study from European lampreys

6.1 ABSTRACT

Ecological speciation mechanisms are widely assumed to play an important role in the early stages of divergence between incipient species, and this is especially true of fishes. In the present study post-zygotic barriers to gene flow between a sympatric, recently diverged lamprey species pair that likely arose through ecological divergence are tested for. Experimental *in vitro* hybridisation between anadromous parasitic *Lampetra fluviatilis* and stream-resident, non-parasitic *Lampetra planeri* resulted in a high proportion of embryos capable of attaining the burrowing pro-larval stage, strongly indicating no post-zygotic barriers to gene flow between these species. A sympatric, locally-adapted freshwater-resident parasitic form of *L. fluviatilis* was also found able to successfully hybridise with both members of this species pair. The consequences of these findings are discussed in the context of petromyzontid speciation.

6.2 INTRODUCTION

Evolutionary divergence in recently derived post-glacial fish populations is one of the most widely studied ecological speciation systems (Rundle & Nosil, 2005). Many of these systems contain species pairs, which display varying degrees of reproductive isolation along a speciation continuum (Hendry *et al*., 2009). This can lead to difficulties in asserting the validity of a species' specific status, particularly where variation among populations is high (Adams & Maitland, 2007). The phylogenetic relationships, specific status and inter-relatedness of lampreys (Petromyzontiformes), for example, have been under debate for decades (Enequist, 1937; Potter & Hilliard, 1987; Docker *et al.,* 1999; Gill, 2003). Contention arises when one considers the repeated parallel evolution of non-parasitic, stream-resident lampreys, commonly termed brook lampreys, from a parasitically-feeding and often migratory ancestor (Docker, 2009). Many parasitic and non-parasitic forms continue to share an overlapping geographic range (Renaud, 1997), and the term "paired species" was coined by Zanandrea (1959) to describe such a situation. Some lamprey species with a wide distribution appear highly variable in their trophic ecology and morphology, resulting in the appearance of divergent phenotypes as adults (Nazarov *et al*., 2011).

Species pairs have been described in seven of the ten petromyzontid genera, where the larvae (ammocoetes) are morphologically and ecologically similar (Goodwin *et al*., 2008) but the adults can be readily distinguished by differing foraging strategies. As a result, specific status is often bestowed on populations based solely on adult trophic ecology (Beamish & Withler, 1986; Potter & Hilliard, 1987). The conventional view is that differences in body size between adults of such species pairs, as a result of increased somatic growth during the feeding phase of parasitic forms, results in a physical barrier to successful mating. During the spawning process a pair forms when a male attaches to a female's head, wraps his tail around her, and with muscular contractions exudes ova from the female's body. Pairings where males and females are not similar in size may not result in successful fertilisation (Beamish & Neville, 1992).

However, this conventional view has always had its opponents (reviewed in Docker, 2009), and evidence of gene flow between species pairs is beginning to accumulate, most convincingly with a lack of genetic differentiation between paired species found in sympatry (Schreiber & Engelhorn, 1998; Blank *et al*., 2008; Docker *et al*, 2012). Communal spawning of paired lamprey species on shared nesting grounds has been reported on occasion (Huggins & Thompson, 1970; Lasne *et al*., 2010), as well as spawning beneath cover, and such behaviours could act to increase the chance of incidental gene flow (Cochran & Gripentrog, 1992). In addition, intra-specific sneak mating tactics by males have been described in at least two genera containing paired species (Malmqvist, 1983; Cochran *et al*., 2008), suggesting that, in some cases, species specific behavioural mating cues may not exist.

The European river lamprey *Lampetra fluviatilis* and European brook lamprey *Lampetra planeri* comprise a species pair with a wide and largely overlapping geographic range (Hardisty & Potter, 1971). Similar to most Northern Hemisphere lampreys, both species spawn in spring and, where found in sympatry, are known to utilise similar spawning habitat (Huggins & Thompson, 1970; Jang & Lucas, 2005). In a few lake systems in Europe, this species pair is further complicated by the expression of a freshwater-resident parasitic form that usually retains the morphology typical of an estuarine and inshore-feeding population (Goodwin *et al*., 2006). In Loch Lomond, Scotland, a freshwater-resident population of parasitic lamprey has been described that differs from both anadromous *L. fluviatilis* and non-parasitic *L. planeri* in a number of morphological and meristic features (Morris, 1989). This population is known to spawn in only one afferent river of the lake. There is no apparent spatial or temporal segregation from sympatric populations of anadromous *L. fluviatilis* or *L. planeri* (Maitland *et al*., 1984; Adams *et al*., 2008; Hume, 2011).

Freshwater-resident *L. fluviatilis* are intermediate in body size between *L. planeri* and anadromous *L. fluviatilis* from Loch Lomond (Morris, 1989) and so it is possible that the freshwater-resident *L. fluviatilis* may enable gene flow between these paired species (Beamish & Neville, 1992). As one part of a larger study on the reproductive ecology of these lamprey populations, the presence of post-zygotic barriers to gene flow between these three groups was tested for. Specifically, to ascertain if anadromous *L. fluviatilis,* freshwater-resident *L. fluviatilis* and *L. planeri* are capable of producing hybrid offspring *in vitro* by analysing survivorship of artificially fertilised eggs, and whether those hybrids are capable of developing to the burrowing stage of the pro-larvae.

6.3 MATERIALS & METHODS

Between October 2010 and April 2011, adult lampreys were collected in static traps (Morris & Maitland, 1987) as they migrated upstream to spawning grounds in the Endrick Water in the Loch Lomond catchment. Live lampreys were held in same-sex, same-type groups of two in 10 litre tanks filled with a constant flow of fresh Loch Lomond water at ambient temperature and exposed to artificial light that tracked the natural photoperiod, until sexual maturity. Ripe lamprey females become swollen with eggs, which are often visible through a patch of translucent skin near the cloaca, and also develop a post-cloacal finfold. Sexually mature male lampreys can be identified by an obvious genital papilla which extends several millimetres from the cloaca.

On April 22nd 2011, six individuals of both sexes from the anadromous *L. fluviatilis* and *L. planeri* populations, and six males and five females from the freshwater-resident *L. fluviatilis* population, were anaesthetised using a benzocaine solution (0.05 g ml^{-1} dissolved in acetone and diluted to 600 ml in water) and hand-stripped to obtain gametes. Gametes were stored in full 1.5 ml microtubes to prevent evaporation of the coelomic fluid. Milt was kept on ice and ova stored at 5°C for less than six hours. Eggs were then fertilised *in vitro* largely in accordance with the methods previously described by Rodríguez-Muñoz & Ojanguran (2002). The ova from individual females were divided into three batches so that each female could be tested with a male of each type, creating 51 families.

For each family, batches of *c*. 100 eggs (95-105; *c*. 100 mg wet weight) from a female were placed in a Petri dish and activated with 50 µl of dechlorinated water and mixed with 50 µl of milt. Gametes were hand stirred for 30 seconds and left to stand for three minutes before being removed to individual containers, which were then placed in an artificial stream containing a constant flow of fresh Loch Lomond water at ambient temperature. Each container had a silver sand substrate filled to a depth of 3 cm. After 28 days at temperatures in the range 11-15°C, viable embryos would have reached the burrowing stage (Yamazaki *et al*., 2003) and so, on May 22nd 2011, all containers were removed and checked for the presence of burrowing pro-larvae, which were examined under a binocular microscope. The hybrid success of each family was scored as the number of burrowing pro-larvae as a percentage of the total number of eggs in each batch (Fig. 6.1).

6.4 RESULTS

Successful hybridisation [(i.e., embryos that developed through to the burrowing stage of the pro-larvae (= Stage 17; Piavis, 1961)], was achieved for all reciprocal crosses between the three groups of *Lampetra*. Overall, survivorship of hybrids between the three groups differed, with some individual batches of eggs failing to fertilise. Only 'freshwater-resident *L. fluviatilis* X anadromous *L. fluviatilis'* and '*L. planeri* X anadromous *L. fluviatilis'* crosses produced significantly more burrowing pro-larvae than the within-group control crosses (ANOVA, Tukey HSD, $P < 0.05$ for both).

Over all crosses combined, eggs from anadromous *L. fluviatilis* produced the highest mean rates of Stage 17 pro-larvae (33.9%); freshwater-resident *L. fluviatilis* were intermediate (10.8%); and *L. planeri* produced the lowest number (4.8%). The mean number of offspring attaining Stage 17 produced from anadromous *L. fluviatilis* eggs when fertilised with anadromous *L. fluviatilis* milt was 12.7% (range 8-23%), compared to 55.7% (range 22-100%) when crossed with freshwater-resident *L. fluviatilis* milt; and 33.8% (range 6-77%) when *L. planeri* milt was used. The proportion of Stage 17 offspring produced from freshwater-resident *L. fluviatilis* females fertilised with milt from male freshwater-resident *L. fluviatilis* had a mean value of 13.2% (range 2-32%), whereas hybrid success was similar (9.6%) when milt from anadromous *L. fluviatilis* (range 2-22%) and *L. planeri* (range 2-16%) was used. Only 3.3% (mean; range 0-12%) of *L. planeri* eggs produced Stage 17 offspring when fertilised with milt from *L. planeri* males, with hybrid success using freshwater-resident *L. fluviatilis* milt 3% (range 0-6%); and 8% when fertilised with anadromous *L. fluviatilis* milt (range 0-20%).

6.5 DISCUSSION

The results obtained in the present study show that there is reproductive compatibility between the gametes from two putative species, *L. fluviatilis* and *L. planeri*, and that those hybrid embryos are capable of attaining at least the stage at which they burrow. Hybrid survivorship following *in vitro* fertilisation between this species pair has been tested previously (Weissenberg, 1925) but, although egg activation was achieved, the larvae failed to develop, suggesting post-zygotic hybrid non-viability. In the present study our knowledge is significantly extended by showing not only that egg activation is possible, but also that hybrid offspring can survive at least through to completion of their larval development (= Stage 18; Piavis, 1961). In addition, this study has provided evidence that a third, and as yet largely uncharacterised, freshwater-resident population of *Lampetra* is reproductively compatible with both sympatric anadromous *L. fluviatilis* and stream-resident, non-parasitic *L. planeri* populations, and that those hybrid offspring also follow a normal developmental trajectory, at least through to the ammocoete stage. The viability of hybrid offspring between other petromyzontid species pairs has been tested previously with varying degrees of success (Cotronei, 1942; Piavis *et al.*, 1970), where only four of a possible 20 heterospecific crosses resulted in offspring attaining the burrowing stage (= Stage 17). No study has reared hybrid ammocoetes through to metamorphosis.

Fertilisation of lamprey eggs gives no indication as to the subsequent viability of the developing embryo (Rodríguez-Muñoz & Ojanguran, 2002), because the period between fertilisation (= Stage 1; zygote) and reaching the blastula stage (= Stage 8) is a critical period in the lamprey life cycle. This is demonstrated by the fact that crosses between five species of lamprey from the Laurentian Great Lakes routinely achieved egg activation, although most failed before Stage 8 (Piavis *et al.*, 1970). Although the storage time of lamprey gametes has been shown to reduce survival rate to the burrowing phase (Ciereszko *et al.*, 2000; Rodríguez-Muñoz & Ojanguran, 2002), all crosses in the present study were carried out in less than six hours, so that the storage effect is likely to be minimal. The low success of same-group control crosses in this study is potentially the result of reduced gamete quality in individuals that were held for a long period in captivity (i.e., beyond the time of their natural spawning period), although this was not explicitly tested. However, this *Lampetra* complex shows no temporal variation in the timing of their spawning migration (Hume, 2011) and so intra-specific differences in the maturation period are unlikely to be a factor resulting in this decline in fertilisation success.

Temporal and spatial barriers to the successful sharing of genes between paired lamprey species in nature are known to be limited in places. For example, heterospecific spawning is particularly well documented in species pairs inhabiting the Laurentian Great Lakes (Morman, 1979; Manion & Hanson, 1980; Cochran *et al.*, 2008), as well as Europe (Huggins & Thompson, 1970; Lasne *et al.*, 2010), and it appears to be common. The presence of intermediate body sizes resulting from alternative foraging strategies (Heard, 1966; Kucheryavyi *et al.*, 2007) could act to narrow body size differences between paired species, the presumed principle mechanism preventing gene flow between parasitic and non-parasitic forms, and is so-far only poorly understood. Sneak male mating tactics have been well documented (Malmqvist, 1983; Cochran *et al.*, 2008; Hume *et al.*, *in press*), and, with the discovery of spawning aggregations in atypical locations such as beneath cover (Cochran & Gripentrog, 1992), it is possible that paired species spawn in close proximity but remain undetected. Given the extended period of viability of lamprey gametes in water (Ciereszko *et al.*, 2000), incidental fertilisation is probable if species pairs are constructing redds communally or nearby in the same gravel patch.

Crucially, clear genetic differences between sympatric lamprey species pairs have yet to be described, and genetic differentiation between isolated stream-resident, non-parasitic populations appears to be reduced when their range is overlapped by a migratory paraistic species (Schreiber & Engelhorn, 1997; Espanhol *et al.*, 2007; Docker *et al.*, 2012). The present study indicates that post-zygotic barriers to gene flow, in the form of gamete incompatibility, between the European lamprey species pair *L. fluviatilis* and *L. planeri* do not exist, supporting the possibility of hybridisation in the wild. In addition, it has also been shown that intermediate phenotypes, such as the freshwater-resident *L. fluviatilis* population from Loch Lomond, are capable of producing hybrid offspring with these paired species.

6.6 FIGURES

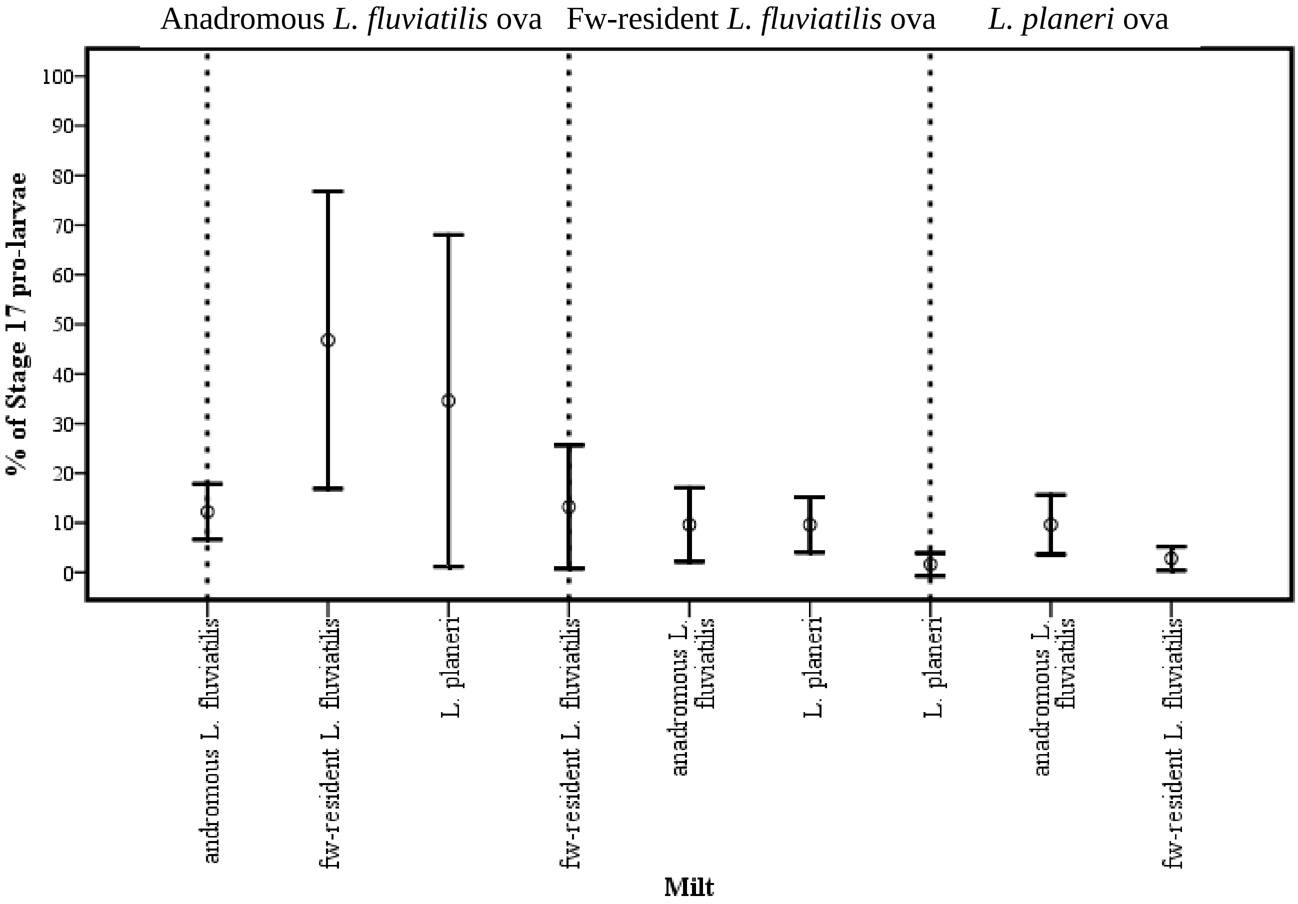


Fig.6.1 The relative success of *in vitro* fertilisation between reciprocal crosses of anadromous *Lampetra fluviatilis*, freshwater-resident *L. fluviatilis* and *L. planeri* from the Endrick Water, Loch Lomond, Scotland as expressed by the percentage of embryos attaining developmental Stage 17. Six batches of ova from different anadromous *L. fluviatilis* and *L. planeri* females, and five from freshwater-resident *L. fluviatilis* were tested against sperm from six different males of each group, creating 51 artificial crosses. Whiskers represent standard error of the mean (± 2). Abbreviations: *fw* = freshwater.

“Petromyzon was like an eel, or a worm, a huge torpid worm. Its body resembled the artificial rubber thing escaped from the fisherman’s hook, magnified, discoloured, sunk in living slovenliness, animated waste-product of the spirit of life.”

Henry Williamson (1935), *Salar the Salmon*

“Orator Hortensius had a house at Bauli…and a fish pond to it belonging: and he took such affection to one lamprey in that pool, that when it was dead (by report) he could not hold but weep for love of it. Within the same pool belonging to the said house, Antonia wife of Drusus (unto whom they fell by inheritance) had so great a liking to another lamprey, that she could find in her heart to deck it, and to hang a pair of golden earrings about the gills thereof. And surely for the novelty of this strange sight, and the name that went thereof, many folks had a desire to see Bauli, and for nothing else.”

Pliny the Elder (77), *Natural History*

Chapter Seven

Resolving the taxonomy of European river (*Lampetra fluviatilis*) and brook (*L. planeri*) lampreys – a synthesised approach

7.1 ABSTRACT

Phenotypically variable taxa often elude attempts at accurate species-level designations. Some fish taxa, for example, are known to be hyper-variable in their phenotypic structuring, exhibiting sub-specific differences within and between disjunct populations. Lampreys are a typical example, expressing alternative life history strategies at the species (parasitic *vs*. non-parasitic) and sub-specific levels (anadromy *vs*. residency). Most lamprey genera contain pairs of genetically and morphologically similar sympatric species exhibiting these alternative life history strategies and their phylogenetic relationships have proven contentious among both traditional taxonomists and molecular ecologists. This study examined the taxonomic and evolutionary relationships of non-parasitic *Lampetra planeri* and parasitic *L. fluviatilis* collected in the U.K. using a suite of traditional morphometric characters, in conjunction with geometric morphometrics, to test for morphological differences between parasitic and non-parasitic forms, and whether those differences related to traditional taxonomic designation. In addition, mitochondrial DNA sequences were used to examine phylogenetic relationships among these non-parasitic and parasitic populations, including sequences collected from elsewhere in Europe, and phylogeographic inferences made regarding these relationships. Morphological examination of parasitic and non-parasitic specimens revealed no consistent morphometric differences between the two forms, indicating traditional taxonomic techniques do not have the power to separate *L. planeri* from *L. fluviatilis*. Relationships among mtDNA sequences revealed that independently derived non-parasitic haplotypes differed by very few mutational steps from haplotypes found in parasitic specimens in different geographic regions. Several haplotypes were also found to be shared between non-parasitic and parasitic individuals. These results, therefore, support the idea that *L. fluviatilis* and *L. planeri* are more likely to represent ecotypes of a single species than *L. planeri* is to represent a discrete species, and suggests *L. planeri* be synonymised with *L. fluviatilis*.

7.2 INTRODUCTION

Despite the ubiquity of its usage when discussing evolutionary processes, the criteria used in defining the taxonomic level known as "species" are surprisingly variable. Due to the consistent inability to formulate a single species definition that can adequately encompass all organisms, some commentators have suggested a unified species concept may no longer be desirable (Butlin *et al*., 2011). One major reason for this is that some groups are inherently difficult to classify into distinct taxa. They may, for example, reproduce asexually, and so reproductively isolated populations will not be readily apparent (Leavitt *et al*., 2011). Similarly, sexually reproducing populations with a wide, but disjunct, geographic range may exhibit significant phenotypic variation between populations that can lead to taxonomic confusion when attempting to allocate such populations to a single widespread species (Etheridge *et al*., 2012; Querci *et al*., 2012). Recently diverged populations in particular can be difficult to adequately describe in terms of discrete taxa, especially if the speciation process occurred sympatrically (Johannesson, 2011). This process may produce continuous variation between populations; from discrete phenotypes sharing the same gene pool at one end, through to complete reproductive isolation at the other (Hendry, 2009). Fishes are one such group of organisms that contain taxa with disjunct populations and have an evolutionary tendency to exhibit high levels of between-population differentiation, either phenotypically or genetically, or both (Adams *et al*., 2008).

Lampreys (Petromyzontiformes), an ancient lineage of fishes, are a relatively understudied group that exemplifies these trends in phenotypic and genetic variation between populations, and this has given rise to some contentious taxonomy (Docker, 2009). However, unlike many other fish groups, lampreys present a tractable problem in that their order contains just 40 currently recognised species distributed in both Northern and Southern Hemispheres (Hardisty & Potter, 1971; Renaud, 2011). All lampreys spend multiple years as microphagous larvae in rivers before undergoing a metamorphosis that produces the adult phenotype (Bird & Potter, 1979). Adult lampreys are broadly categorised as being either parasitic (feeding on the tissue of their hosts), or non-parasitic (do not feed following metamorphosis), and these polarised life history strategies have been described as being species specific (Hubbs & Potter, 1971). Many parasitic and non-parasitic lampreys share an overlapping geographic range, are morphologically inseparable as larvae, and appear phenotypically similar as adults. Thus, they have been termed "paired species" (Zanandrea, 1959). Non-parasitic lampreys are, however, typically small as adults (*c*. 140 mm), while parasitic lampreys tend to be larger (*c*. 300 mm) as a result of the increased somatic growth during post-metamorphic feeding (Beamish, 1980). Due to this body-size discrepancy it is believed that reproductive isolation has arisen between paired species as a function of the difficulty in achieving fertilisation of eggs between size mismatched partners, yet this assumption has rarely been tested (Beamish & Neville, 1992).

In addition, recent molecular evidence questions whether the species status of geographically proximal parasitic and non-parasitic forms is warranted. Based on mitochondrial DNA (mtDNA) and nuclear genes no species specific markers have been found between sympatric forms (Schreiber & Engelhorn, 1998; Docker *et al*., 1999; Espanhol *et al*., 2007; Blank *et al*., 2008; Pereira *et al*., 2011), and where examined, these co-occurring forms do not exhibit microsatellite-based allele frequency differences (Boguski *et al*., 2012; Docker *et al*., 2012). These results suggest that paired species of lampreys are not reciprocally monophyletic and that there may be contemporary gene flow between both life history strategies. This would instead indicate that paired lamprey species represent two different ecotypes of a single species (Enequist, 1937) and are, therefore, comparable to the divergent ecotypes of many other fishes (Taylor, 1999).

Although generally accepted that non-parasitic lampreys have evolved from parasitic ancestors (Hubbs & Potter, 1971), the phylogenetic relationships between these so-called paired species are not well resolved (Docker, 2009). One major hindrance in classifying lampreys is a relative lack of morphological features useful to traditional taxonomists (e.g., bony elements), and a historic over-reliance on characters susceptible to misinterpretation (e.g., degenerative dentition, or adult body proportions that may be confounded by allometric growth) (Beamish, 2010). Taxonomically discrete variables between paired species are particularly scarce; therefore, trophic differences inherent to the non-parasitic or parasitic adult stage have been considered to be the only robust species-specific differences (Hardisty & Potter, 1971). However, some parasitic lampreys exhibit high levels of phenotypic variability, typically in the form of reduced adult body size in response to shortened periods of parasitic feeding, so that in certain geographic locations the relationship between paired species may be further convoluted by the presence of an intermediate phenotype (Kucheryavyi *et al*., 2007), which could mediate gene flow between parasitic and non-parasitic forms (Salewski, 2003).

Intermediate phenotypes resulting from variable parasitic forms remain an understudied, yet crucial factor, in understanding the mechanisms driving the evolution of non-parasitism among petromyzontids, as there is suggestion such intermediates could act as a physiological stepping stone between parasitic and non-parasitic life history strategies (Beamish 1985; Salewski, 2003; Hardisty, 2006). Within the U.K. one such morphologically and ecologically variable parasitic lamprey, the European river lamprey *Lampetra fluviatilis*, is paired with a non-parasitic form currently recognised as *L. planeri*. Both lampreys are widespread, and whereas *L. fluviatilis* is typically parasitic in estuaries and coastal seas, *L. planeri* remains exclusively in fresh water and is non-trophic. Two of the largest lake systems in the U.K.; Loch Lomond, Scotland and Lough Neagh, Northern Ireland, are known to contain freshwater-resident populations of *L. fluviatilis* (Adams *et al*., 2008; Inger *et al*., 2010), as well as sympatric anadromous *L. fluviatilis* and populations of *L. planeri*. Additionally, the River Bladnoch in southwest Scotland contains a population of so-called "praecox" anadromous *L. fluviatilis* that exhibit a much reduced adult body size (*sensu* Berg, 1948), and is likely the result of a truncated period of foraging at sea. Therefore, within Scotland alone *L. fluviatilis* is known to feed in marine environments for both long and short periods, as well as being able to feed exclusively within freshwater environments, and these populations can be found sympatrically with non-parasitic *L. planeri*.

As a result of the extensive variation in adult life history strategy and morphology among populations of *L. fluviatilis*, and the current uncertainty regarding the validity of *L. planeri* as a distinct taxa, the objective of this study was to determine whether variability among populations of lampreys exhibiting alternative life history strategies conforms to traditional species classification based on morphological and/or molecular variation. The specific aims were to: a) quantify differences in phenotype using geometric morphometric shape analysis, in conjunction with those taxonomic characters traditionally used in the description of lamprey species, to assess whether there is discrete variation between life history strategies (parasitism *vs.* non-parasitism) that can be mapped onto currently recognised species (*L. fluviatilis* & *L. planeri*) and 2) whether patterns of variation in mtDNA genes are concordant with ecotypes or species designations.

7.3 MATERIALS & METHODS

7.3.1 *Terminology*

Throughout this study two currently recognised species of lamprey are considered; *Lampetra fluviatilis* and *L. planeri*. As life history strategy is regarded as being species specific (i.e., parasitic *vs*. non-parasitic), and to simplify discussion of a complex group of life histories, throughout this study *L. fluviatilis* will therefore be synonymised with "parasitic" populations, and *L. planeri* with "non-parasitic" populations. A "population" refers to a group of lampreys collected from one site only and belonging to the same life history strategy (e.g., the "population" of freshwater-resident *L. fluviatilis* collected in… is separate from the anadromous *L. fluviatilis* "population" also collected in…).

7.3.2 *Sampling*

To establish the applicability of using traditional taxonomic keys in classifying phenotypically variable lamprey species, detailed morphometric descriptions of adult and larval specimens of both *L. fluviatilis* and *L. planeri* were made. To obtain adult lampreys for comparative study a broad sampling strategy was employed by using static, double-funnel traps (Morris & Maitland, 1987) installed in rivers throughout Scotland between October 2009 and April 2012. These traps target actively migrating lampreys moving upstream towards spawning sites. However, only three rivers provided suitable numbers of individuals for morphological examination, and so the number of collection localities was increased by using two additional methodologies known to capture adult lampreys: removing individuals that become impinged on the water intake screens of power stations, and lampreys caught as by-catch in other fisheries. All adult lamprey specimens collected in this study were euthanised using a lethal dose of anaesthetic (benzocaine) and frozen at -18°C to -30°C to preserve morphological features for future examination. Adult lampreys collected from all sites were then classified to species (*L. fluviatilis* or *L. planeri*) using published keys (Morris, 1989; Gardiner, 2003; Renaud, 2011).

Adult anadromous (n = 18) and freshwater-resident *L. fluviatilis* (n = 34), as well as non-parasitic *L. planeri* (n = 40), were collected from the Endrick Water, Loch Lomond catchment, approximately 14 km upstream of the point of discharge into the lake (56°3'17·3" N; 4°27'16·2" W). Adult *L. planeri* (n = 8) were also collected from the River Falloch, which drains into the north basin of Loch Lomond (56°3’06·7” N; 4°72’19·12” W), approximately 30 km from the Endrick Water. Adult praecox *L. fluviatilis* (*n* = 8) were obtained from the River Bladnoch (54°8’88·9” N; 4°55’81·3” W), which drains into the Solway Firth, southwest Scotland. Adult anadromous *L. fluviatilis* were obtained from the Forth Estuary (*n* = 30), located in east-central Scotland, from the water intake screens of Longannet power station (56°4’84·5” N; 3°68’85·7” W). Specimens of adult freshwater-resident *L. fluviatilis* (Goodwin *et al.*, 2006) from Lough Neagh (*n* = 27), Northern Ireland were collected in Toome Bay (54° 44’ N; 6°29’ W) as by-catch from a local silver eel (*Anguilla anguilla*) fishery.

In addition to adult lamprey specimens, collections of *Lampetra* spp. ammocoetes (*n* = 300) were made between June and August in 2010 and 2011 from the Endrick Water, Loch Lomond using backpack electrofishing equipment. This site is known to contain spawning adults of both *L. fluviatilis* and *L. planeri* and it was expected that mixed collections would provide larval specimens of both for comparison. All ammocoetes were euthanised by a lethal dose of anaesthetic (benzocaine) and stored in 70% ethanol.

7.3.3 *Phenotypic Analysis*

To evaluate overall body shape differences between adult lamprey populations collected from different sites, thin-plate spline geometric morphometric software was used, utilising images captured by a Canon EOS 1100D digital camera. Lampreys were photographed in lateral view (left side). Images were compiled using tpsUtil (Rohlf, 2006*a*) and 16 landmarks on each lamprey (Fig. 7.1) were located and digitised using tpsDig2 (Rohlf, 2006*b*). Generalised least squares procrustes superimposition was used to translate, scale, and rotate digitised landmarks to minimise the summed and squared inter-landmark distance among individual lampreys (Rohlf & Slice, 1990). This removes the effect of body size on the position of landmarks, and produces partial warp scores for each landmark on every lamprey (Rohlf, 2007). Principal component analysis of partial warp scores from each individual was used to reduce the number of informative variables and analysis of variance (ANOVA) performed on component scores to examine whether body shape differed between parasitic and non-parasitic populations.

The taxonomic descriptions made in this study (Appendix 7.1; Table 7.1) were based on the morphological and meristic characters of both ammocoetes (where available) and adult specimens, and largely follow the convention and methodology of Renaud (2011). Shape analysis (see above) was not employed in these descriptions to enable direct comparisons with traditional morphometric studies for other lamprey species. Linear measurements (± 0.01 mm) of specimens (Fig. 7.2) were taken using the digitised images created during the analysis of shape (using tpsDig2) that employed a scale factor calculated on each individual image. Linear measurements included: total length (L_T); pre-branchial length (L_{PB}); branchial length (L_B); trunk length (L_{TR}); tail length (L_{TL}); eye diameter (L_{ED}); disc length (L_D) and length of the male urogenital papilla (L_U). L_U was measured by hand using a binocular microscope, and the numbers of trunk myomeres were counted using a binocular microscope or hand lens. Linear measurements were converted to a proportion of L_T for each specimen.

Examination of the dentition of adult specimens (Fig. 7.3) included the following counts: marginals; anterial and posterial rows; exolaterals; velar tentacles; oral papillae and fimbriae. The pattern and counts of endolaterals, infra- and supraoral lamina were also recorded. In addition, the extent of pigmentation in the dorsal and caudal fins, and the intensity of pigmentation in the iris and the lateral line neuromasts, was recorded using the following criteria: for extent, - = absent, + = < 25%, ++ = 25 – 75%, +++ = > 75%; for intensity: unpigmented, light or darkly pigmented. Morphometric data were compared between populations using ANOVA and post-hoc Tukey's tests to examine whether differences between parasitic and non-parasitic populations conformed to current species boundaries using traditional taxonomic criteria.

7.3.4 *Genetic Analysis*

To evaluate whether genetic divergence was concordant with life history strategy, species designation, or geographic location, mtDNA sequences of parasitic and non-parasitic individuals were compared for several of the populations used in the morphometric analysis, as well as from a wider geographic range. DNA was extracted from 40 specimens collected from eight localities during sampling (Table 7.2) and mtDNA haplotypes compared with published sequences of *L. fluviatilis* and *L. planeri* retrieved from GenBank, representing 21 additional localities throughout Europe (Espanhol *et al.*, 2007; see Table 7.3 for accession numbers).

DNA was extracted from fin tissue using the DNeasy Blood & Tissue Kit (QIAGEN Sample & Assay Technologies, Copenhagen, Denmark). Polymerase chain reaction (PCR) amplification of 1221 bp of the cytochrome *b* (cyt *b*) gene used the primers LA (5'-GCGACTTGAAAAACCACCGTT-3') and PRO (5'-TAGATACAGAGGTTTGAATCCC-3') (Espanhol *et al*., 2007), with the internal sequencing primers LB (5'-CTGCAGCTACTGCTTTCGTTGG-3') and CB2H (5'-CCCTCAGAATGATATTTGCCCTCA-3') used to sequence through the entire product. Amplification and sequencing of 856 bp of the ATPase subunit 6 and 8 genes used the primers ATPfor (5'-CCTTTTAAGCTGAAGAAGATGGGTG-3') and ATPrev (5'-TGGTATGCGTGAGCTTGGTGGG-3') (Espanhol *et al*., 2007). The ND3 gene (423 bp) was amplified and sequenced using the primers ND3-L (5'-ACGTGAATTCTATAGTTGGGTTCCAACCA-3') and ND3-H (5'- ATGCGGATCCTTTTGAGCCGAAATCA-3') (Docker *et al*., 1999).

Each 20 µl reaction contained 1.5 mM $MgCl_2$, 0.2 mM DNTPs, 0.5 µM of each primer, 1 unit of *Taq* DNA polymerase (Invitrogen Inc., Paisley) and 1x of the supplied buffer. Reactions were run in a Peltier Thermal Cycler beginning with an initial denaturation period of 3 min at 94°C. Reactions consisted of 30 cycles of denaturation at 94°C for 1 min followed by annealing at 60°C and extension at 72°C for 2 min and a final 10 min 72°C extension. PCR products were purified using ExoSAP-IT PCR Product Cleanup (USB Products, Affymetrix, Inc., California) prior to sequencing. PCR products were sequenced in both directions using ABI 3730 automated sequencers at the GenePool (University of Edinburgh).

Mitochondrial DNA sequences were aligned and base-calling errors corrected using Sequencher v4.5 (Gene Codes Corp., Ann Arbour) and matched to published haplotype sequences in GenBank using megaBLAST. For the new sequences generated in this study, all three genes were concatenated to evaluate haplotype distribution among the Scottish and Irish populations sampled. Unique haplotypes were identified using Collapse v1.2 (Posada, 2004) and a statistical-parsimony based haplotype frequency network was reconstructed using TCS v1.8 (Clement *et al*., 2000). To assess population differentiation, pairwise F_{ST} (distance method: pairwise difference) was calculated between complete sequences (ATPase 6/8, cyt *b*, and ND3), and an analysis of molecular variance (AMOVA) used to calculate the genetic variation among and within populations, as implemented in Arelquin v3.5 (Excoffier, 2009). Since cyt *b* and ATPase are contiguous, published concatenated sequences were available for 33 specimens described by Espanhol *et al*. (2007) (see Table 7.3). Thus ND3 was removed from the concatenated alignment of the Scottish and Irish sequences, and these sequences aligned with those downloaded from GenBank using ClustalX v2 (Larkin *et al*., 2002). A minimum spanning network employing an uncorrected *p*-distance matrix was then used to reconstruct relationships among this broader geographic sample, as implemented in Splits Tree v4.12 (Huson & Bryant, 2006).

7.4 RESULTS

7.4.1 *Phenotypic Analysis*

All adult and larval specimens examined in this study, except for a single ammocoete of *Petromyzon marinus* (Appendix 7.2), belonged to the genus *Lampetra*. Existing keys could not be used to classify ammocoetes collected in the Endrick Water to either parasitic or non-parasitic populations, and so further comparative discussion among populations relates only to adult specimens. Overall, very few phenotypic characters (morphometrics, dentition or pigmentation) could be used to distinguish specimens of parasitic from non-parasitic lampreys collected and examined in this study (Appendix 7.1 for detailed descriptions; summarised in Table 7.1). When grouped by population (i.e., a life history strategy collected from a single site) it was not possible to separate lampreys into current species designations using body proportions, as both parasitic and non-parasitic populations overlapped widely in all examined characters (Fig. 7.4*a*-*i*).

In general, all *Lampetra* specimens examined in this study (Table 7.1) possessed a large median cusp on the transverse lingual lamina, had velar tentacles and exhibited three endolateral teeth on both sides of the mouth, typically in a 2-3-2 pattern (84% of specimens). No specimens possessed exolateral teeth, and posterials were recorded in only four individuals (n = 1, freshwater-resident parasitic from Endrick Water; n = 3, non-parasitic from Endrick Water). The number of anterial rows was typically 1 or 2, although the frequency differed between populations. The lateral line neuromasts were darkly pigmented in 68% of all specimens, and was the dominant character state in all populations, while non-parasitic populations alone tended to possess a prominent dark blotch on the apex of the second dorsal fin (84% of specimens).

Between the seven populations examined (five parasitic and two non-parasitic), adult body size (L_T) differed widely (ANOVA, d.f. = 6, 169, $F = 170.4$, $\eta^2 = 0.90$, $P < 0.01$). Non-parasitic populations were significantly smaller than all parasitic populations (Appendix 7.3), yet within the Endrick Water some overlap is still evident between the non-parasitic population and the freshwater-resident parasitic population also found there (Fig. 7.4*a*). Disc length (L_D) was similarly variable (ANOVA, d.f. = 6, 169, $F = 94.4$, $\eta^2 = 0.78$, $P < 0.01$), and again non-parasitic populations had significantly smaller discs in proportion to body size compared to all parasitic populations (Appendix 7.3). Both non-parasitic populations in this study, however, contained specimens that had oral discs that overlapped in size with several parasitic populations (Fig. 7.4*g*). No other morphometric variable tested in this study differed consistently between the non-parasitic populations and all of the parasitic populations examined (Appendix 7.3), indicating they are not robust criteria for separating parasitic and non-parasitic populations into distinct species (*L. fluviatilis vs. L. planeri*).

Discriminant analysis (based on nine morphometric characters: L_T; L_{PB}; L_B; L_{TR}; L_{TL}; L_{ED}; L_D; L_U; trunk myomeres) that assigned individuals to one of the seven populations examined, revealed six discriminant functions (DF). The first (DFI) explained 62.7% of the variance (canonical $R^2 = 0.93$) and the second (DFII) explained 21.6% (canonical $R^2 = 0.83$) (Fig. 7.6). Together, all six DFs significantly differentiated between all populations examined (Wilks' Lambda = 0.013; $\chi^2 = 333.7$, d.f. = 48, $P < 0.01$), indicating that only 1.3% of the total variability was not explained (Fig. 7.6). However, although DFI explained the majority of variation between populations, it alone could not separate non-parasitic and parasitic populations (Fig. 7.7). Although classification success (i.e., allocation of an individual lamprey to its population of origin based on phenotypic traits) was generally high; 75.3% of cross-validated grouped cases were identified correctly, there were, however, many incorrect assignments of individuals to populations other than their collection locality, including mismatches between parasitic and non-parasitic populations (Table 7.4). For example, non-parasitic lampreys collected from the Endrick Water were sometime classified as belonging to the freshwater-resident parasitic population collected from the same site (15%), and specimens of the anadromous parasitic population from the Forth Estuary were classified as belonging to the non-parasitic populations of both the Endrick Water (6.7%) and River Falloch (6.7%).

Principal component (PC) scores were derived from a PCA of partial warp scores calculated during overall body shape analysis to detect more subtle morphological variation between parasitic and non-parasitic populations. Twenty-nine PCs were derived and cumulatively the first two factor loadings explained 72.5% of the variation in landmark position. The first (corresponding to L_{TL} and L_{ED}) explained 42.3% of the total variation in shape between specimens, while the second (corresponding to L_D and myomere counts) explained 30.2%. Both of these loadings explained a significant amount of between-population variation (ANOVA, PC1 d.f. = 6, 169, $F = 57.7$, $P < 0.01$; PC2 d.f. = 6, 169, $F = 23.3$, $P < 0.01$). Although clustering was observed within both parasitic and non-parasitic populations, there was still overlap between both life history strategies (Fig. 7.5), indicating that some individuals could not be clearly distinguished into one group or the other based on overall body shape.

7.4.2 *Genetic Analysis*

Sequencing 2077 bp across the ND3, cyt *b* and ATPase 6/8 genes from the Scottish and Irish lamprey specimens revealed 12 haplotypes (Table 7.2) defined by 16 polymorphic sites; 13 of which were found in cyt *b* and ATPase 6/8. A haplotype frequency network (Fig. 7.8) indicated the presence of a single common haplotype (H1). Although most derived haplotypes only differed from this sequence by a single bp, two divergent haplotypes were identified (H4 and H5). Both of these haplotypes were identified in anadromous *L. fluviatilis* specimens collected from the River Bladnoch (but were not praecox specimens) (Table 7.2) and their level of divergence from other haplotypes found in *L. fluviatilis* in the network (five mutational steps) indicates these individuals could have migrated into the River Bladnoch from out-with the sampling regions of Scotland and Ireland. Most haplotypes were restricted to a single collection locality (Table 7.2) but H2 and H3 were shared between non-parasitic and parasitic individuals. Haplotype 2 was shared between freshwater-resident parasitic and non-parasitic individuals collected from the Endrick Water, while H3 was shared between freshwater-resident parasitic individuals from Lough Neagh and non-parasitic specimens from the Endrick Water. These patterns indicate that freshwater-resident parasitic forms in Loch Lomond and Lough Neagh could have derived independently from a single ancestral anadromous *L. fluviatilis* population that entered both lake basins, and that non-parasitic lampreys (e.g., H7 and H8) have also diverged independently in different river systems from a *L. fluviatilis* ancestor. Among the Scottish and Irish populations examined, F_{ST} values were low (Table 7.5), and the mean number of pairwise differences between populations was 1.792 ± 1.062 S.D. The AMOVA showed that the proportion of genetic variation attributable to within-populations differences was high (66%, $P = 0.00$), whereas 34% of the variation was among populations ($P = 0.01$).

When the cyt *b*-ATPase 6/8 concatenated alignment from Scottish and Irish sequences was combined with haplotypes described by Espanhol *et al*. (2007), 32 haplotypes were observed. Seven haplotypes were found across two or more localities, and six of these contained individuals belonging to both parasitic and non-parasitic populations, indicating shared haplotypes between both life history strategies (Table 7.3). The most common haplotype (labelled as 18 in Table 7.3 and Fig. 7.9) was seen in 14 populations from across Europe and was found in both parasitic and non-parasitic specimens. A phylogenetically informative tree could not be resolved as the majority of sequences differed by only a single mutation from one another, and so a minimum spanning network (MSN) utilising uncorrected *p*-distances was used to best illustrate relationships (Fig. 7.9).

The MSN indicates the broad geographic patterning of haplotypes, and this pattern corresponds to the repeated derivation of non-parasitic populations within regions from widespread parasitic populations. In only one case was the evolutionary direction reversed, where a haplotype sampled from a parasitic individual (haplotype 15) appeared to have been derived from a non-parasitic haplotype (haplotypes 14) (Fig. 7.9). However, this may be the result of incomplete sampling that failed to discover one of these haplotypes common to the other life history strategy. The most common haplotype (18), for example, can be seen to have derived several non-parasitic haplotypes in both Portugal (24-26) and France (14) (Fig. 7.9; Table 7.3). Haplotype 22 was found in a parasitic specimen collected in Poland, and can be seen to have derived a non-parasitic haplotype also collected in Poland (haplotype 23; Fig. 7.9). A similar pattern was seen in haplotype 29, which was found in parasitic specimens from Scotland, where two non-parasitic haplotypes have been derived from this common haplotype also within Scotland [haplotypes 30 and 31; (Fig. 7.9)]. These patterns, whereby non-parasitic populations in different parts of Europe are more closely related to geographically proximal parasitic populations than they are to other non-parasitic populations, could indicate that each non-parasitic population was derived independently within each broad geographic region.

7.5 DISCUSSION

This study confirms that the paired lampreys *L. fluviatilis* and *L. planeri* should not be considered as separate species; rather, these results suggest that both represent alternative ecotypes of a single species (Enequist, 1937; Schreiber & Engelhorn, 1998). Morphological examination of the seven lamprey populations collected in Scotland and Ireland; five of which were classified as *L. fluviatilis* and two as "*L. planeri*" according to current taxonomic keys, failed to robustly and consistently distinguish between parasitic and non-parasitic forms (Appendix 7.1). Body proportions used by taxonomists could not separate parasitic and non-parasitic populations (Fig. 7.4*a-i*), and neither could comparisons of their dentition (Table 7.1). A discriminant function analysis based on nine key morphometric features failed to consistently assign individual specimens of both parasitic and non-parasitic forms to their population of origin (Table 7.4 and Fig. 7.7), and although subtle differences in overall body shape were the most reliable way in which to separate both life history strategies, parasitic and non-parasitic populations were still not clearly distinguished using geometric morphometrics (Fig. 7.5). The genetic relationships among Scottish and Irish lamprey populations, as well as specimens collected more widely in Europe, did not conform to a bifurcating pattern whereby parasitic and non-parasitic forms diverged at a single point and followed separate evolutionary pathways (Fig. 7.8 & Fig. 7.9). Instead, analysis of mtDNA revealed the presence of multiple, independently derived non-parasitic populations in separate geographic regions, suggesting they had arisen repeatedly from more wide ranging parasitic ancestral populations.

Petromyzontid taxonomy has traditionally employed a relatively small set of morphometric characters, many of which are restricted to the adult stage only, and as a result generic and species level differences are far from well resolved (Gill *et al*., 2003; Docker, 2009). Comparing traditional morphometric characters measured in the present study, and used in describing the paired species *L. fluviatilis* and "*L. planeri*" (derived from Renaud, 2011), it was not possible to reliably distinguish between parasitic and non-parasitic forms, as there was significant overlap between both. For example, several morphometric characters previously believed to be species specific (e.g., L_T & L_D) were in fact found to differ significantly among parasitic populations exhibiting alternative forging strategies, as well as between parasitic and non-parasitic populations. Other supposedly rigorous species specific differences [e.g., L_{ED} (Gardiner, 2003)] were found to be erroneous, as they could not distinguish one life history strategy from the other. Dentition was similarly unsuitable for distinguishing individual specimens into *L. fluviatilis* or "*L. planeri*", as tooth patterning and the range of tooth counts varied widely between parasitic and non-parasitic populations. One reason for this may be that the dentition of non-parasitic forms is not under selective pressure to remain fixed given that they no longer have a functional role in feeding (Hardisty, 2006).

Many of the phenotypic traits used in describing adult petromyzontids rely on body proportions (see Renaud, 2011 for development of taxonomic keys), but which are subject to allometric change as the individual grows throughout its life, and their continued use in lamprey taxonomy should be considered critically (Svärdson, 1950; Beamish, 2010). Although body shape variation was evident among lamprey populations collected from different localities and examined in this study (Fig. 7.5), this variation reflected significant differences among parasitic populations, as well as between parasitic and non-parasitic populations. As phenotypic traits in fish are known to respond plastically to environmental pressure, particularly foraging opportunities (Etheridge *et al*., 2010; Garduño-Paz *et al*., 2010), this is likely to be one reason why phenotypic variation among the parasitic lamprey populations was greater than that seen between non-parasitic populations (Fig. 7.6). Thus, it is highly debatable whether *L. fluviatilis* and "*L. planeri*" should be considered as two separate species, based solely on a small number of morphological differences currently in use, that have been shown to vary significantly among populations of *L. fluviatilis*, and rarely distinguish "*L. planeri*" from *L. fluviatilis*.

The geographical patterns of mtDNA haplotypes observed between both parasitic and non-parasitic specimens were inconsistent with two independent evolutionary lineages corresponding to *L. fluviatilis* and "*L. planeri*". In contrast, the star-phylogeny that was observed was consistent with multiple independent origins of non-parasitic haplotypes from parasitic haplotypes that were more found in more geographically widespread specimens (Fig. 7.9). Parasitic lampreys feeding in freshwater lakes in both Scotland and Ireland exhibited a similar evolutionary pattern, whereby each freshwater parasitic population was derived independently in each lake from a common haplotype (Fig. 7.8). The repeated independent divergence of freshwater populations from anadromous populations is a common evolutionary trait among fishes (Schluter & Nagel, 1995), and has been described in a variety of taxa, including ecotypes of stickleback *Gasterosteus aculeatus* (Hendry, 2009), trout *Salmo trutta* (Bernatchez *et al*., 1992), whitefish *Coregonus lavaretus* (Siwertsson *et al*., 2012) and other lampreys (Taylor *et al*., 2012).

The star-phylogeny observed in this study, whereby a single high frequency ancestral haplotype was evident with numerous low frequency haplotypes separated from this by only a few mutational steps, is consistent with rapid geographical expansion. Much of Europe has experienced repeated glaciation since the late Pliocene (*c*. 3 million years ago), where ice has extended south and then retreated north as the climate warmed over periods of tens to hundreds of thousands of years (Webb & Bartlein, 1998; Golledge *et al*., 2008). In central and northern Europe, lamprey populations (*L. fluviatilis* and "*L. planeri*") exhibit low nucleotide diversity and little phylogeographical structure (Espanhol *et al*., 2007), while in the Iberian Peninsula, much further south, populations exhibit far higher nucleotide diversity and significant phylogeographic structuring (Pereira *et al*., 2011). This is suggestive of the Iberian Peninsula being a glacial refuge for lamprey populations over successive ice ages, with range expansion northwards as the ice retreated. Those anadromous parasitic forms at the leading edge of the expansion would have been likely to find themselves in freshwater bodies, such as post-glacial lakes, as they searched for either suitable spawning grounds or foraging opportunities (Bell & Andrews, 1997), and this may have led to the evolution and maintenance of freshwater-resident parasitic forms within those lake basins. Given their restricted distribution in Europe, specific ecological conditions are likely to have been necessary for the evolution of freshwater-resident parasitic forms, and a simplified fish community with abundant available hosts is one potential explanation why Loch Lomond (Adams, 1994) and Lough Neagh (Kelly & King, 2001) alone contain these forms within the U.K.

During interglacial periods when lamprey populations where expanding north, those populations in lower latitudes could have remained in freshwater, eventually abandoning the anadromous life history in favour of a non-parasitic life history strategy, due to the high costs related to migration, and increased predation risk during the adult phase (Salewski, 2003; Docker, 2009). It seems likely that highly variable lampreys, such as *L. fluviatilis*, are finely balanced between the fitness advantages of being large and highly fecund (provided by an anadromous and parasitic life history strategy), or being small and less fecund but avoiding the fitness costs of anadromy (by adopting a freshwater-resident parasitic or non-parasitic strategy) (Hardisty, 2006). Lampreys have traditionally been described as different species based on these alternative adult feeding strategies because body size differences between them are believed to result in a barrier to reproduction (Beamish & Neville, 1992). However, communal spawning sites frequently result in the appearance of *L. fluviatilis* and "*L. planeri*" in the same nests (Huggins & Thompson, 1970; Lasne *et al*., 2010), and inter-specific sneak male mating tactics between both forms (Hume *et al*., *in press a*) have the potential to result in viable hybrid offspring (Hume *et al*., *in press b*).

Therefore, the morphological and molecular data presented in this study strongly indicate that the European river and brook lamprey reflect a situation more similar to the ecotypes of many other fish taxa (McDermid *et al*., 2010; Woods *et al*., 2013) than either is to being a discrete species. Despite differences related to their adult trophic status (parasitic *vs.* non-parasitic) it appears neither life history strategy represents an independent evolutionary lineage, or can be distinguished based on traditional morphometric comparisons. This would indicate that European river and brook lampreys should be considered under a single Latin binomial. As *fluviatilis* (L. 1758) was used prior to *planeri* (Bloch 1784), the species name for both would be *Lampetra fluviatilis* in compliance with the Principle of Priority (Article 23.1) in the International Code of Zoological Nomenclature (ICZN, 1999).

7.6 FIGURES & TABLES

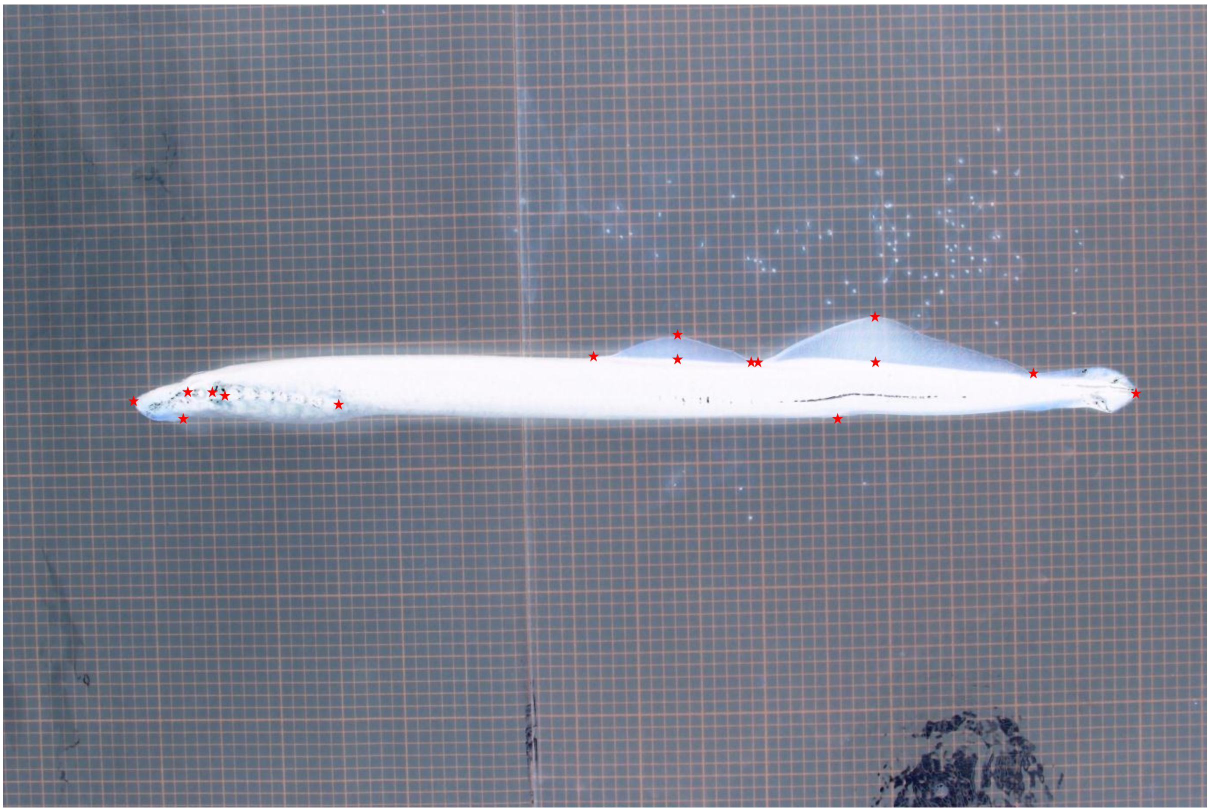


Fig.7.1 Negative image of an adult non-parasitic specimen (= “*Lampetra planeri*”) indicating the position of 16 digitised landmarks used to analyse shape variation among parasitic and non-parasitic populations. Landmarks were homologous to all specimens. Negative images were often employed for clarity of landmark sites.

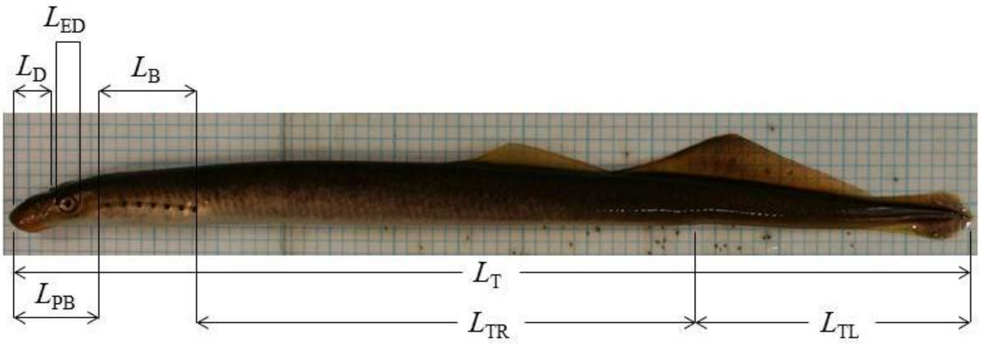


Fig.7.2. Image of an adult non-parasitic form (= "*Lampetra planeri*") indicating some of the major linear measurements used in the morphometric examination of parasitic and non-parasitic populations. Abbreviations: L_T = total length; L_{TR} = trunk length; L_{TL} = tail length; L_B branchial length; L_{PB} = pre-branchial length; L_D = disc length; L_{ED} = eye diameter.

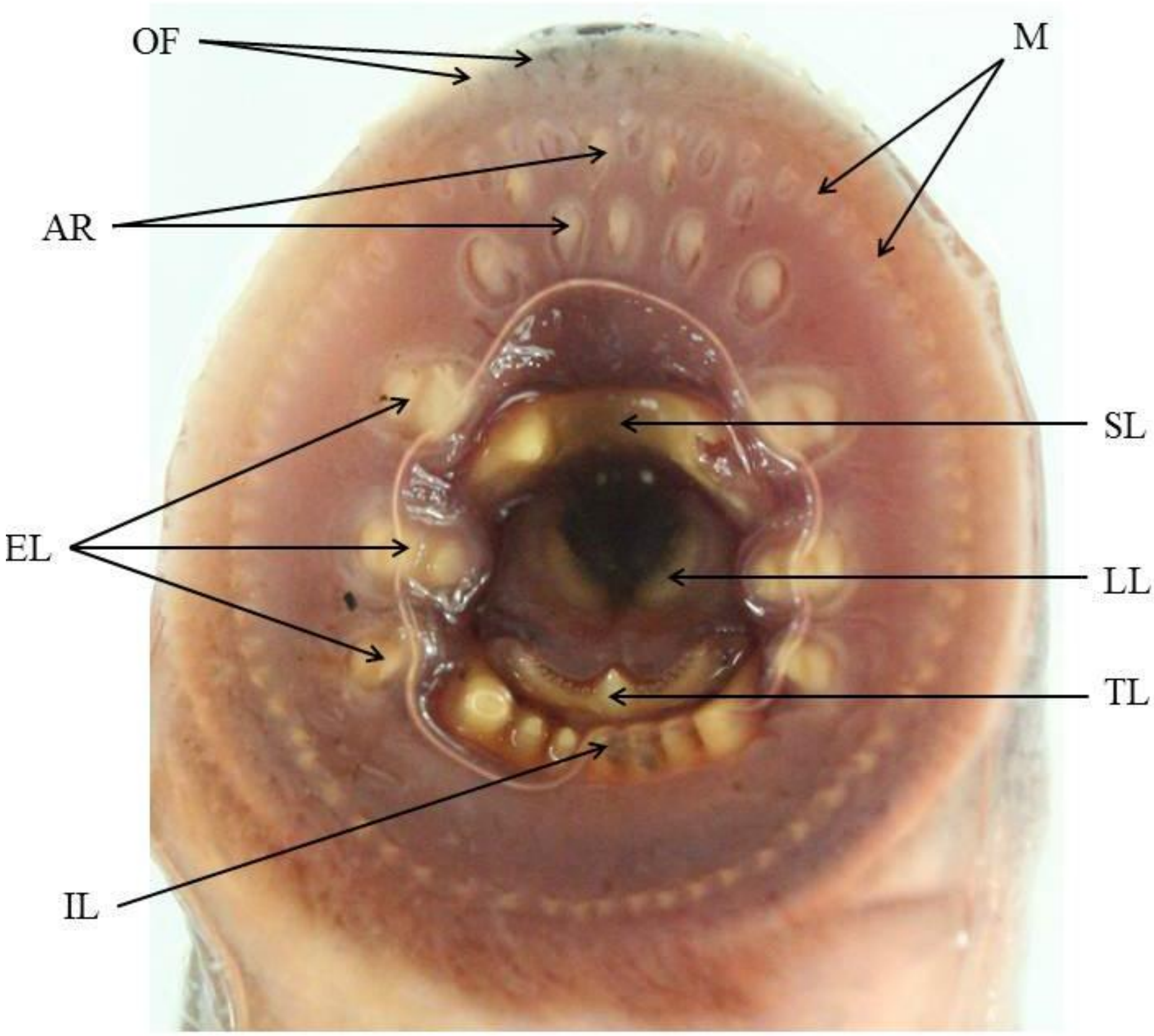


Fig.7.3 Oral disc of an adult parasitic form (= *Lampetra fluviatilis*) indicating some of the major dentition used in the morphometric examination of parasitic and non-parasitic populations. Abbreviations: IL = Infra-oral lamina; EL = endolaterals; AR = anterior rows; OF = oral fimbriae; M = marginals; SL = supra-oral lamina; LL = longitudinal lingual lamina; TL = transverse lingual lamina.

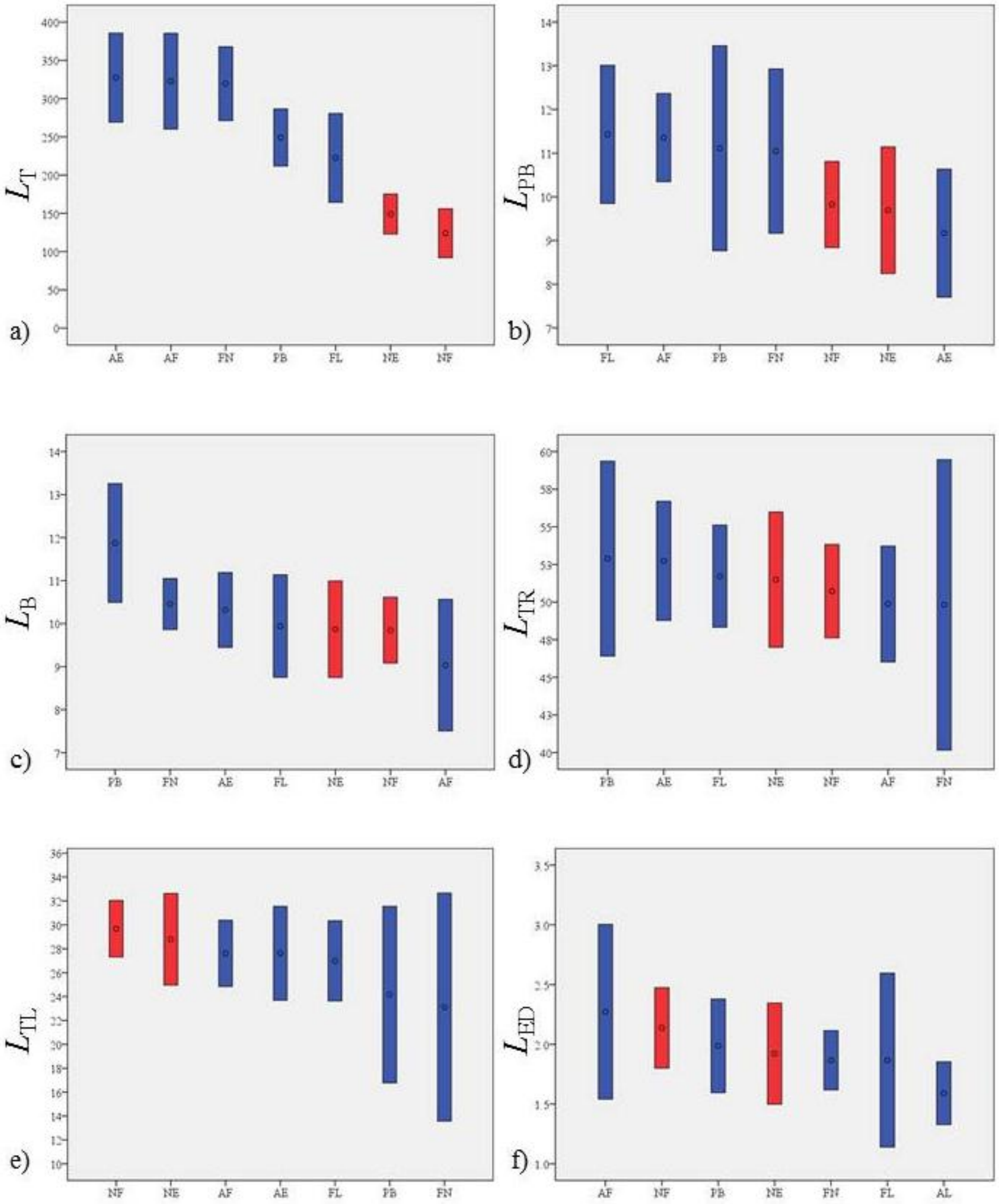

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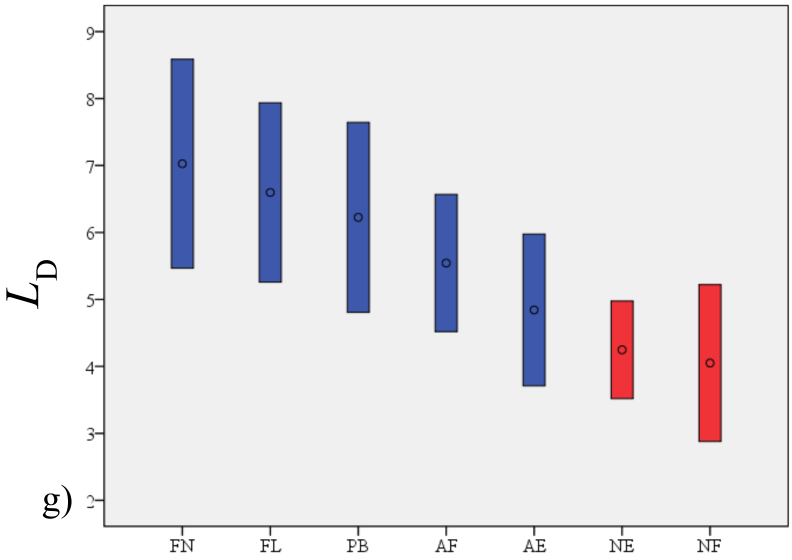
Fig.7.4 (previous page) Mean (± S.D.) of *a*) total length (L_T) (mm); and *b*) pre-branchial length (L_{PB}), *c*) branchial length (L_B); *d*) trunk length (L_{TR}); *e*) tail length (L_{TL}), and *f*) eye diameter (L_{ED}) as proportions of $L_{T,}$ from adult parasitic and non-parasitic specimens collected from seven localities in Scotland and Ireland. Blue boxes represent values of parasitic forms, red boxes represent non-parasitic forms. Values for non-parasitic forms overlap extensively with those of parasitic forms indicating these populations cannot be distinguished from populations of parasitic lampreys using these morphometric criteria. Abbreviations: AE = anadromous, Endrick Water; AF = anadromous, Forth Estuary; FN = freshwater-resident, Lough Neagh; PB = praecox, River Bladnoch; FL = freshwater-resident, Loch Lomond; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch.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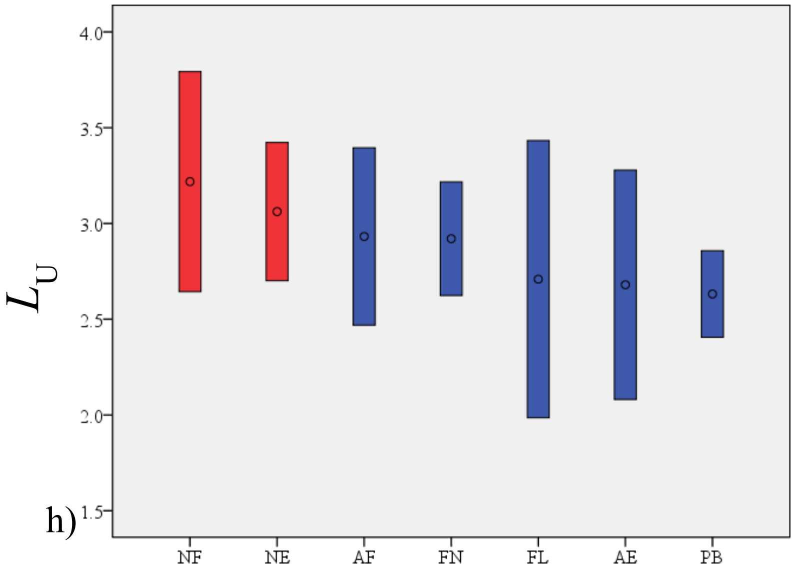
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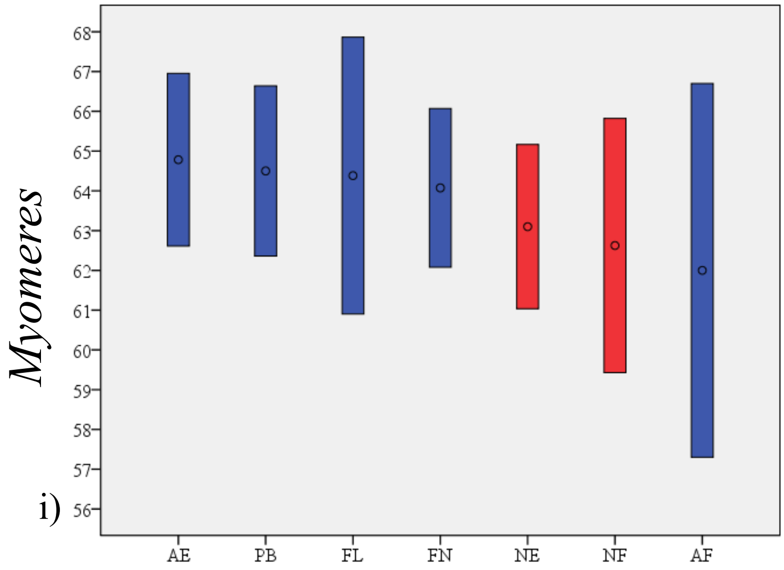
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Fig.7.4 (*cont.*) (previous page) Mean (± S.D.) of *g*) disc length (L_D); and *h*) urogenital papilla length (L_U) as proportions of L_T; and *i*) myomere counts.

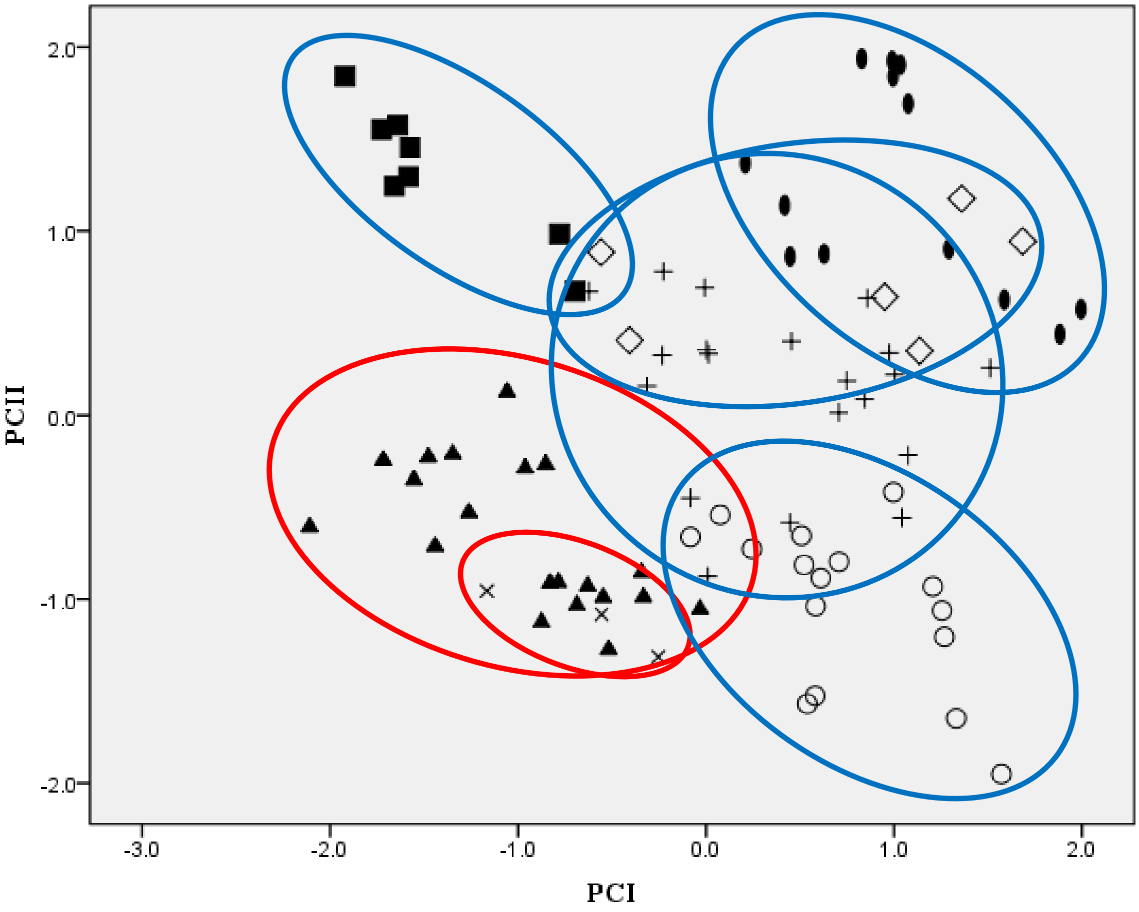

Fig.7.5 Variation in PC1 and PC2 scores (cumulative variance = 72.5%) describing overall body shape differences (based on 16 landmarks) between parasitic and non-parasitic forms collected from: (○) anadromous, Forth Estuary; (■) anadromous, Endrick Water; (x) non-parasitic, River Falloch; (▲) non-parasitic, Endrick Water; (+) freshwater-resident, Loch Lomond; (⬮) freshwater-resident, Lough Neagh; and (◊) praecox, River Bladnoch. Red circles indicate non-parasitic forms while blue circles indicate parasitic forms. Overlapping between these circles suggests some individuals fall within the variation in body shape exhibited by alternative life history strategies.

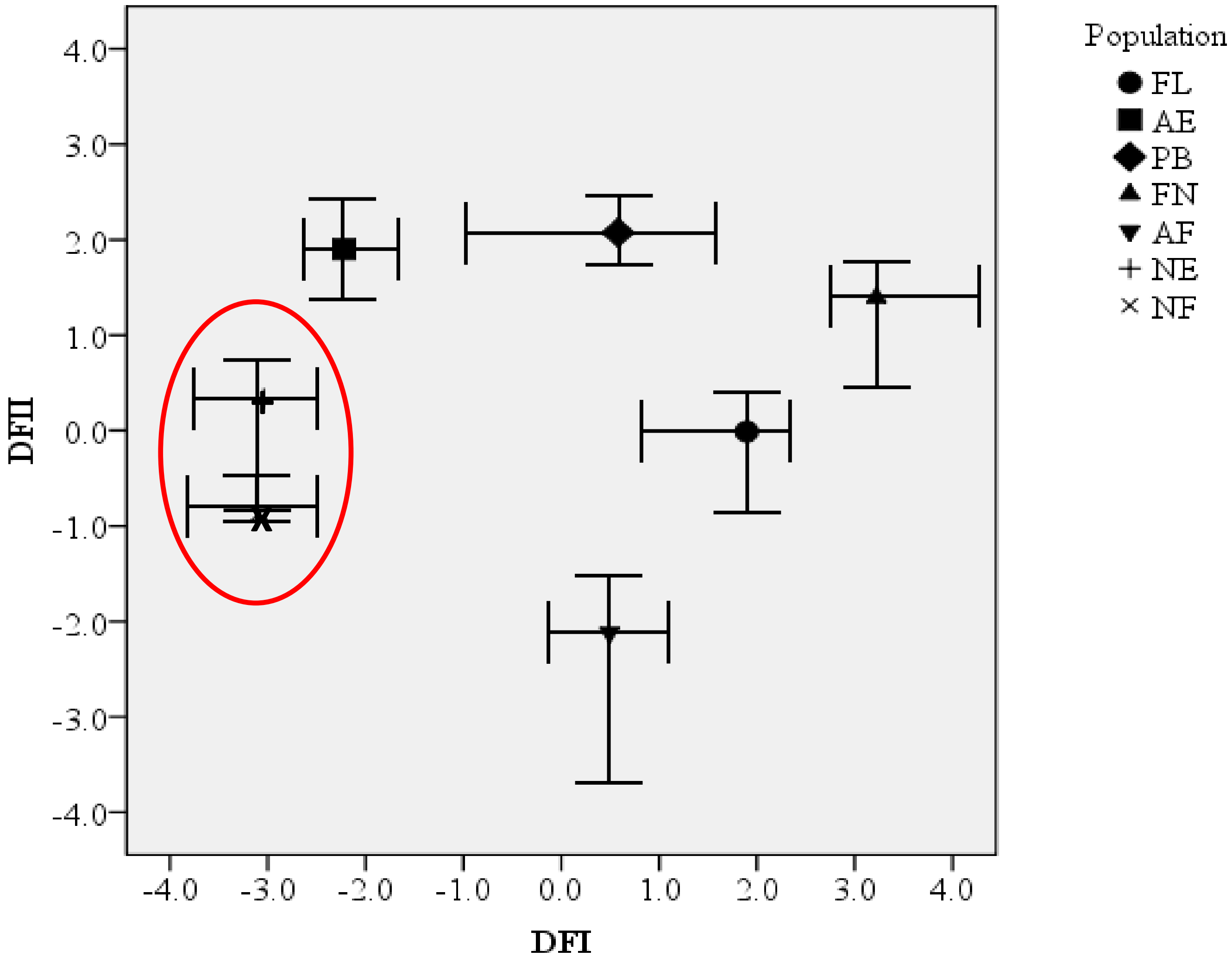

Fig.7.6. Median discriminant function scores (DFI and DFII) (based on the morphometric measures L_{PB}, L_{B}, L_{TR}, L_{ED}, L_{D}, L_{U} and myomere counts) of parasitic and non-parasitic populations collected from Scotland and Ireland. Lower bars indicate the 25 percentile; upper bars indicate the 75 percentile. The red circle indicates the position of non-parasitic populations, all others are parasitic populations. Abbreviations: FL = freshwater-resident, Loch Lomond; AE = anadromous, Endrick Water; PB = praecox, River Bladnoch; FN = freshwater-resident, Lough Neagh; AF = anadromous, Forth Estuary; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch. Non-parasitic forms cluster tightly, with significant overlap between them, indicating a high degree of similarity in the morphometric measures and an inability to discriminate between them using the DF. Parasitic forms display no clustering, indicating that there are high levels of morphometric differences between these populations that enable clear separation when using the DF.

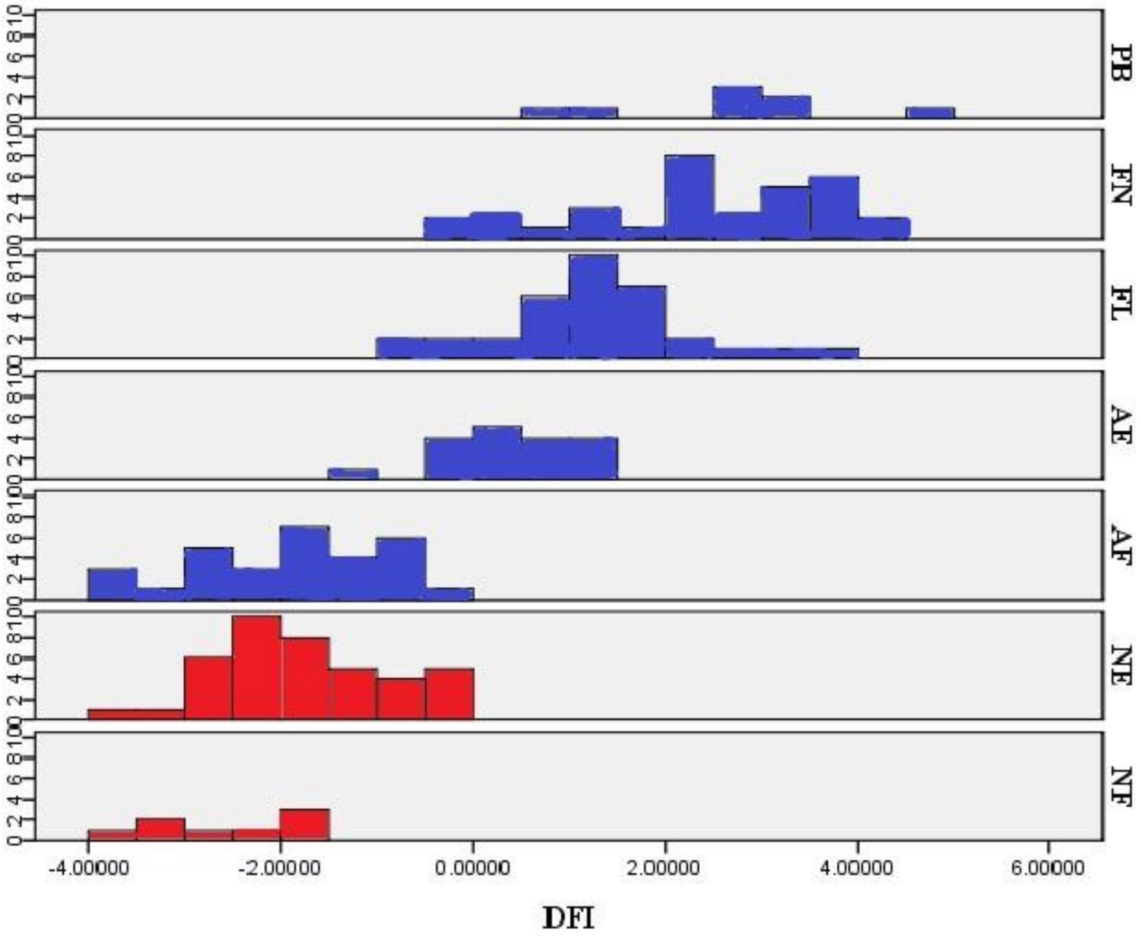

Fig.7.7. Distribution of DFI scores from six discriminant functions (based on the morphometric measures L_{PB}, L_B, L_{TR}, L_{ED}, L_D, L_U and myomere counts). Red panels represent non-parasitic populations, blue panels represent parasitic populations. The overlapping distribution of scores between non-parasitic and parasitic forms indicates that there was not a clear separation of life history strategies using the most powerful discriminant function (DFI). Abbreviations: PB = praecox, River Bladnoch; FN = freshwater-resident, Lough Neagh; FL = freshwater-resident, Loch Lomond; AE = anadromous, Endrick Water; AF = anadromous, Forth Estuary; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch.

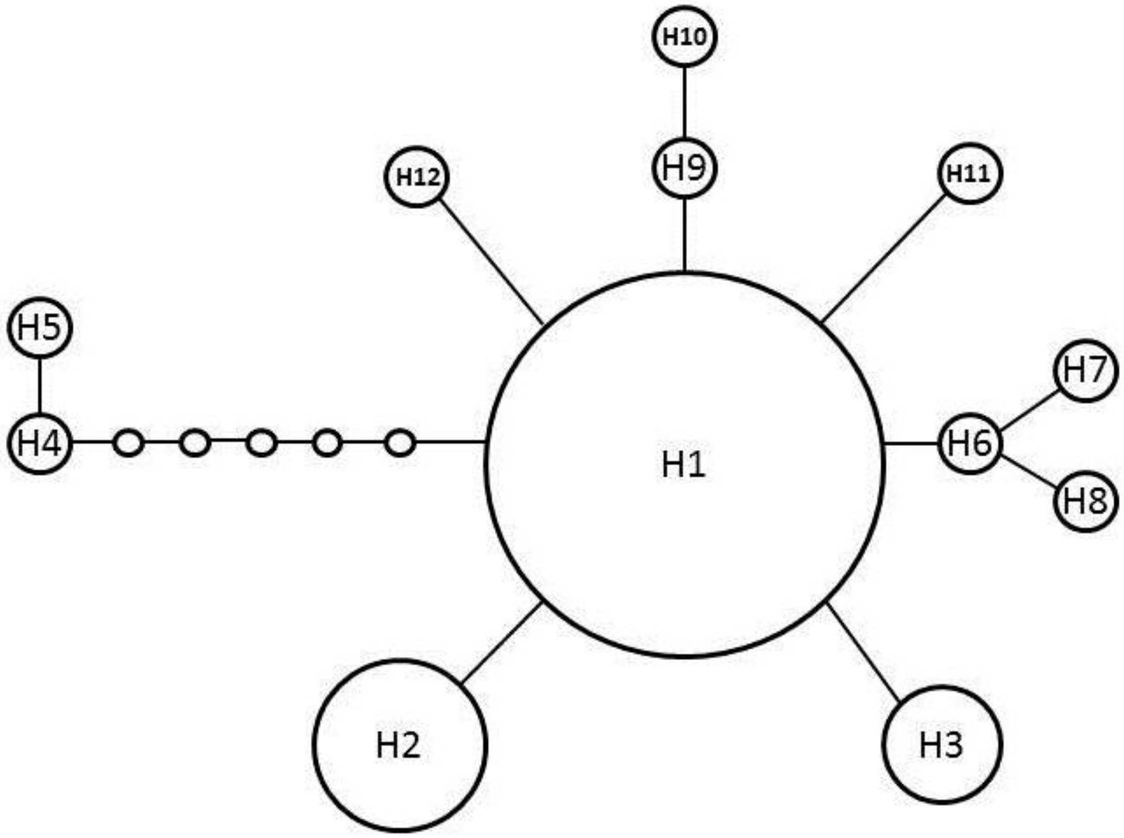

Fig.7.8 Haplotype frequency network of concatenated sequences (ATPase 6/8, cyt *b* and ND3) of 2077 bp of mtDNA for 40 lamprey specimens collected from seven localities. Haplotypes are identified in Table 7.2. Haplotype frequencies are proportional to the area of the circle. Each line represents one mutational step. Hollow circles indicate intermediate haplotypes not observed in the sample. H1 contains parasitic and non-parasitic specimens, while the most divergent haplotypes (H4 & H5) were from parasitic specimens alone. H2 and H3 contain the freshwater-resident parasitic populations of Loch Lomond and Lough Neagh respectively, as well as non-parasitic specimens from the Endrick Water, Loch Lomond, suggesting that both freshwater parasitic populations and non-parasitic forms derived independently from a common anadromous parasitic ancestor (H1).

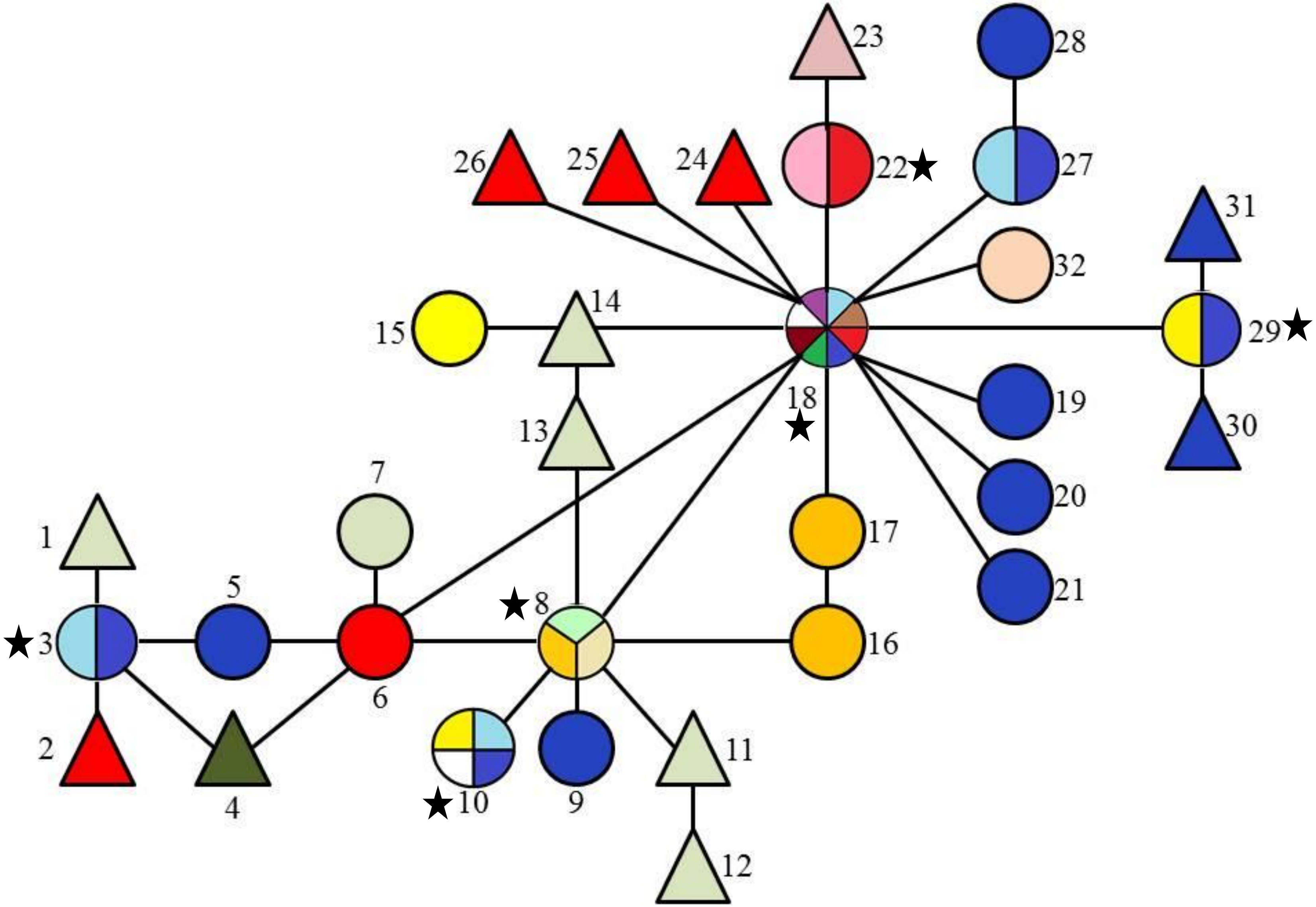
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Fig.7.9 (previous page) Minimum spanning network (MSN) of haplotypes based on concatenated sequences (ATPase 6/8 and cyt *b*) indicating relationships among *L. fluviatilis* (= parasitic) and "*L. planeri*" (= non-parasitic) populations. Each line represents a single mutational step; solid circles are haplotypes found only in parasitic specimens, while triangles represent haplotypes found only in non-parasitic specimens. A black ★ beside the haplotype number indicates shared haplotypes between parasitic and non-parasitic specimens. Collection locality of each haplotype is noted in Table 7.3 and broad geographic locations are indicated by colour: dark blue = Scotland; pale blue = Sweden; dark red = Slovakia; pale red = Portugal; dark green = Czech Republic; pale green = France; purple = Northern Ireland; brown = Germany; white = Norway; yellow = England; pink = Poland; peach = Holland; orange = Denmark. Haplotype 18 is the most probable ancestral haplotype as it was common to several populations collected across Europe and has the largest number of connections to other haplotypes. Note that in all cases, except for haplotype 15, non-parasitic haplotypes appear to be derived from those found in parasitic forms. However, non-parasitic haplotypes from the same geographic location do not always cluster together e.g., haplotypes 24-26 and haplotype 2 were all from Portuguese specimens and yet haplotype 2 is more highly diverged, indicating that this specimen could have derived from a separate parasitic ancestor to that giving rise to haplotypes 24-26.

Table 7.1 Morphometrics, pigmentation and dentition of adult specimens of parasitic (= *Lampetra fluviatilis*) and non-parasitic (= “*L. planeri*”) forms from Scotland and Northern Ireland. For morphometrics, data represent the mean ± standard deviation (S.D.) (see Fig. 7.2 for an explanation of linear measurements). For dentition, values represent the mean with the range in parentheses, or counts where applicable (see Fig. 7.3 for an explanation of dentition). Blue columns represent data recorded from parasitic forms, red columns from non-parasitic forms. Abbreviations: AF = anadromous, Forth Estuary; AE = anadromous, Endrick Water; PB = praecox, River Bladnoch; FN = freshwater-resident, Lough Neagh; FL = freshwater-resident, Loch Lomond; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch; L_T = total length, L_{PB} = pre-branchial length, L_B = branchial length, L_{TR} = trunk length, L_{TL} = tail length, L_{ED} = eye diameter, L_D = disc length, L_U = urogenital papilla length, *u*, unicuspid; *b*, bicuspid; *n/a*, not available; *d*, dark; *li*, light; *p*, pigmented; *unp*, unpigmented.

Source character	**AF (*n* = 30)**	**AE (*n* = 23)**	**PB (*n* = 8)**	**FN (*n* = 27)**	**FL (*n* = 34)**	**NE (*n* = 40)**	**NF (*n* = 8)**
L_T	322.8 ± 30.8	327.4 ± 28.4	249.1 ± 17.5	319.6 ± 23.7	222.5 ± 28.6	149.1 ± 12.9	124.0 ± 14.9
L_{PB}	11.4 ± 0.5	9.2 ± 0.7	11.1 ± 1.1	11.0 ± 0.9	11.4 ± 0.8	9.7 ± 0.7	9.8 ± 0.5
L_B	9.0 ± 0.8	10.3 ± 0.4	11.9 ± 0.6	10.5 ± 0.3	9.9 ± 0.6	9.9 ± 0.6	9.9 ± 0.4
L_{TR}	49.9 ± 1.9	52.7 ± 1.9	52.9 ± 3.0	48.1 ± 9.6	51.7 ± 1.7	51.5 ± 2.2	50.7 ± 1.5
L_{TL}	27.6 ± 1.4	27.6 ± 1.9	24.2 ± 3.5	23.1 ± 4.7	27.0 ± 1.7	28.8 ± 1.9	29.7 ± 1.1
L_{ED}	2.3 ± 0.4	1.6 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	1.9 ± 0.4	1.9 ± 0.2	2.1 ± 0.2
L_D	5.5 ± 0.5	4.8 ± 0.6	6.2 ± 0.7	7.0 ± 0.8	6.6 ± 0.7	4.2 ± 0.4	4.0 ± 0.5
L_U	2.9 ± 0.2	2.7 ± 0.3	2.6 ± 0.08	2.9 ± 0.1	2.7 ± 0.4	3.1 ± 0.2	3.2 ± 0.2
Trunk myomeres	62.0 (58-66)	64.8 (63-66)	64.5 (63-66)	64.1 (62-66)	64.4 (61-67)	63.1 (62-66)	62.6 (60-65)
Marginals	78.4 (64-96)	81.1 (69-96)	n/a	87.1 (72-108)	84.8 (76-100)	69 (51-82)	70.8 (58-82)
Supraoral lamina	1-1(30)	1-1(30)	n/a	1-1(27)	1-1(34)	1-1(40)	1-1(8)
Infraoral	4u2b(3),5u2b(1	5u2b(3),6u1b	n/a	5u2b(15),6u1b(	3u1b(1),3u2b	3u2b(2),4u2b(1),5u(1),5u	5u1b(1),6u1b(3),

lamina	6),6u1b(5),6u2b(1)	(2),7u(3)		10),7u(2)	(2),4u2b(3), 5u2b(5),6u1b(1),7u(3)	1b(4),6u(1),6u1b(2), 7u(11)	7u(4)
Endolaterals	2-2-2(6), 2-3-2(19)	2-3-2(10)	n/a	2-2-2(5), 2-3-2(21)	2-3-2(16)	2-2-1(4),2-2-2(3), 2-3-2(23)	2-3-2(8)
Anterial rows	1(8),2(18)	1(2),2(8)	n/a	1(14),2(11), 3(1)	1(10),2(7)	1(7),2(18),3(1)	1(2),2(6)
Exolaterals	0	0	n/a	0	0	0	0
Posterials	0	0	n/a	0	0,1(1)	0,1(2),2(1)	0
Velar tentacles	6(7),7(4),8(2)	6(3),7(2), 8(4),9(1)	n/a	5(1),6(6),7(3), 8(3)	5(1),6(8), 7(5),8(1)	4(5),5(10),6(13),7(2)	4(1),5(3),6(4)
Dorsal fin pigmentation	++(30)	+(1),++(2), +++(20)	+(3),++(2), +++(3)	++(2),+++(23)	+++(34)	+(4),++(20),+++(16)	+(5),++(3)
Iris colouration	li(30)	d(5),li(18)	li(8)	li(27)	d(34)	d(1),li(39)	d(2),li(6)
Lateral line neuromast pigmentation	p(21),unp(9)	p(16),unp(7)	p(5),unp(3)	p(15),unp(12)	p(34)	p(31),unp(9)	p(5),unp(3)
Caudal fin pigmentation	++(30)	+++(23)	+(1),++(2), +++(5)	++(1),+++(26)	+++(34)	+(1),++(15),+++(24)	+(3),++(2),+++(3)

Table 7.2 Haplotypes (Fig. 7.8) assigned to Scottish and Irish samples of parasitic and non-parasitic forms collected in the present study, indicating collection locality and traditional species designation. Blue cells represent parasitic forms, red cells represent non-parasitic forms.

Haplotype	**Species**	**Collection Locality**
H1	*L. planeri*	River Falloch, Loch Lomond, Scotland
	L. planeri	Endrick Water, Loch Lomond, Scotland
	L. planeri	River Bladnoch, Galloway, Scotland
	L. fluviatilis	Endrick Water, Loch Lomond, Scotland
	freshwater-resident *L. fluviatilis*	Endrick Water, Loch Lomond, Scotland
	praecox *L. fluviatilis*	River Bladnoch, Galloway, Scotland
	L. fluviatilis	Fruin Water, Loch Lomond, Scotland
	L. fluviatilis	Finlas Water, Loch Lomond, Scotland
	L. fluviatilis	Forth Estuary, Scotland
H2	*L. planeri*	Endrick Water, Loch Lomond, Scotland
	freshwater-resident *L. fluviatilis*	Endrick Water, Loch Lomond, Scotland
H3	*L. planeri*	Endrick Water, Loch Lomond, Scotland
	freshwater-resident *L. fluviatilis*	Lough Neagh, Northern Ireland
H4	*L. fluviatilis*	River Bladnoch, Galloway, Scotland
H5	*L. fluviatilis*	River Bladnoch, Galloway, Scotland
H6	*L. planeri*	Endrick Water, Loch Lomond, Scotland
H7	*L. planeri*	Endrick Water, Loch Lomond, Scotland
H8	*L. planeri*	River Falloch, Loch Lomond, Scotland
H9	*L. fluviatilis*	Endrick Water, Loch Lomond, Scotland
H10	*L. fluviatilis*	Endrick Water, Loch Lomond, Scotland
H11	*L. fluviatilis*	Luss Water, Loch Lomond, Scotland
H12	*L. fluviatilis*	Fruin Water, Loch Lomond, Scotland

Table 7.3 Collection localities of all adult specimens sequenced in this study, including sequences retrieved from GenBank (Espanhol *et al*., 2007). Traditional taxonomic designation and their corresponding accession numbers are indicated. * denotes misidentified specimens in GenBank. Haplotype names used in Fig. 7.9 are indicated. Blue cells indicate parasitic forms, red cells indicate non-parasitic forms.

Haplotype	**Species**	**Collection Locality**	**Accession Number**
1	*Lampetra planeri*	River Allier, France	AJ937933.1
2	*Lampetra planeri*	River Anços, Portugal	AJ937950.1
3	*Lampetra fluviatilis*	River Bladnoch, Scotland	n/a (this study)
	Lampetra fluviatilis	River Ricklean, Sweden	AJ937925.1
4	*Lampetra planeri*	River Blanice, Czech Republic	AJ937953.1
5	*Lampetra fluviatilis*	River Bladnoch, Scotland	n/a (this study)
6	*Lampetra fluviatilis**	River Sorraia, Portugal	AJ937954.1
7	*Lampetra fluviatilis*	Garonne Estuary, France	AJ937921.1
8	*Lampetra fluviatilis*	Wadden Sea, Netherlands	AJ937927.1
	Lampetra fluviatilis	River Lilleaa, Denmark	AJ937935.1
	Lampetra planeri	River Loire, France	AJ966336.1
9	*Lampetra fluviatilis*	Forth Estuary, Scotland	AJ937938.1
10	*Lampetra fluviatilis*	River Ricklean, Sweden	AJ937924.1
	Lampetra fluviatilis	Endrick Water, Scotland	n/a (this study)
	Lampetra planeri	River Vikedalseva, Norway	AJ966334.1
	*Lampetra planeri**	River Ouse, England	AJ937941.1
11	*Lampetra planeri*	River Ain, France	AJ937931.1
12	*Lampetra planeri*	River Ain, France	AJ937930.1
13	*Lampetra planeri*	River Loire, France	AJ937922.1
14	*Lampetra planeri*	River Loire, France	AJ937932.1
15	*Lampetra fluviatilis**	River Ouse, England	AJ937942.1
16	*Lampetra fluviatilis*	River Lilleaa, Denmark	AJ937935.1

17	*Lampetra fluviatilis*	River Lilleaa, Denmark	AJ937936.1
18	*Lampetra fluviatilis*	River Ricklean, Sweden; River Elbe, Germany; Tejo Estuary, Portugal	AJ937926.1
	Lampetra fluviatilis	Fruin Water, Scotland	n/a (this study)
	praecox *Lampetra fluviatilis*	River Bladnoch, Scotland	n/a (this study)
	Lampetra fluviatilis	Endrick Water, Scotland	n/a (this study)
	Lampetra fluviatilis	Finlas Water, Scotland	n/a (this study)
	Lampetra fluviatilis	Forth Estuary, Scotland	n/a (this study)
	freshwater-resident *Lampetra fluviatilis*	Endrick Water, Scotland	n/a (this study)
	freshwater-resident *Lampetra fluviatilis*	Lough Neagh, Northern Ireland	n/a (this study)
	Lampetra planeri	River Vikedalseva, Norway; River Tollense, Germany; River Blanice, Czech Republic	AJ966335.1
	Lampetra planeri	River Poprad, Slovakia	AJ937945.1
	Lampetra planeri	Endrick Water, Scotland	n/a (this study)
	Lampetra planeri	River Falloch, Scotland	n/a (this study)
	Lampetra planeri	River Bladnoch, Scotland	n/a (this study)
19	*Lampetra fluviatilis*	Forth Estuary, Scotland	AJ937940.1
20	*Lampetra fluviatilis*	Luss Water, Scotland	n/a (this study)
21	*Lampetra fluviatilis*	Forth Estuary, Scotland	AJ937939.1
22	*Lampetra fluviatilis*	Wadden Sea; Tejo Estuary, Portugal	AJ937929.1
	Lampetra planeri	River Grzmiace, Poland	AJ966337.1
23	*Lampetra planeri*	River Grzmiace, Poland	AJ937934.1
24	*Lampetra planeri*	Esmoriz Basin, Portugal	AJ937946.1
25	*Lampetra planeri*	River Anços, Portugal	AJ937923.1
26	*Lampetra planeri*	River Sorraia, Portugal	AJ937951.1
27	*Lampetra fluviatilis*	River Bladnoch, Scotland	n/a (this study)
	Lampetra planeri	River Ricklean, Sweden	AJ937925.1

28	*Lampetra fluviatilis*	Endrick Water, Scotland	n/a (this study)
29	*Lampetra fluviatilis*	River Ouse, England	AJ937943.1
	Lampetra planeri	Endrick Water, Scotland	n/a (this study)
30	*Lampetra planeri*	Endrick Water, Scotland	n/a (this study)
31	*Lampetra planeri*	River Falloch, Scotland	n/a (this study)
32	*Lampetra fluviatilis*	Wadden Sea, Netherlands	AJ937928.1

Table 7.4. Classification success of the discriminant function analysis. Figures in bold indicate the proportion of individuals allocated by the analysis to the "predicted" population based on all six discriminant function scores. Average classification success placing an individual within its population of origin was 75.3%. Blue cells represent parasitic forms, red cells represent non-parasitic forms. Abbreviations: AE = anadromous, Endrick Water, FL = freshwater-resident, Loch Lomond; PB = praecox, River Bladnoch; FN = freshwater-resident, Lough Neagh; AF = anadromous, Forth Estuary; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch.

		Predicted Population Membership							
		AE	FL	PB	FN	AF	NE	NF	Total
Percentage	AE	**79.4**	5.9	0	2.9	11.8	0	0	100
	FL	0	**88.9**	0	5.6	0	5.6	0	100
	PB	0	12.5	**75**	12.5	0	0	0	100
	FN	15.6	15.6	0	**68.8**	0	0	0	100
	AF	3.3	0	0	0	**83.3**	6.7	6.7	100
	NE	0	15	0	0	5	**80**	0	100
	NF	0	0	0	0	0	100	**0**	100

Table 7.5 Pairwise population F_{ST} (distance method: pairwise differences) between all pairs of parasitic and non-parasitic populations collected from Scotland and Ireland and examined genetically in this study (below diagonal). Values above the diagonal refer to approximate geographic distance between collection localities (kilometres). Red cells indicate non-parasitic forms, blue cells indicate parasitic forms. Abbreviations: 1 = River Falloch; 2 = Endrick Water; 3 = River Bladnoch; 4 = Endrick Water; 5 = freshwater resident, Endrick Water; 6 = praecox, River Bladnoch; 7 = Luss Water; 8 = Fruin Water; 9 = Finlas Water; 10 = Forth Estuary; 11 = freshwater-resident, Lough Neagh.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	0.00000	30	165	30	30	165	26	35	32	67	215
2	-0.10345	0.00000	133	0	0	133	13	12	12	46	199
3	-1.00000	-0.84000	0.00000	133	133	0	139	131	134	141	131
4	0.07368	0.10000	-0.73333	0.00000	0	133	13	12	12	46	199
5	0.48430	0.26906	0.50000	0.49953	0.00000	133	13	12	12	46	199
6	0.46953	0.49338	-0.07692	0.55435	0.61670	0.00000	139	131	134	141	131
7	0.33333	0.16364	1.00000	0.42222	0.77778	0.12500	0.00000	8	5	59	195
8	0.00000	-0.02632	-1.00000	0.09434	0.57655	0.31618	0.33333	0.00000	3	58	189
9	-1.00000	-0.08400	0.00000	-0.73333	0.50000	-0.07692	1.00000	-1.00000	0.00000	58	192
10	-0.26316	-0.22124	0.00000	-0.16418	0.62264	0.32258	1.00000	0.00000	0.00000	0.00000	232
11	0.52941	0.25405	1.00000	0.55429	0.82684	0.44371	1.00000	0.66667	1.00000	1.00000	0.00000

Appendix 7.1

Taxonomic descriptions of parasitic (= *Lampetra fluviatilis*) and non-parasitic (= "*L. planeri*") lampreys from Scotland and Ireland; including two freshwater-resident parasitic populations and a praecox parasitic population

Anadromous parasitic (= *Lampetra fluviatilis*) (Linnaeus 1758) (Fig. 7.10*a* & 7.11*a*)

Taxonomic Remarks: common name – European river lamprey.

Diagnostic Features: Ammocoetes: Given the difficulties in separating ammocoetes of this form from non-parasitic populations (= *L. planeri*) (Potter & Osborne, 1975; Gardiner, 2003) the following description likely applies to both. Maximum size attained: 146 mm total length (L_T). Body proportions as a percentage of L_T (based on 144 specimens measuring 56 - 146 mm L_T): prebranchial length**,** 6.6 – 9.1; branchial length, 11 – 13.1; trunk length, 49.2 – 53.7; tail length, 25.6 – 28.9. Trunk myomeres: range 55 – 66. Body colouration: dorsal surface brown and lateral aspects brownish-yellow, ventral surface lighter. Pigmentation: upper lip, - (100% of specimens); lower lip - (100%); between upper lip and cheek, +++ (100%); cheek, + (90%) or ++ (10%); subocular, ++ (69%) or +++ (31%); upper prebranchial, - (95%) or + (5%); lower prebranchial, - (92%) or + (8%); upper branchial, + (100%); lower branchial, - (30%) + (70%); ventral branchial, - (100%); caudal fin, - (74%) or + (26%); tongue precursor bulb, ++ (100%); elastic ridge, ++ (100%). Lateral line neuromasts unpigmented. Caudal fin shape: rounded.

Metamorphosing Ammocoetes: 82 – 141 mm L_T.

Adults: 97 – 374 mm L_T. Body wet weight in individuals 257 – 374 mm L_T, 28 – 81 g. Body proportions, as a percentage of L_T (based on 18 specimens measuring 269 – 369 mm L_T): prebranchial length, 8.2 – 12.4; branchial length, 7.8 - 11.2; trunk length, 48.8 – 56.8; tail length, 24.3 – 31.7; eye length, 1.4 – 3.0; disc length, 3.7 – 6.5. The urogenital papilla length in six spawning males 297 – 350 mm L_T, 9.2 – 9.8 mm. Trunk myomeres, range 58 – 66. Dentition: marginals, 64 – 96; supraoral lamina, 2 unicuspid teeth; infraoral lamina, 7 – 9 either all unicuspid, or one or both lateral-most are bicuspid with the others unicuspid; 3

endolaterals on each side; endolateral formula, 2-3-2, rarely 2-2-2; 1 - 2 rows of anterials; first row of anterials, 4 - 7 unicuspid teeth; exolaterals absent; posterials absent; transverse lingual lamina, 10 – 17 unicuspid teeth, the median enlarged; longitudinal lingual laminae, straight or parentheses-shaped and each with 8 – 12 unicuspid teeth. Velar tentacles: 6 – 9, with tubercles; no velar wings. Oral fimbriae: 96 – 113; oral papillae, not recorded. Body colouration, in recently metamorphosed individuals: silver on dorsal aspect to white on ventral aspect; in early spawning migrants: slate grey to bronze and pale brown on dorsal aspect to cream on ventral; in spawning individuals: dark brown or yellowish on dorsal aspect and cream on ventral aspect. Dorsal fins are typically +++, and rarely ++ or +. Iris colour is typically light, rarely dark. Lateral line neuromasts are darkly pigmented or unpigmented. Caudal fin pigmentation, + in recently metamorphosed individuals and +++ in mature adults. Caudal fin shape: typically spade-like and rarely rounded.

Non-parasitic (= *Lampetra planeri*) (Bloch 1784) (Fig. 7.10*c* & 7.11*d*)

Taxonomic Remarks: common name – European brook lamprey. The specific status of this non-parasitic lamprey is contentious, with some authors believing instead that it represents a stream-resident population of *L. fluviatilis* (e.g., Enequist, 1937). Molecular genetic evidence indicates that *L. planeri* is not monophyletic; instead populations have evolved repeatedly and independently from *L. fluviatilis* (e.g., Schreiber & Engelhorn, 1998). These data suggest either ongoing gene flow with *L. fluviatilis*, or very recent divergence. Populations of *L. planeri* exhibit high degrees of morphological variation across its range, including adults of a much reduced size in the Scottish Hebridian Islands (ERA, 2005).

Diagnostic Features: Ammocoetes: Given the difficulties in separating ammocoetes of this form from parasitic populations (= *L. fluviatilis*) (Potter & Osborne, 1975; Gardiner, 2003) the following description likely applies to both. Maximum size attained ≥ 200 mm L_T given maximum adult L_T. Body proportions as a percentage of L_T (based on 198 specimens measuring 33 - 176 mm L_T): prebranchial length**,** 6.8 – 9.1; branchial length, 10.3 – 13.1; trunk length, 49.2 – 54.5; tail length, 25.5 – 29.7. Trunk myomeres: range 54 – 66. Body colouration: dorsal surface brown and lateral aspects brownish-yellow, ventral surface lighter. Pigmentation: upper lip, - (100% of specimens); lower lip - (100%); between upper lip and cheek, +++ (100%); cheek, + (81%) or ++ (19%); subocular, - (25%) ++ (51%) or +++ (24%); upper prebranchial, - (92%) or + (8%); lower prebranchial, - (85%) or + (15%); upper branchial, + (100%); lower branchial, - (34%) + (66%); ventral branchial, - (100%); caudal fin, - (80%) or + (20%); tongue precursor bulb, + (15%) or ++ (85%); elastic ridge, ++ (100%). Lateral line neuromasts unpigmented. Caudal fin shape: rounded.

Metamorphosing Ammocoetes: 118 – 200 mm L_T.

Adults: 103 – 195 mm L_T. Body wet weight in individuals 103 – 195 mm L_T, 1 – 13 g. Body proportions, as a percentage of L_T (based on 48 specimens measuring 103 – 176 mm L_T): prebranchial length, 8.0 – 11.4; branchial length, 7.7 - 10.8; trunk length, 47.1 – 56.2; tail length, 24.3 – 32.2; eye length, 1.5 – 2.4; disc length, 2.9 – 4.9. The urogenital papilla length in 21 spawning males measuring 113 – 176 mm L_T, 4 – 5 mm. Trunk myomeres: range 54 – 66. Dentition: marginals, 51 – 82; supraoral lamina, 2 unicuspid teeth; infraoral lamina, 5 – 7 either all unicuspid, or one or both lateral-most are bicuspid with the others unicuspid; 3 endolaterals on each side; endolateral formula, 2-3-2, rarely 2-2-2 or 2-2-1; 1 - 2 rows of anterials; first row of anterials 4 - 7 unicuspid teeth; exolaterals absent; posterials absent, rarely 1 – 3 unicuspid teeth; transverse lingual lamina, 9 – 14 unicuspid teeth, the median enlarged; longitudinal lingual laminae, straight or parentheses-shaped and each with 8 – 12 unicuspid teeth. Velar tentacles: 4 – 7, with tubercles; no velar wings. Oral fimbriae: not recorded; oral papillae, not recorded. Body colouration, in recently metamorphosed individuals: similar to ammocoete colouration; spawning individuals very variable: mostly dark brown to pale brown on dorsal aspect and cream on ventral aspect with strong countershading, though some are mottled on ventral aspect. Dorsal fins are +++, ++ or + and have a prominent dark blotch on apex of second dorsal (84%). Iris colour is typically light, rarely dark. Lateral line neuromasts are darkly pigmented or unpigmented. Caudal fin pigmentation, + in recently metamorphosed individuals and +, ++ or +++ in mature adults. Caudal fin shape: spade-like (74%) or rounded (16%).

Freshwater-resident parasitic "Loch Lomond" (= *L. fluviatilis*) (Morris, 1989) (Fig. 7.10*b* & 7.11*c*)

Taxonomic Remarks: This freshwater-resident parasitic population (= *L. fluviatilis*) is known only from Loch Lomond, Scotland and has been referred to variously as "freshwater-feeding *L. fluviatilis*" (Morris, 1989), "small body size *L. fluviatilis*" (Maitland *et al*., 1994; Adams *et al*., 2008) or "dwarf river lamprey" (Bond, 2003). However, previous nomenclature is deemed to be unsuitable. Instead the common usage of the prefix "freshwater-resident" is proposed, as this denotes the population's life-history strategy and distinguishes it from the sympatric anadromous form.

Diagnostic Features: Ammocoetes: It has not been possible to distinguish between ammocoetes of this population and anadromous parasitic (= *L. fluviatilis*) or non-parasitic (= *L. planeri*) populations from same site (see previous descriptions).

Metamorphosing Ammocoetes: lengths unrecorded.

Adults: 149 – 277 mm L_T. Body wet weight in individuals 149 – 269 mm L_T, 4.45 – 30.7 g. Body proportions, as a percentage of L_T (based on 34 specimens measuring 149 – 277 mm L_T): prebranchial length, 9.8 – 12.9; branchial length, 8.6 - 11.2; trunk length, 47.9 – 55.2; tail length, 24.1 – 31.3; eye length, 1.3 – 3.1; disc length, 5.4 – 8.4. The urogenital papilla length in 19 spawning males measuring 149 – 277 mm L_T, 4.9 – 6.2 mm. Trunk myomeres: range 61 – 68. Dentition: marginals, 76 – 100; supraoral lamina, 2 unicuspid teeth; infraoral lamina, 4 – 7 either all unicuspid, or one or both lateral-most are bicuspid with the others unicuspid; 3 endolaterals on each side; endolateral formula, 2-3-2; 1 - 2 rows of anterials; first row of anterials 3 - 8 unicuspid teeth; exolaterals absent; posterials absent, rarely 1 – 2 unicuspid teeth; transverse lingual lamina, 8 – 15 unicuspid teeth, the median enlarged, rarely only large median is present; longitudinal lingual laminae, straight or parentheses-shaped and each with 10 – 13 unicuspid teeth. Velar tentacles: 5 – 8, with tubercles; no velar wings. Oral fimbriae: not recorded; oral papillae, not recorded. Body colouration is uniformly black, with some individuals having a thin section of silver on the ventral aspect. Dorsal fins are black (+++). Iris colour is dark. Lateral line neuromasts are darkly pigmented. Caudal fin pigmentation: +++. Caudal fin shape: spade-like.

Freshwater-resident parasitic "Lough Neagh" (= *L. fluviatilis*)

Taxonomic Remarks: This freshwater-resident parasitic population (= *L. fluviatilis*) is known from Lough Neagh, Northern Ireland. It remains in fresh water for the duration of the parasitic phase (*c*. 12 months) (Goodwin *et al*., 2006; Inger *et al*., 2010).

Diagnostic Features: Ammocoetes: It has not been possible to distinguish between ammocoetes of this population and anadromous parasitic (= *L. fluviatilis*) or non-parasitic (= *L. planeri*) from the same site (see previous descriptions).

Metamorphosing Ammocoetes: lengths unrecorded.

Adults: 118 – 391 mm L_T. Body wet weight in individuals 194 – 363 mm L_T, 10.3 – 138.2 g. Body proportions, as a percentage of L_T (based on 27 specimens measuring 271 – 357 mm L_T): prebranchial length, 10.3 – 14.2; branchial length, 9.7 – 10.8; trunk length, 40.3 – 56; tail length, 17.3 – 28.7; eye length, 1.6 – 2.1; disc length, 5.5 – 7.7. The urogenital papilla length in 13 spawning males measuring 276 – 357 mm L_T, 8.9 – 9.7 mm. Trunk myomeres: range 62 – 66. Dentition: marginals, 72 – 108; supraoral lamina, 2 unicuspid teeth; infraoral lamina, 7 either all unicuspid, or one or both lateral-most are bicuspid with the others unicuspid; 3 endolaterals on each side; endolateral formula, 2-3-2, rarely 2-2-2; 1-2 rows of anterials; first row of anterials 4 - 7 unicuspid teeth; exolaterals absent; posterials absent; transverse lingual lamina, 10 – 13 unicuspid teeth, the median enlarged; longitudinal lingual laminae, straight or parentheses-shaped and each with 10 – 14 unicuspid teeth. Velar tentacles: 5 – 8, with tubercles; no velar wings. Oral fimbriae: 102 - 112; oral papillae, not recorded. Body colouration ranges from grey through to dark brown on the dorsal aspect, and cream to white on the ventral aspect. Dorsal fins: +++, rarely ++. Iris colour is light. Lateral line neuromasts are darkly pigmented or unpigmented. Caudal fin pigmentation: +++, rarely ++. Caudal fin shape: spade-like.

Praecox parasitic (= *L. fluviatilis*) (Berg, 1931, 1948) (Fig. 7.11*b*)

Taxonomic Remarks: Some river systems are known to contain sympatric populations of *L. fluviatilis* that have been termed "*forma typica"* and "*forma praecox"*. The latter attain a smaller adult body size, are believed to mature and spawn earlier, and have a lower fecundity than the former. Such populations have been described from the Neva River and Lake Ladoga in the Russian Federation (Berg, 1938), the River Severn (Abou-Seedo & Potter, 1979), River Tywi (Huggins & Thompson, 1970) and the River North Esk (P. Maitland, *pers. com.*) in the U.K. A population of *L. fluviatilis "forma praecox"* was identified in a collection of *L. fluviatilis* from the River Bladnoch, Scotland and is described below.

Diagnostic Features: Ammocoetes: It has not been possible to distinguish between ammocoetes of this population and anadromous parasitic (= *L. fluviatilis*) or non-parasitic (= *L. planeri*) populations from the same site (see previous descriptions).

Metamorphosing Ammocoetes: lengths unrecorded.

Adults: 229 – 273 mm L_T. Body wet weight in individuals 229 – 273 mm L_T, 28 – 51 g. Body proportions, as a percentage of L_T (based on 8 specimens measuring 229 – 273 mm L_T): prebranchial length, 9.5 – 13; branchial length, 10.7 – 13.1; trunk length, 47.8 – 57.1; tail length, 19.2 – 28.7; eye length, 1.8 – 2.4; disc length, 5.1 – 7.1. The urogenital papilla length in four spawning males measuring 229 – 270 mm L_T was 5.6 – 6.1 mm. Trunk myomeres: range 63 – 66. Dentition: not recorded. Oral fimbriae: not recorded; oral papillae, not recorded. Body colouration in spawning individuals is grey on the dorsal aspect and cream on the ventral aspect, some individuals are mottled. Dorsal fins are grey (+, ++ or +++). Iris colour is light. Lateral line neuromasts are darkly pigmented or unpigmented. Caudal fin pigmentation: +++, rarely + or ++. Caudal fin shape: spade-like.

FIGURES

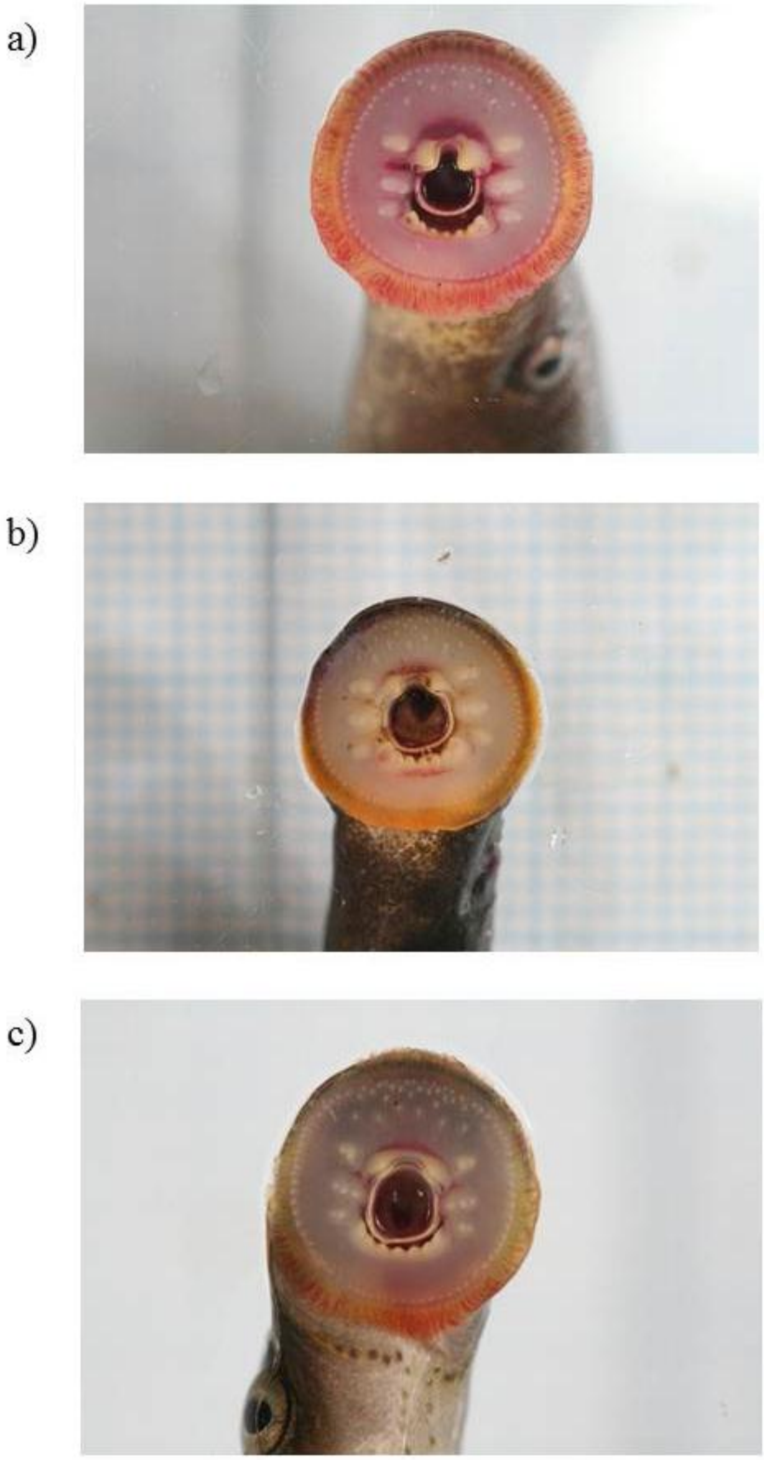

Fig.7.10 Oral discs of: *a*) anadromous parasitic (= *Lampetra fluviatilis*); *b*) freshwater-resident parasitic (= *L. fluviatilis*); *c*) non-parasitic (= *L. planeri*) adult specimens from the Endrick Water, Loch Lomond.

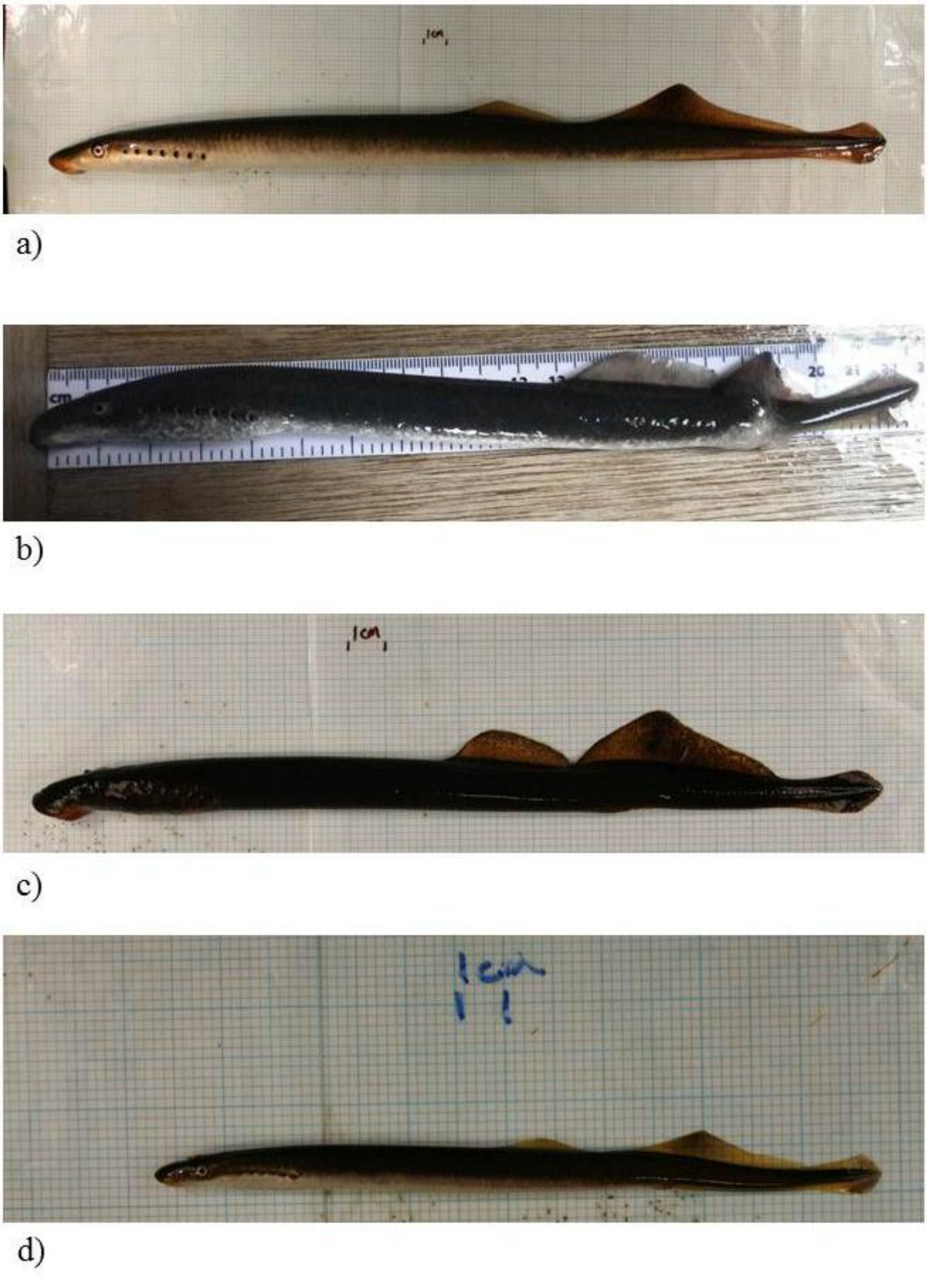

Fig.7.11 Post-metamorphic specimens of: *a*) anadromous parasitic (= *Lampetra fluviatilis*) adult from the Endrick Water, Loch Lomond; *b*) praecox parasitic (= *L. fluviatilis*) adult from the River Bladnoch, Galloway; *c*) freshwater-resident parasitic (= *L. fluviatilis*) adult from the Endrick Water, Loch Lomond; and *d*) non-parasitic (= *L. planeri*) adult from the Endrick Water, Loch Lomond.

Appendix 7.2

First record of larval sea lamprey *Petromyzon marinus* L. in the Endrick Water, Loch Lomond

Three lamprey species are known to occur in Scotland: European river lamprey *Lampetra fluviatilis,* European brook lamprey *L planeri*, and the sea lamprey *Petromyzon marinus*. Although detailed records of their distribution remain scarce, lampreys have been sampled from 79 Scottish regions (ERA, 2005). The sea lamprey is the rarest species in both records and surveys and has been recorded nationally in just 35 rivers, although their continuing presence in some is uncertain (ERA, 2005).

The Endrick Water drains the south east catchment of Loch Lomond into its southern basin. This river contains scientifically important populations of brook and river lamprey, and has been designated a Special Area of Conservation (SAC) and Site of Special Scientific Interest (SSSI) as a result (Bond, 2003). Although several lamprey surveys have been conducted in recent years (Maitland *et al*., 1994; Gardiner *et al*., 1995; Gardiner & Stewart, 1997, 1999; Forth Fisheries Foundation, 2004; Hume, 2011; Watt *et al*., 2011) adult *P. marinus* have been recorded only very occasionally in the Endrick Water, and they have not been observed since the 1960s (Hunter *et al*., 1959; Maitland, 1966). Spawning is believed to be restricted to the efferent River Leven between the barrage (NS 393 894) and footbridge (NS 394 793) in the town of Balloch (Maitland *et al*., 1994; Gardiner *et al*., 1995). Despite extensive sampling of larval habitat around the Loch Lomond basin in recent years, *P. marinus* ammocoetes have until now only been recorded in the River Leven.

On March 21st 2012 a single *P. marinus* ammocoete was collected immediately downstream of Drymen Bridge on the Endrick Water (NS 473 874) in static traps designed to capture adult lampreys on their upstream spawning migration. This individual measured 151 mm in total length (L_T) and was 4.6 g wet weight. Positive identification as *Petromyzon* as opposed to *Lampetra* spp. was confirmed from the following meristic and morphometric characteristics (Fig. 7.12): trunk myomeres 71 (*P. marinus* range 67 - 74; *Lampetra* spp. range 58 - 64), oral hood fully pigmented (*Lampetra* spp. upper/lower lip unpigmented), caudal fin spade-like (*Lampetra* spp. typically rounded), robust head region (*Lampetra* spp.

distinct pre-nostril region) (Renaud, 2011). Sea lamprey larval duration is typically five years, although it can be as long as 19 years as growth rates vary enormously, so an accurate age estimate of just one individual is fraught with uncertainty. Based on typical values from other U.K. populations this individual is likely to be 3 - 5 years old, indicating that spawning took place in the Endrick Water at sometime between May/June 2007 - 2009 (Hardisty, 1969; Bird *et al*., 1994).

Throughout Scotland larval *P. marinus* are recorded in very low densities compared to *Lampetra* spp., even in rivers known to contain strong adult spawning populations (APEM, 2004; ERA, 2004; Watt *et al*., 2008). There remains the possibility that *P. marinus* spawns in the Endrick Water in small numbers, but; that adults are either not detected because trapping methodology excludes their larger body size, or *P. marinus* ammocoetes are not detected during routine surveys due to their inherent scarcity or are not identified from among collections of *Lampetra* spp. Currently, the Endrick Water is a stronghold for lampreys in Scotland, with both *L. fluviatilis* and *L. planeri* populations being of international conservation importance (Bond, 2003). If indeed this isolated record of larval *P. marinus* represents the first indication that the species now maintains a spawning population within the Endrick Water, there is an implication that the conservation strategy for this river should be modified to include sea lamprey as a qualifying feature of the SAC.

FIGURE

Fig.7.12 Ammocoete of *Petromyzon marinus* collected from the Endrick Water, Loch Lomond.

Appendix 7.3

Table 7.6 Mean differences of nine morphometric features of parasitic and non-parasitic populations examined morphologically. Means displaying significant differences (at the 0.05 level) between populations, as derived from a Tukey's post hoc test of Analysis of Variance, are indicated by *. See Fig. 7.2 for explanation of morphometric measures. Blue cells represent parasitic forms, red cells represent non-parasitic forms. Abbreviations: FL = freshwater-resident, Loch Lomond; AE = anadromous, Endrick Water; PB = praecox, River Bladnoch; FN = freshwater-resident, Lough Neagh; AF = anadromous, Forth Estuary; NE = non-parasitic, Endrick Water; NF = non-parasitic, River Falloch.

Variable	Population(a)	Population(b)	Mean Difference (a-b)	Variable	Population(a)	Population(b)	Mean Difference (a-b)
L_T	FL	AE	-104.8983*	L_{PB}	FL	AE	2.2598*
		PB	-26.6103			PB	.3169
		FN	-97.0408*			FN	.3813
		AF	-100.2853*			AF	.0727
		NE	73.3897*			NE	1.7344*
		NF	98.5147*			NF	1.6044*
	AE	FL	104.8983*		AE	FL	-2.2598*
		PB	78.2880*			PB	-1.9429*
		FN	7.8575			FN	-1.8786*
		AF	4.6130			AF	-2.1871*
		NE	178.2880*			NE	-.5254
		NF	203.4130*			NF	-.6554
	PB	FL	26.6103		PB	FL	-.3169
		AE	-78.2880*			AE	1.9429*
		FN	-70.4306*			FN	.0644
		AF	-73.6750*			AF	-.2442

		NE	100.0000*			NE	1.4175*
		NF	125.1250*			NF	1.2875*
	FN	FL	97.0408*		FN	FL	-.3813
		AE	-7.8575			AE	1.8786*
		PB	70.4306*			PB	-.0644
		AF	-3.2444			AF	-.3085
		NE	170.4306*			NE	1.3531*
		NF	195.5556*			NF	1.2231*
	AF	FL	100.2853*		AF	FL	-.0727
		AE	-4.6130			AE	2.1871*
		PB	73.6750*			PB	.2442
		FN	3.2444			FN	.3085
		NE	173.6750*			NE	1.6617*
		NF	198.8000*			NF	1.5317*
	NE	FL	-73.3897*		NE	FL	-1.7344*
		AE	-178.2880*			AE	.5254
		PB	-100.0000*			PB	-1.4175*
		FN	-170.4306*			FN	-1.3531*
		AF	-173.6750*			AF	-1.6617*
		NF	25.1250			NF	-.1300
	NF	FL	-98.5147*		NF	FL	-1.6044*
		AE	-203.4130*			AE	.6554
		PB	-125.1250*			PB	-1.2875*
		FN	-195.5556*			FN	-1.2231*
		AF	-198.8000*			AF	-1.5317*
		NE	-25.1250			NE	.1300
L_B	FL	AE	-.3733	L_{TR}	FL	AE	-1.0185

		PB	-1.9309*			PB	-1.1669
		FN	-.5114*			FN	1.9095
		AF	.9075*			AF	1.8473
		NE	.0741			NE	.2306
		NF	.0941			NF	.9956
	AE	FL	.3733		AE	FL	1.0185
		PB	-1.5576*			PB	-.1484
		FN	-.1382			FN	2.9280*
		AF	1.2807*			AF	2.8658*
		NE	.4474*			NE	1.2491
		NF	.4674			NF	2.0141
	PB	FL	1.9309*		PB	FL	1.1669
		AE	1.5576*			AE	.1484
		FN	1.4194*			FN	3.0764
		AF	2.8383*			AF	3.0142
		NE	2.0050*			NE	1.3975
		NF	2.0250*			NF	2.1625
	FN	FL	.5114*		FN	FL	-1.9095
		AE	.1382			AE	-2.9280*
		PB	-1.4194*			PB	-3.0764
		AF	1.4189*			AF	-.0622
		NE	.5856*			NE	-1.6789
		NF	.6056			NF	-.9139
	AF	FL	-.9075*		AF	FL	-1.8473
		AE	-1.2807*			AE	-2.8658*
		PB	-2.8383*			PB	-3.0142
		FN	-1.4189*			FN	.0622

		NE	-.8333*			NE	-1.6167
		NF	-.8133*			NF	-.8517
	NE	FL	-.0741		NE	FL	-.2306
		AE	-.4474*			AE	-1.2491
		PB	-2.0050*			PB	-1.3975
		FN	-.5856*			FN	1.6789
		AF	.8333*			AF	1.6167
		NF	.0200			NF	.7650
	NF	FL	-.0941		NF	FL	-.9956
		AE	-.4674			AE	-2.0141
		PB	-2.0250*			PB	-2.1625
		FN	-.6056			FN	.9139
		AF	.8133*			AF	.8517
		NE	-.0200			NE	-.7650
L_{TL}	FL	AE	-.6306	L_{ED}	FL	AE	.2763*
		PB	2.8287			PB	-.1199
		FN	3.8727*			FN	.0010
		AF	-.6222			AF	-.4057*
		NE	-1.8013*			NE	-.0549
		NF	-2.6838			NF	-.2699
	AE	FL	.6306		AE	FL	-.2763*
		PB	3.4592*			PB	-.3962*
		FN	4.5032*			FN	-.2754*
		AF	.0084			AF	-.6820*
		NE	-1.1708			NE	-.3312*
		NF	-2.0533			NF	-.5462*
	PB	FL	-2.8287		PB	FL	.1199

		AE	-3.4592*			AE	.3962*
		FN	1.0440			FN	.1208
		AF	-3.4508*			AF	-.2858
		NE	-4.6300*			NE	.0650
		NF	-5.5125*			NF	-.1500
	FN	FL	-3.8727*		FN	FL	-.0010
		AE	-4.5032*			AE	.2754*
		PB	-1.0440			PB	-.1208
		AF	-4.4948*			AF	-.4067*
		NE	-5.6740*			NE	-.0558
		NF	-6.5565*			NF	-.2708
	AF	FL	.6222		AF	FL	.4057*
		AE	-.0084			AE	.6820*
		PB	3.4508*			PB	.2858
		FN	4.4948*			FN	.4067*
		NE	-1.1792			NE	.3508*
		NF	-2.0617			NF	.1358
	NE	FL	1.8013*		NE	FL	.0549
		AE	1.1708			AE	.3312*
		PB	4.6300*			PB	-.0650
		FN	5.6740*			FN	.0558
		AF	1.1792			AF	-.3508*
		NF	-.8825			NF	-.2150
	NF	FL	2.6838		NF	FL	.2699
		AE	2.0533			AE	.5462*
		PB	5.5125*			PB	.1500
		FN	6.5565*			FN	.2708

		AF	2.0617			AF	-.1358
		NE	.8825			NE	.2150
L_D	FL	AE	1.7536*	L_U	FL	AE	.0289
		PB	.3721			PB	.0774
		FN	-.4289			FN	-.2115
		AF	1.0537*			AF	-.2232
		NE	2.3496*			NE	-.3531*
		NF	2.5471*			NF	-.5095*
	AE	FL	-1.7536*		AE	FL	-.0289
		PB	-1.3815*			PB	.0485
		FN	-2.1824*			FN	-.2404
		AF	-.6999*			AF	-.2521
		NE	.5960*			NE	-.3820*
		NF	.7935*			NF	-.5384*
	PB	FL	-.3721		PB	FL	-.0774
		AE	1.3815*			AE	-.0485
		FN	-.8009*			FN	-.2889
		AF	.6817			AF	-.3006
		NE	1.9775*			NE	-.4305*
		NF	2.1750*			NF	-.5869
	FN	FL	.4289		FN	FL	.2115
		AE	2.1824*			AE	.2404
		PB	.8009*			PB	.2889
		AF	1.4826*			AF	-.0117
		NE	2.7784*			NE	-.1415
		NF	2.9759*			NF	-.2980
	AF	FL	-1.0537*		AF	FL	.2232

		AE	.6999*			AE	.2521
		PB	-.6817			PB	.3006
		FN	-1.4826*			FN	.0117
		NE	1.2958*			NE	-.1299
		NF	1.4933*			NF	-.2863
	NE	FL	-2.3496*		NE	FL	.3531*
		AE	-.5960*			AE	.3820*
		PB	-1.9775*			PB	.4305*
		FN	-2.7784*			FN	.1415
		AF	-1.2958*			AF	.1299
		NF	.1975			NF	-.1565
	NF	FL	-2.5471*		NF	FL	.5095*
		AE	-.7935*			AE	.5384*
		PB	-2.1750*			PB	.5869
		FN	-2.9759*			FN	.2980
		AF	-1.4933*			AF	.2863
		NE	-.1975			NE	.1565
Myomere Count	FL	AE	-.400				
		PB	-.118				
		FN	.308				
		AF	2.382*				
		NE	1.282*				
		NF	1.757				
	AE	FL	.400				
		PB	.283				
		FN	.709				
		AF	2.783*				

		NE	1.683*
		NF	2.158*
	PB	FL	.118
		AE	-.283
		FN	.426
		AF	2.500*
		NE	1.400
		NF	1.875
	FN	FL	-.308
		AE	-.709
		PB	-.426
		AF	2.074*
		NE	.974
		NF	1.449
	AF	FL	-2.382*
		AE	-2.783*
		PB	-2.500*
		FN	-2.074*
		NE	-1.100*
		NF	-.625
	NE	FL	-1.282*
		AE	-1.683*
		PB	-1.400
		FN	-.974
		AF	1.100*
		NF	.475
	NF	FL	-1.757

	🟥	AE	-2.158*
		PB	-1.875
		FN	-1.449
		AF	.625
		NE	-.475

“According to Lanzing (1959), lamprey were used up to 1915 as bait. From 1660 to 1961 there was in Vlaardingen even a large holding tank (prikkenwater) in which lamprey were held for this and other purposes. Every ship’s crew included a “lamprey biter” who killed the animal by a bite to the head thus destroying the brain. The paralysed lamprey was then placed on a hook.”

Gunther Sterba (1963), *Die Neunaugen*

Chapter Eight

Implications for the conservation and management of *Lampetra fluviatilis* populations in the U.K.

The relevance of results obtained during the studies outlined in Chapters Two through Seven are discussed here in the context of how they impact the current conservation strategy of European river lamprey in the U.K.

8.1 *Is the European brook lamprey Lampetra planeri no longer a "real" species?*

The idea that non-parasitic lampreys are not considered to be discrete species from larger ancestral parasitic types is not a novel idea. The concept of pairs of morphologically similar, yet ecologically distinct, forms of lamprey within river systems, and referred to variously as ecotypes (Enequist, 1939), paired species (Zanandrea, 1959), or stem-satellite species (Vladykov & Kott, 1979) has been considered for many decades (reviewed in Hubbs & Potter, 1971; Salewski, 2003; Docker, 2009). Two schools of thought have arisen in consideration of the available evidence: one group believes that adult life history strategy (i.e., parasitic *vs*. non-parasitic) is not a species specific trait and is instead a case of phenotypic plasticity, whereby a single gene pool is capable of producing alternative morphologies in response to environmental pressures. The other group believes such differences in life history strategy precludes the ability for genes to be shared between both forms, as non-parasitic lampreys are often significantly smaller than parasitic forms, and therefore both life history strategies are reproductively isolated.

The former group is dominated by molecular ecologists and evolutionary theorists; the latter is dominated by morphological taxonomists. In the case of the paired species *Lampetra fluviatilis* and *L. planeri* in Europe, what the molecular evidence tells us is that where found sympatrically it is not possible to distinguish between either form, indicating contemporary or on-going gene flow (Schreiber & Engelhorn, 1998; Espanhol *et al*., 2007; Chapter Seven). Where *L. planeri* has been found to be genetically divergent, such as populations collected in the Iberian Peninsula (Mateus *et al*., 2012), *L. fluviatilis* has been extirpated and likely has not penetrated such river systems for hundreds to thousands of years and so non-parasitic population have been evolving in isolation. What the morphological data tells us is that *L. planeri* can only be distinguished from *L. fluviatilis* on the basis of overall adult size, which is itself a direct result of whether or not the individual has fed parasitically not fed following metamorphosis (Chapter Seven).

Therefore, if both parasitic and non-parasitic populations of lampreys are sharing the same gene pool, and are inseparable using morphological taxonomy, what criteria are being used to split these forms into distinct species? The findings presented in this Thesis have eroded the strength of arguments against the separation of *L. fluviatilis* and *L. planeri* based solely on body size, as within the relatively small geographical region under consideration two populations of parasitic lamprey have been described that are intermediate in size between this pair (Chapter Seven), which have the potential to mitigate gene flow in both directions (Chapter Four). Observations of sneak male mating tactics between both parasitic and non-parasitic forms have for the first time presented the possibility body size among sexually mature petromyzontids will pose no barrier to gene flow whatsoever (Chapter Five), particularly in light of the fact hybrid offspring do not appear to be selected against during development (Chapter Six).

Thus, it is not readily apparent what criterion can or could be used to distinguish *L. planeri* from *L. fluviatilis* throughout its European range. As a result of these findings, conservation and management decisions regarding lampreys in the U.K. should in future consider *L. planeri* to be a non-parasitic form of the European river lamprey *L. fluviatilis*. Both life history strategies could, however, continue to be conserved separately as Evolutionarily Significant Units (de Guia & Saitoh, 2007) considered under the single Latin binomial *L. fluviatilis*.

8.2 *How can we best protect lamprey diversity in the U.K.?*

Currently anadromous forms of *L. fluviatilis* are included in Annex II and V of the EU Habitats Directive, Appendix III of the Bern Convention, and the U.K. Biodiversity Priority List. The freshwater-resident form found in Loch Lomond is additionally protected under the Wildlife & Countryside Act 1981 (as amended) by its inclusion as a qualifying feature of the local Site of Special Scientific Interest (SSSI) and Special Area of Conservation (SAC). Non-parasitic forms, however, have attracted relatively little scientific attention or conservation interest, presumably as a result of their seeming ubiquity in the U.K. (ERA, 2005). Given their commonality, *L. planeri* was not included in the U.K. Biodiversity Action Plan, despite the inclusion of *L. fluviatilis*. Conservation legislature guided by thinking at the species level potentially fails to adequately protect the full extent of diversity in lower levels of organisation. Certainly there is provision for protecting infra-specific diversity in current legislation as the guidelines for designating Sites of Special Scientific Interest, for example, state that ecotypic or genetically distinctive fish populations are considered during the selection of suitable sites.

Lampetra fluviatilis exhibits significant phenotypic and ecological sub-structuring that is of conservation importance throughout the U.K. The repeated evolution of non-parasitic populations from the anadromous parasitic type, either in response to geological (e.g., waterfalls, river capture) or anthropogenic factors (e.g., hydroelectric dams, pollution) will have had a marked effect on the genetic relationships among these populations. To ensure *L. fluviatilis* is represented fully by conservation legislation, genetically as well as ecologically, isolated populations of non-parasitic lampreys (e.g., on islands or above impassable barriers) should be surveyed. Additionally, populations such as the recently discovered praecox form in the River Bladnoch, Galloway, should be critically evaluated to ensure all habitats used by these populations are adequately protected, and that such biodiversity is recognised in management decisions.

Attempts could be made at incorporating adult lamprey surveys into routine monitoring of river systems using static traps (Chapter Three), especially where similar survey methodologies are already in use for species such as eels (*Anguilla anguilla*) and Atlantic salmon (*Salmo salar*). Ammocoete surveys remain a critical source of information regarding recruitment, but they provide no information on the diversity of adult life history strategies produced within each river system. Environmental variables common to rivers producing large numbers of anadromous forms may be useful in identifying other such systems, ensuring there is an accurate geographic representation of sites under protection.

It is unlikely that freshwater-resident parasitic forms are currently supported in other lakes throughout the U.K. given the relatively conspicuous effects of their foraging strategy (Chapter Two), and their appearance in just two of the largest lakes available (Lough Lomond, Scotland and Lough Neagh, Northern Ireland). As a result of the pernicious introduction of non-native fish species to these lakes, the continued presence of this rare and scientifically valuable foraging strategy is under threat. Consideration should be given to the possibility of translocating representatives of these populations to other lakes containing suitable host species (e.g., Etheridge *et al*., 2010*a*), with access to afferent rivers containing spawning grounds, and suitable larval habitat to ensure the long-term survival of these life history variants.

8.3 *The Endrick Water SAC as a case study: knowledge gaps and recommendations*

Within the Loch Lomond basin *L. fluviatilis* expresses significant infra-specific diversity. This is represented by a large anadromous parasitic form, a small stream-resident non-parasitic form, and an intermediate freshwater-resident parasitic form (Chapter Three). The Endrick Water SAC reflects the importance of this site to the maintenance of that diversity by listing all three forms as qualifying features. Each of these three life history strategies, however, requires discrete conservation measures at particular points in their life cycle, reflecting differential habitat use. The anadromous form must have unrestricted access to the Clyde Estuary *via* the River Leven in order to forage in marine environments, and this foraging strategy demands immediate attention as the trophic interactions of this population and local fish fauna have not yet been considered. The freshwater-resident form requires suitable hosts within the lake, and an understanding of the importance of non-native fish to this population is a high priority and should be investigated (Chapter Two).

Although spawning sites for anadromous and freshwater-resident parasitic forms are believed to be restricted to between the village of Drymen and the Pots of Gartness in the Endrick Water mainstem, and as far as the village of Dumgoyne in the Blane Water, this remains to be confirmed (Chapter Three). The identification of sites used by these forms during spawning is crucial in protecting both populations, and will provide greater insight into their spawning interactions on communal spawning grounds (Chapter Four). The use of acoustic or visual tags is advised in tracking migrating lampreys trapped at Drymen and released above the trapping site. The adult population size of all three forms remains unknown, although the non-parasitic form numerically dominates collections of migrating adults. A repeated attempt at a mark-recapture study at this site (Chapter Three) may prove a useful management strategy. Alternatively, effective population size may be estimated from analysis of genetic variation derived from ammocoete collections.

The availability of spawning habitat appears to be the limiting factor within the Endrick Water, as extensive areas of suitable larval habitat can be found along most of the river's length as well as the littoral zone of the lake itself, adjacent to the point of discharge. The addition of gravel to sections of the river upstream of Drymen would be a novel method of improving numbers of spawning adults, and additionally could provide an opportunity to observe lampreys spawning at this site. Ammocoetes are known to attract sexually maturing adults into rivers by releasing pheromones derived from bile acids, and there appears to be a causal relationship between the number of ammocoetes and the number of migrating adults within that river. Therefore, with the provision of greater areas available for spawning adults, increased recruitment to the larval population is expected to loop back into attracting increasing numbers of adults the following year. As adult lampreys do not home to natal rivers, any increase in the concentration of larval pheromones released from the Endrick Water could be expected to attract lampreys from a wider area, potentially resulting in increased recruitment to other afferent rivers of the lake basin.

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