Eco-evolutionary feedback in fish–zooplankton communities on the Scottish island of North Uist

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Dedication

To my wife

To my children

To the memory of my mother

Abstract

'Eco-evolutionary feedbacks' occur when evolution of organismal traits causes environmental change that drives further evolution. Predator and prey interactions provide good examples of eco-evolutionary feedbacks. Here I examine the potential for eco-evolutionary feedbacks between three-spined sticklebacks (*Gasterosteus aculeatus*, hereafter 'stickleback') and their zooplankton prey in lochs (lakes) on the Scottish island of North Uist. Many lochs on the island were colonised by sticklebacks after the last glaciation, approximately 10,000-15,000 years ago. Previous work has shown that sticklebacks in different lochs have diverged greatly from each other in response to local environments. On the other hand, apart from several very old studies on the occurrence of some zooplankton species, there have been no previous in depth studies on the population dynamics of zooplankton on North Uist.

I investigated first the diversity and abundance of zooplankton groups and the most common species across all North Uist lochs. Thirty-nine species were classified from three main groups (Rotifera, Cladocera and Copepoda). Species abundance in the presence of fish was relatively more even, while the abundance of zooplankton groups was not generally related to fish presence except for Cladocera in a subset of locations. The effects of predation on the life-history of zooplankton were also examined by comparing reproductive traits of the dominant cladoceran species (*Bosmina* and *Daphnia*) in lochs with and without fish.

Cladocerans in lochs with fish have more rapid reproductive cycles and higher fecundity parameters, probably in response to the increased threat of predation. These effects remain evident in *Bosmina* after they have been

raised through three generations in the laboratory, supporting the hypothesis that they have a genetic basis and are not a plastic response to predator presence. Life-history variables of cladocerans were also related to abiotic and biotic variation among lochs, including depth, pH, chlorophyll levels and the concentration of alkaline metals.

I also demonstrate that stickleback have diversified greatly among lochs in functional trophic traits that determine the efficiency of feeding on different types of prey, which could affect total primary production and the structure of prey communities. Ancestral stickleback populations have adapted according to the type of habitat colonised. Fish feed on benthic prey in shallow lochs, which requires greater effort for successful foraging than that required by fish that feed on planktonic prey. I found that variation in stickleback trophic traits was related to both abiotic and biotic variation among lochs, including loch depth and the mean contributions of planktonic and benthic prey to diet.

The results presented in this thesis suggest the possibility of eco-evolutionary feedbacks in these simple ecosystems on North Uist based on the significant responses in fish and zooplankton communities. The thesis also provides a basis for further studies on fish-zooplankton interactions on North Uist and contributes to the wider body of knowledge concerning the relevance of natural variation in shaping the foraging mechanisms of animals.

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Contributions

A number of people made a contribution to some chapters in my own presented work in this thesis:

Chapter 2: Andrew MacColl helped to select populations to sample. D'Agostino and Shaun Robertson gave a valuable support in collecting zooplankton samples during the fieldwork on North Uist.

Chapters 4: Andrew MacColl helped to design the laboratory life-table experiments and suggest the populations of zooplankton samples.

Chapter 5: Michael Clifford and Modhara Sunil helped to use Matlab R2016a to calculate the force and kinematic transmission of four-bar system of fish jaw. Matthew McGee gave also advice on measuring the fish jaw opening inlever.

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Chapter 1. General Introduction

Species within ecological communities are involved in many different kinds of interactions (Hendry & Kinnison, 1999; Yoshida *et al.*, 2003). Evolution, adaptation and speciation can be caused by these interactions. In turn, this evolution can lead to changes in the structure of the community (Haloin & Strauss, 2008). The study of these interactions is a major component of the science of ecology and the basic understanding of the optimal integration of ecology and evolution (Pelletier *et al.*, 2009), resulting in the persistence of biodiversity in changing environments (Hanski, 2012).

If evolutionary changes can happen at the same rate as ecological processes, then evolution can affect ecology and the opposite is also true (Laland *et al.*, 1999; Post & Palkovacs, 2009). This bidirectional transformation interlinks ecology and evolution in modern-day life and results in so-called eco-evolutionary dynamics. Only one of these directions of causality has been well researched namely that ecological variation can cause characteristic evolution over years to decades (contemporary evolution) (Hendry & Kinnison, 1999). While it is widely recognised that ecological variation can bring about evolution, ecologists have traditionally considered that evolution takes place over time scales too great to affect the processes they study (Lewontin, 2001; Lively, 2012). More recently, researchers have begun to realise that this may not be true, and that the evolution brought about by ecological interactions can in turn cause feedbacks on ecological processes over short time scales (Pelletier *et al.*, 2009; Palkovacs & Hendry, 2010).

It is generally understood that the structure and dynamics of prey communities can be changed and affected by predators and this in turn

would lead to evolution in the predator populations (Palkovacs & Post, 2008). The well-established essential differences in communities can be observed at different levels of population dynamics , feeding behaviour and morphology (Post & Palkovacs, 2009; Kahilainen *et al.*, 2011; Walsh & Post, 2011; Korosi *et al.*, 2013; Turcotte *et al.*, 2013; Palkovacs *et al.*, 2014).

Furthermore, variations in ecological and evolutionary diversity appear to be a result of different strengths of eco-evolutionary feedbacks inside predator-prey communities (Palkovacs & Post, 2009). It is generally considered that the relationships between predators and prey will provide good examples of eco-evolutionary feedbacks in an ecosystem (Palkovacs & Hendry, 2010).

The simple communities in northern temperate lakes, formed after the last glacial period 10,000-15,000 years ago provide especially useful locations for studies of such effects (Waples *et al.*, 2008; Lees, 2016). Recurrent waves of effective physical and environmental habitat alterations have encouraged evolutionary modifications within many organisms such as fish and zooplankton (McPhail & Lindsey, 1986; Waples *et al.*, 2008; Korosi *et al.*, 2013; MacColl *et al.*, 2013)

In this project I studied the eco-evolutionary relationships between a fish species (the three-spined stickleback, *Gasterosteus aculeatus*) and zooplankton communities in 33 lochs on the Scottish island of North Uist. Sticklebacks exhibit unique evolutionary phenotypic divergence since the Last Glacial Maximum, including rapid contemporary evolution of some traits (Klepaker, 1993; Yoshida *et al.*, 2003; Bell *et al.*, 2004; Kristjánsson, 2005).

On the other hand, zooplankton are an important food source for fish and are the main energy linkage in the food web of freshwater aquatic ecosystems.

1.1 Eco-evolutionary feedback dynamics

Eco-evolutionary feedbacks are a two way interaction that takes place between ecology and evolution; thus any attempts to merely integrate ecology into evolution or the contrary would not describe the whole dynamic process (Lewontin, 2001; Odling-Smee *et al.*, 2013). Under eco-evolutionary feedbacks, organisms bring about a modification in their environment and these environmental modifications, in turn, cause further evolution in organisms (Palkovacs & Post, 2009).

It is clearly understood now that organisms can affect their environment by predation (Holzman *et al.*, 2008; Post *et al.*, 2008; Palkovacs & Post, 2009; Walsh & Post, 2012), environmental alterations, nutrient secretion and habitat modification (Smith & Knapp, 2003; Bohlen *et al.*, 2004; Post *et al.*, 2008). In addition, organisms evolve gradually in response to the changes in the ecology at time-scales consistent with ecological variation (Post & Palkovacs, 2009). Two influential theories are applicable to the concept of eco-evolutionary feedbacks. They come from two different perspectives. First, the theory of adaptive radiation grounded in evolutionary biology, proposes that niche availability is defined by the biotic and abiotic environment and that until existing niches are occupied, diversification continues to occur in lineages (Schluter, 2001; Grant & Grant, 2006; Calsbeek *et al.*, 2007). In contrast, the theory of niche construction is grounded in the domain of ecology. It argues that organisms by their

activities, bring changes to the biotic and abiotic milieu that shape their own niches.

Eco-evolutionary feedbacks occur at the intersection of the processes identified by these two theories. Informed by this combined conceptual framework, it is appropriate to suggest that the niche-modifying abilities of organisms cannot only shape adaptive diversification, but can also be shaped by it (Odling-Smee *et al.*, 2013).

1.2 Implications of fish-plankton interaction in standing water systems

According to the trophic cascade hypothesis, it is predictable that nutrient and trophic variation in food webs can be attributed to physical factors and interactions between the predators and prey within the ecosystem (Post *et al.*, 2008). Accordingly, predation by fish determines the presence of herbivorous zooplankton, which in turn leads to changes in the biomass of phytoplankton (Sarvala *et al.*, 1998). Strong predation by fish may cause cascading interactions which alter plankton communities (Carpenter *et al.*, 1987; Leavitt *et al.*, 1989; Pace *et al.*, 1999). Algae may increase when predation on zooplankton reduces grazing pressure (Strock *et al.*, 2013).

McQueen *et al.* (1989) consider how the influence of top-down consumer controlled versus bottom-up (producer controlled) processes depends mainly on nutrient availability, and this bottom-up regulation is strongest at the lowermost level of the food web and decreases with every upward step as energy moves up the food web (McQueen *et al.*, 1989).
On the other hand, at the top of the food web, top-down (predation) interactions are strong, but these decrease with every downward step in the food web (McQueen *et al.*, 1989; Hessen & Kaartvedt, 2014). However, it

has been suggested that the influence of planktivorous fish on lower trophic levels in freshwater lakes can be seen in different patterns of species composition, size frequency, morphology and life histories of zooplankton, together with the development of vertical migration (Brooks & Dodson, 1965; Walls *et al.*, 1990; Post *et al.*, 2008; Palkovacs & Post, 2009). Moreover, these interactions in a predator-prey system, may result in patterns of ecological and evolutionary diversity, such as adaptive divergence, divergence in predator foraging traits and variation in ecological dynamics of prey communities (Palkovacs & Post, 2008).

There may also be differences in average body size and phytoplankton biomass due to the structuring of populations of herbivorous zooplankters and fish (Walls *et al.*, 1990; Mazumder, 1994). It has also been found that top-down cascading has similar effects on zooplankton body size in both oligotrophic and eutrophic lake systems, while cascading effects on phytoplankton were only observed in oligotrophic lakes (Strock *et al.*, 2013). Furthermore, the essential differences in fish-plankton interaction can be evaluated in community structure and body size and feeding morphology (Palkovacs & Post, 2008).

1.3 The role of zooplankton in eco-evolutionary feedback

Interactions between environmental change and natural selection from the core of ecology and evolutionary biology. Eco-evolutionary feedbacks require that organisms alter their environment to construct a niche, and that those alterations in the environment in turn influence the ensuing evolution of the organism (Fussmann *et al.*, 2007). The study of zooplankton communities is important in examining eco-evolutionary feedbacks. Zooplankton communities have been shown to influence their

environment through habitat modification, nutrient excretion, predation, and their evolution in response to alterations in their environment (Smith & Knapp, 2003; Bohlen *et al.*, 2004; Post *et al.*, 2008). Therefore, their studies are important in examining eco-evolutionary feedbacks.

One of the ways in which zooplankton can affect the eco-evolutionary process is through their abundance which has been shown to affect trophic cascading. This may appear when environmental factors influence the number of organisms in the ecosystem (Chang *et al.*, 2014). For instance, in areas where there are fewer predators of zooplankton, the number of zooplankton greatly increases (Barnes, 2014). The high density of the zooplankton means that there is an increased demand for food, which consists mainly of other organisms in the environment. As a result, zooplankton feeding on these organisms may lead to decrease in their numbers. This creates a top-down cascade which is also shown to increase considerably with the collapsing of the predator in ecosystems (Casini *et al.*, 2009).

1.4 Evolution of foraging behaviour and strategy in predator-prey interactions

The optimal foraging hypothesis predicts that predators tend to target the prey that provide a higher level of energy as compared to the energy expenses of catching, ingesting and digesting the prey (Charnov & Orians, 1973). Prey determination is in this way dictated by the relative profitability of specific sorts and sizes of prey, with greatest benefit presented by prey sizes and types to give a maximum energy reward per unit of time (Nunn *et al.*, 2012). It has been well established that sticklebacks vary in prey

selection depending on different criteria like the colour, shape, size and the movement of the prey (Ibrahim & Huntingford, 1989).

There is an impact on the predator when there is a change of prey availability driven by predation. In some circumstances when zooplankton become dominated by smaller organisms, or by those organisms that are not easily preyed upon, then the fish have no other option but to feed sub-optimally. This may affect fish growth, productivity and continued existence. On the other hand, when there is a low concentration of fish predation on the zooplankton (Perrow & Irvine, 1992), fish have the ability to feed on large prey and to get a high rate of return of energy (Dill, 1983; Fossette *et al.*, 2011). In general zooplanktivorous predators like fish select large adults and gravid females carrying eggs while invertebrates prefer to prey on juveniles, small organisms and non-vigorous females (Zaret, 1980; Carpenter *et al.*, 1985).

Predators can control the structure of the community and some ecosystem processes through modification of complex foraging traits: gill raker spacing, gape width and prey size-selectivity (Post *et al.*, 2008; Harmon *et al.*, 2009; Palkovacs & Post, 2009; Palkovacs *et al.*, 2014). On the opposite side, the costs of predation pressure result in different effects on the population dynamics of the prey community. For example, early maturation and increased fecundity of zooplankton individuals may occur (Walsh & Post, 2011). The consequences of fish predation may also affect the proportions of adults and the maximum size of the body, while Manca *et al.* (2008) noticed a reduction in the number of juveniles under the impact of invertebrate predation may also change.

In addition, vertical and horizontal migration is considered as an adaptive behaviour of zooplankton to escape predation (Dodson, 1988; Burks *et al.*, 2002; Arcifa *et al.*, 2016). Horizontal migration is mainly important for planktonic cladocerans (Lauridsen *et al.*, 1996). Cladoceran species may shelter in macrophyte bed edge, which is suitable place to avoid predators (Arcifa *et al.*, 2016). However, macrophyte is not common in most of lochs and during most of the year on North Uist and therefore it may not play important roles in determining the distribution of zooplankton (MacColl, A. D., personal communication).

1.5 Evolutionary and plastic trait changes in response to environmental change

It has been largely assumed that population divergence and speciation are based on differences related to genetic factors. However, it is likely that some of the differences among populations are related to phenotypic plasticity (Price *et al.*, 2003).

It is not yet clear to what extent organisms can evolve to deal with environmental changes instead of relying on plastic changes to mitigate the loss of fitness (Gabriel *et al.*, 2005). Pfennig *et al.* (2010) showed that plastic response is more widespread in the environment. However, plastic mechanisms may be considered to be very costly in terms of energy consumption in the long-term (DeWitt *et al.*, 1998; Pigliucci, 2005; Seebacher *et al.*, 2005). Furthermore, it may not provide the means for the continuity of animal populations necessary to keep up with large environmental changes that may require evolutionary responses (Mäkinen *et al.*, 2015). Cladoceran populations are considered to be a good model for the examination of evolutionary and plastic response under environmental

variability such as that resulting from selective predation (Stibor, 1992). Different phenotypic and life-history responses were assumed to be in accordance with one of the two following hypotheses: they were either the result of a succession of different genotypes or of a phenotypic plasticity (Lampert & Wolf, 1986). Recent evidence has shown that combining both plasticity and evolution is more effective in reaching optimisation under rapid environmental variation (Phillimore *et al.*, 2010). Plasticity may also rapidly evolve, so plasticity and evolutionary changes should not be considered as independent processes (West-Eberhard, 2003; Lande, 2009; Crispo *et al.*, 2010). Therefore, understanding how populations deal with rapid environmental requires a quantification of plasticity and evolution to trait changes (Gienapp *et al.*, 2008; Merilä & Hendry, 2014).

1.6 Evolutionary diversification of three-spined stickleback

The three-spined stickleback (*Gasterosteus aculeatus*) is a small fish that exhibits unique phenotypic variation and offers scientists widespread opportunities for surveillance in the field as well as manipulation in the laboratory (Bell & Foster, 1994).

Three-spined sticklebacks are originally marine fish that have invaded freshwaters in many parts of the Northern Hemisphere. Differences in biotic and abiotic environments between freshwaters and the ocean, including in predator–prey interactions, in physical and chemical environmental factors and in the competitor composition, coupled with opportunities for isolation in freshwater bodies have meant that the invasion of freshwater often results in phenotypic divergence (Walker, 1997; Schluter, 2000; Walker & Bell, 2000). In freshwater three-spined sticklebacks can be found in various habitats, including permanent flowing waters of different sizes and

freshwater lakes, where the fish can sometimes be categorized into benthic and limnetic ecotypes. Benthic habitats often comprise moderately eutrophic lakes while limnetic ecotypes are usually found in oligotrophic lakes (Östlund-Nilsson *et al.*, 2006; Shaw *et al.*, 2007).

Three-spined sticklebacks are generalist feeders whose diet includes limnetic and littoral invertebrates and zooplankton. Benthic ecotypes tend to feed on invertebrates that dwell on the bottom or on the vegetation of the littoral zone, including crustaceans as well as larval insects. In contrast, limnetic species in lentic systems feed on zooplankton (Walker, 1997; Cresko *et al.*, 2007).

1.7 Eco-evolutionary evidence in ecosystems

Eco-evolutionary feedbacks are defined as the recurring interactions that takes place between ecology and evolution (Turcotte *et al.*, 2013). This cyclical interaction brings about changes in the traits of different organisms which in turn, also cause alterations in these ecological interactions. This process is a continual occurrence (Koch *et al.*, 2014). Variation within a species or population can change the species' role in the environment and hence change the strength of eco-evolutionary interactions. On the other hand, community composition can also affect the role played by individual species (Turcotte *et al.*, 2013). Species that exist in species-rich communities are likely to have less effect on their environments, but may in turn have strong effects in communities with fewer species. For instance, when islands with fewer species are occupied by mainland species, it can result in strong evolutionary responses (Sax & Gaines, 2008).

It has been well demonstrated that population dynamics can modify the direction and strength of selection during a few generations and maintain

the diversity of the species (Yoshida *et al.*, 2003). Furthermore, in spite of many studies on the rapid, adaptive evolution in predator-prey populations, understanding the potential of predator-prey dynamics or the full consequences of eco-evolutionary feedbacks still needs more work (Koch *et al.*, 2014).

On the basis of examples of contemporary evolution in both natural and human-disturbed habitats, many reviews have questioned the belief that evolution is very slow to have an effect on contemporary ecological interactions (Reznick & Ghalambor, 2001). An increasing number of empirical studies that assess the reasons for changes in ecological dynamics are inspired by these examples of contemporary evolution (Bone & Farres, 2001; Terhorst *et al.*, 2010). There are many examples that have produced a variety of evidence for feedbacks in natural systems. I showed below some examples that may reveal main aspects in eco-evolutionary feedbacks.

1.7.1 Evolution of gill-raker spacing in alewife

Palkovacs *et al.* (2014) investigated evidence for the evolution of a trophic trait following an ecological experiment. They looked at the morphology of gill-raker: segmentally reiterated sets of dermal bones which serve as sieves to trap tiny prey (Langeland & Nøst, 1995; Friedland *et al.*, 2006) of anadromous alewife (*Alosa pseudoharengus*) fish that had been isolated from the ocean in an inland lake (Crystal Lake) in the USA in 1955 (Brooks & Dodson, 1965). They compared old and recent samples from the isolated population with recent samples of anadromous fish and fish isolated in freshwater for much longer periods of time. The old samples from Crystal Lake had been preserved in a museum soon after the introduction of the

fish to the lake. The gill-raker spacing of the fish at the time of the introduction was similar to that of samples of anadromous populations of alewife collected near to the lake. Recent samples from Crystal Lake provided evidence that a rapid decrease in gill-raker spacing had occurred together with a dramatic decline in zooplankton size relative to the observations made by Brooks & Dodson (1965) before (in 1942) and after (in 1964) the introduction.

Palkovacs *et al.* (2014) suggested that these two changes had been caused by the beginning of alewife predation. Their study demonstrates that such predator trophic impacts may mould predator trophic morphology through a feedback process.

1.7.2 Landlocked and anadromous alewife

Among alewives (*Alosa pseudoharengus*) systems in eastern North America, there is strong potential for differences in feedbacks between ecology and evolution. There are two forms of alewives: anadromous fish that migrate between the ocean and freshwater and landlocked fish that spend their whole life in freshwater lakes. These changes in the movement from one place to another may result in alteration in the habit of alewife making the opportunity for eco-evolutionary feedbacks very strong (Palkovacs & Post, 2008; Post & Palkovacs, 2009; Jones *et al.*, 2013). Presence of anadromous alewives in spring has an ecological effect (removes large zooplankton), but is insufficient to have an evolutionary effect (Palkovacs & Post, 2008), while, the presence of landlocked alewives has an ecological and an evolutionary effect, since their continuous presence results in intense predation that removes large zooplankton and has caused evolution of smaller body size of zooplankton. This in turn has resulted in closer spacing

of gill-rakers in Crystal Lake, suggesting that both prey and predator have evolved in response to each other in contemporary time scales (Palkovacs & Post, 2008). Therefore, in lakes with landlocked alewives a very strong eco-evolutionary feedback can be seen, whereas a weak eco-evolutionary feedback can be seen in the lakes with anadromous alewives. The main point of this research is that variation in strength of complex trophic interactions can be a significant way of shaping intraspecific phenotypic variation in a predator and allow us to understand how eco-evolutionary feedbacks work. (Palkovacs & Post, 2008; Palkovacs & Post, 2009).

1.7.3 Trinidadian guppies and nutrient cycling

It is well established that the life history of guppies (*Poecilia reticulata*) varies greatly depending on the level of predation in their locality (El-Sabaawi *et al.*, 2012). To study the effect of guppy life-history evolution on ecosystem processes, (El-Sabaawi *et al.*, 2015) performed a mesocosm experiment.

Guppies from high-predation populations such as the pike cichlid (*Crenicichla spp*) and the wolfish (*Hoplias malabaricus*), excreted nitrogen and phosphorus at twice the rate of guppies from low-predation populations (Bassar *et al.*, 2010; El-Sabaawi *et al.*, 2015) in relation to the levels of nutrient in source water from the adjacent stream: high-predation guppies led to an increase of 11% in nitrogen and a decline of 83% in phosphorus, while guppies from populations with low-predation led to an increase of 6% nitrogen and an increase of 64% phosphorus.

A noticeable rise of algal biomass in the treatment with high-predation guppies was caused by this change in excretion. Various changes in evolution in other guppy qualities may have further influenced algal

biomass, including dietary preferences and trophic morphology. This change of algal biomass subsequently influenced guppy evolution. For example, sensitivity to the quantity of carotenoids in algae available in the environment is found in the colour patterns of male guppies under both natural and sexual selection (Grether, 2000; Grether *et al.*, 2005). Thus, changes in evolution because of biotic factors such as predation may have an unexpected influence on ecological impacts at various levels of organisation leading to further evolutionary feedback.

1.7.4 Evolution of foraging traits in seed predators

Darwin's finches (*Geospiza fortis*) in the Galápagos Islands have been considered the most famous example for evaluating how fluctuations in ecology have affected evolution and vice versa (Palkovacs & Post, 2008). With changes in seed availability rapid evolution in beak size and shape could be seen as a response (Grant & Grant, 1995; Grant & Grant, 2006). Variation in beaks increases the chances of birds to feed differently when food is scarce during the non-breeding season (Grant & Grant, 1995; Grant, 1999; Grant & Grant, 2006). In this system, eco-evolutionary interactions have been concluded to be important for both ecological and evolutionary dynamics. Beak size reacts evolutionarily to variations in seed availability which in turn responds ecologically to the intense predation of finch (Grant & Grant, 2006). These feedbacks are enabled by rapid evolution in finches' beak size (Hairston *et al.*, 2005).

1.8 Evolution of mechanical properties in feeding mechanisms of fish

A fundamental objective of functional morphology is to understand the transmission of velocity and force in musculoskeletal systems. In general,

velocity and force properties of musculoskeletal systems in vertebrates are calculated based on geometric model data of muscles and bones (Westneat, 2004). Most recent modelling studies have focused mainly on the development of vertebrate jaw systems (Erickson *et al.*, 1996; Westneat, 2003).

Fish skulls (Fig. 1.1) have been of great interest as good models for the investigation of the functional morphology of feeding mechanisms. Twenty or more independent mobile skeletal features have been identified in the skulls and pharyngeal apparatus of fish as being responsible for cranial motion in many teleosts (Westneat, 2004). Linkages and levers are fundamental components in models used for the analysis of biomechanical models in fish skulls (Fig 1.1). Simple levers were used to understand factors that control opening and closing of the lower jaw (Westneat, 1994; Wainwright & Richard, 1995). On the other hand, lower jaw depression in teleosts can be modelled with the more dynamic and complex 'opercular four-bar' system. In this system, muscular force and motion are transmitted through simplest movable closed chain that regulates jaw opening and closing in fish (Anker, 1974; Westneat, 2003).

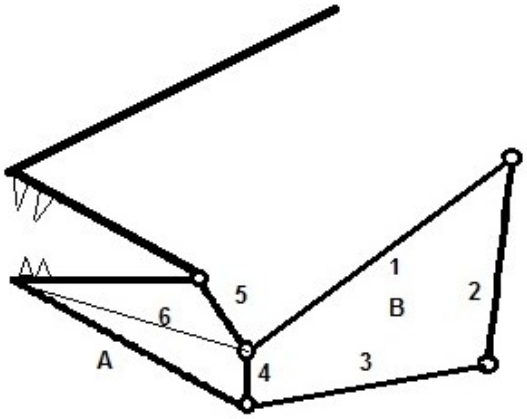


Figure 1.1. Linkages and levers components of biomechanical models in fish skulls. Simple levers A (4-6) in controlling opening and closing the lower jaw. Opercular four-bar system B (1-4) in which force and kinematic transmit and regulate jaw opening and closing through simple mobile closed chain according to Anker (1974) and Westneat (1994).

1.9 Trophic trait evolution in three-spined stickleback

The eco-morphological hypothesis suggests that variation in foraging traits of fish strongly affects diet and differences in feeding ability and prey selection (Wainwright & Richard, 1995). In fish, the composition of a population's diet and the size of the prey is often correlated with gill-raker patterns. Feeding specialization is predicted by the number of gill rakers. A greater number of gill rakers is correlated with feeding on zooplankton (Robinson, 2000; Rösch *et al.*, 2013). On the other hand, a lesser number of gill rakers is associated mainly with feeding on benthos (Rösch *et al.*, 2013). Freshwater sticklebacks generally feed on larger prey items, whereas marine sticklebacks feed on small zooplankton that are suspended in the water column (Kislalioglu & Gibson, 1977; Gross & Anderson, 1984). The functional component of trophic traits number come under strong natural selection in freshwater across the Northern Hemisphere (Glazer *et al.*, 2014). Sticklebacks of limnetic and benthic ecotypes that co-occur in a

series of British Columbian lakes (Fig. 1.2) are a good example of the speciation of post-glacial freshwater fishes associated with trophic divergence (McPhail, 1993; Schluter, 1994; Baker *et al.*, 2005). British Columbian lakes formed within the last ten thousand years by retreating glaciers (Schluter & McPhail, 1992; Schluter, 1994; Rundle *et al.*, 2003). These two examples of sticklebacks' species demonstrate the ecological effects of evolutionary diversification during adaptive radiation. (Harmon *et al.*, 2009).

On the bottom of the lakes, the large benthic ecotype that has a big mouth, resides and feeds on large invertebrates while the limnetic ecotype has a small mouth with longer and more numerous gill-rakers and usually feeds on small prey including pelagic zooplankton (Robinson, 2000; Baker *et al.*, 2005). In addition, a significant variation in trophic apparatus among stickleback species in some aquatic ecosystems was associated with higher prey capture success (Lavin & McPhail, 1986). Limnetic sticklebacks, which feed on fast-moving zooplankton like copepods, have a functional system adapted to utilise greater speed in capturing prey items (Holzman *et al.*, 2012; McGee *et al.*, 2013). On the other hand, stickleback species that feed on benthic invertebrate need a strong but a slower effort to handle large benthic prey (McGee *et al.*, 2013).

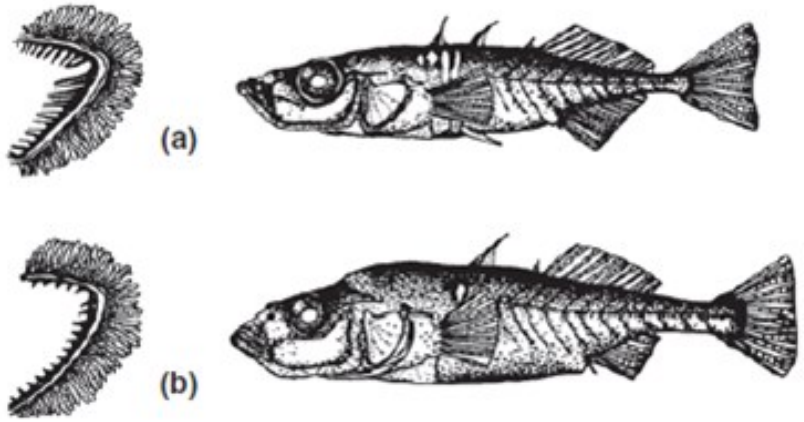


Figure 1.2. Morphological differences in gill-rakers of a stickleback species pair (limnetic and benthic forms) from Cranby Lake in British Columbia. The limnetic species (a) are specialised for feeding on plankton and have a more slender body and a smaller mouth with many long gill-rakers, while the benthic species (b) feed on macroinvertebrates and have deep body depth and a larger mouth with few short gill-rakers for filtering ingested items (Smith & Smith, 2012)

1.10 The scientific impact

Eco-evolutionary feedback studies are significant to evolutionary biologists as they can recognise the fact that the environment is shaped by organisms and that this in turn influences evolution. Ecologists on the other hand should also be interested in the extent to which phenotypic evolutionary changes can affect ecological processes and patterns. Evolution and ecology are therefore unified through bidirectional interactions, hence the significance of eco-evolutionary feedback studies. These eco-evolutionary feedbacks produce phenotypic variations which are essential in understanding the initial stages of adaptive radiation and ecological speciation (Habets *et al.*, 2006). This investigation will mainly target clades that display diversification in habitat, foraging and life-history traits.

1.11 Aims and objectives

The aim of this study is to understand the interactions between predator and prey communities in simple temperate lake communities and the potential for eco-evolutionary dynamics (effects of predator on prey and of prey on predator, and feedback between the two). Studies have already revealed some aspects of the eco-evolutionary interactions between fish and zooplankton. However, interactions between the three-spined stickleback (*Gasterosteus aculeatus*) and zooplankton on North Uist have not been examined in this manner.

Therefore, I collected evidence relevant to these ideas and tested hypotheses on how cyclical interaction between ecology and evolution brings about changes in the traits of different organisms which in turn, cause alterations in these ecological interactions. The main objectives of this study were as follows:

1- To describe and evaluate taxonomic assemblages of prey communities (zooplankton). The reason for that are in order to examine eco-evolutionary feedbacks in zooplankton communities, it is essential to know what those communities are. In addition, testing prey diversity across different gradients of fish presence gives a good account of differences and distributions of individuals within and between populations.

2- To examine specific traits of zooplankton, such as age at maturity and fecundity rate, and to examine how these relate to fish predation in order to reveal whether there are any evolutionary responses between the two components under eco-evolutionary dynamics.

3- To investigate whether the expected effects of predation on zooplankton life history are the result of plastic or evolutionary changes.

4-To quantify how the evolution of predator foraging traits and functional feeding systems of the main foraging apparatus is related to variation in prey communities. Additional analysis will also examine whether variation in prey communities resulting from the effects of (fish) predators can feedback to shape evolution in the predator population.

1.12 Study areas and sampling plan

Fish samples and zooplankton were collected from lochs on the island of North Uist, Western Isles, Scotland (57°35”N; 7°18”W). North Uist is a small island with an area of 303 km^2 (Haswell-Smith, 2008). Ice covered all of the Western Isles during the period of the Last Glacial Maximum around 25,000 years ago (Ballantyne & Hallam, 2001). Freshwater organisms must therefore have invaded lochs on these islands after the ice receded approximately 10,000-15,000 years ago (Jordan *et al.*, 2010). There are more than 180 lochs (lakes) and many smaller lochans (ponds), on the island (Giles, 1981). The lochs are largely geographically independent and isolated from the sea, although some are tidal or have streams running to the sea. In the west of North Uist the surface geology is shell-rich sand with high-levels of $CaCO_3$ (Kneale & Viles, 2000). This has resulted in a short grassland habitat rich in plant species (machair’ habitat), and lochs are basic in water chemistry. In the central and eastern parts of the island lochs are acidic. This contrast in water chemistry has contributed to an ecological diversity unusual for such a small area (MacColl *et al.*, 2013). The water bodies on North Uist also vary greatly in size (0.12-53.9 ha), but are for the most part shallow (0.6-4.24 m mean depth).

Thirty freshwater lochs (Fig. 1.3) on North Uist were involved in this study. Another three lochs (Tros, Obse and Faik) were added to investigate the

differences in sticklebacks' trophic traits in freshwater and marine habitats. Fish and zooplankton populations were chosen across a range of ecological factors, namely those of resources, competition and predation.

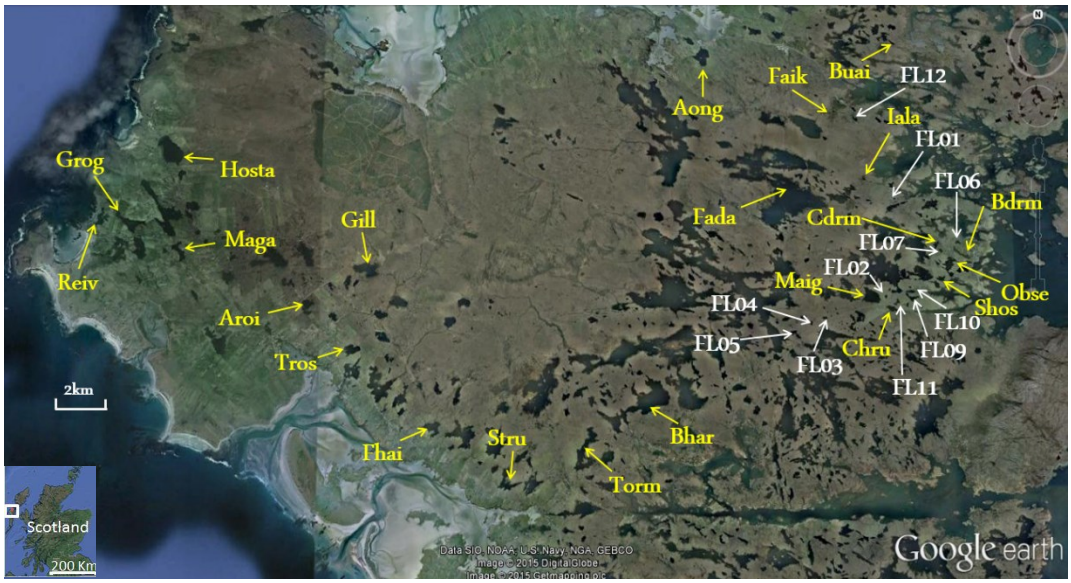


Figure 1.2. Map of North Uist showing the 33 of freshwater and marine lochs that were involved in this study. White arrows represent lochs without fish and yellow arrows represent lochs with fish. The map was adapted from Google earth, access date 19th Jun. 2016.

Chapter 2. Composition and distribution of crustacean zooplankton and Rotifera along a gradient of fish presence on North Uist, UK

2.1 Abstract

While previous studies have examined zooplankton communities in single, especially larger Scottish lakes, little information is available for smaller lakes, or how communities might vary between such lakes in close proximity. In this study, samples were taken from 30 lochs on the Scottish island of North Uist, which shows large biotic and abiotic variation among its lochs. In particular the sampling included lochs with and without fish in different quantities.

This study has examined how physical and chemical factors and fish presence shape the diversity and abundance of zooplankton. Thirty-nine species were classified from three main groups: Rotifera, Cladocera and Copepoda. Zooplankton abundance were found to vary greatly between lochs. Rotifera was the most abundant group, followed by Copepoda. No strong correlation was recorded between fish density and zooplankton abundance, although a positive relationship was recorded between Cladocera density and fish abundance. Species richness was higher in those lochs without fish than in those with fish. Biodiversity indices exhibited significant differences related to fish presence; species abundance in the presence of fish was comparatively more even. Zooplankton abundance and species richness exhibited significant relationships with pH and loch depth but not with chlorophyll a or concentrations of alkaline metals.

2.2 Introduction

Zooplankton play a critical role in energy flow and nutrient cycling in aquatic ecosystems (James, 2010). They are considered a key component in aquatic food webs by linking primary producers and the consumers in the higher trophic levels such as macroinvertebrates and fish (Chavez *et al.*, 2011). Zooplankton populations showed a worldwide distribution in many aquatic ecosystems (Pociecha *et al.*, 2016). The abundance of zooplankton communities is structured by numerous biological interactions and abiotic environmental factors (Irigoien *et al.*, 2004). Depth and area of water bodies are two major factors that structure community composition (Horváth *et al.*, 2017). Temporal variation, in both abundance and distribution of zooplankton, also affects community composition (Harding, 2001; Sanful *et al.*, 2017). In general, seasonal cycles of zooplankton are characterised by a maximum peak in spring followed by a small decrease thereafter until phytoplankton blooms lead to further increases in the summer and autumn (d'Alcalà *et al.*, 2004). However, control zooplankton diversity and community at the local and regional scales are not well understood (Aranguren-Riaño *et al.*, 2011); many elements of zooplankton community structure in some water bodies remain unknown.

Zooplankton are also influenced by predation. They are important food resources for predators in freshwater systems where fish are the dominant top predator (Elser & Goldman, 1991; Pintar & Resetarits, 2017). Direct consumption obviously reduces zooplankton abundance (Neves *et al.*, 2003), but changes in the abundance of top predators can lead to positive, negative, or neutral effects on diversity through both direct and indirect effects (Shurin & Allen, 2001; Pintar & Resetarits, 2017).

The natural resources found in different ecosystems also play different potential roles in shaping zooplankton population dynamics (Nicolle *et al.*, 2011). Resource availability in freshwater can be estimated from chlorophyll a concentration (a measure of algal biomass) and water pH. Low zooplankton biomass has been reported in lakes with low pH due to lower phytoplankton density and shortage of nutrients (Ross, 2015). Many examples have been documented in which physicochemical factors have affected zooplankton communities (Xiong *et al.*, 2017). Zooplankton have been noted to be very sensitive to the predominant ionic composition of water (Arnott *et al.*, 2017). An increase in salt levels in freshwater ecosystems affects the osmoregulation of Rotifera (Bielańska-Grajner & Cudak, 2014). Calcium concentration is another important element in determining crustacean zooplankton abundance and richness (Jeziorski *et al.*, 2008; Bielańska-Grajner & Cudak, 2014). Decreased calcium concentrations delay reproduction and reduce both the survival and fecundity of crustacean zooplankton (Ashforth & Yan, 2008; Jeziorski *et al.*, 2008). Increased salt (NaCl) levels have also been found to reduce the population abundance of freshwater Rotifers and cladocerans (Sarma *et al.*, 2006).

In the last few decades, large-scale field studies of zooplankton ecology have primarily focussed on large Northern Hemisphere aquatic systems, which is a region where many species invaded new freshwater environments following the most recent glaciation (Dynesius & Jansson, 2000). Such studies in northern Europe have investigated a wide range of habitats. They have documented the phenology and the community structure of zooplankton (Beaugrand *et al.*, 2003; Vadadi-Fülöp & Hufnagel, 2014) and biodiversity and species assemblage indicators (Beaugrand *et al.*,

2002). Other studies have focussed on how zooplankton communities change in structure in response to climatic variability (Planque & Taylor, 1998), eutrophication (May & O'Hare, 2005) and fish and invertebrate colonisation of waterbodies (Mehnera & Thielb, 1999; McNaught *et al.*, 2004; Pothoven *et al.*, 2012).

Among these studies, surprisingly little information is available about zooplankton communities in small freshwater bodies in the Northern Hemisphere, even though other aspects of small lakes, especially the ecology and evolution of their fish populations, have been scrutinised (MacColl *et al.*, 2013; Magalhaes *et al.*, 2016). Numerous studies have examined the zooplankton communities of large lochs (lakes) in Scotland. These studies have shown that most Scottish lochs are clear, rich in resource (Maitland, 1981; Bresnan *et al.*, 2015) and dominated by Rotifera among other zooplankton groups of Cladocera and Copepoda (Romo, 1990). The zooplankton community structure of these lochs can be driven by fish predation (Pomeroy, 1994). Scotland has a multitude of small lochs, the zooplankton communities of which remain understudied. This is especially true of the Western Isles, the low-lying areas of which harbour many small lakes with diverse ecologies (Waterston, 1981). Here I examine the zooplankton communities of lochs on the island of North Uist in the Western Isles. A few very old studies have mentioned several species of crustacean zooplankton on North Uist (Scott, 1894; Scott, 1895), but no recent study has specifically focussed on the composition of these groups. The lochs on North Uist vary greatly in water chemistry, from acid lochs lying on top Lewisian gneiss and peat in the east to alkaline lochs underlain by shell-rich machair sand in the west (Waterston, 1981). The fish communities are simple in most of the lochs but completely absent from others. The present

study has used variations in pH and alkaline metals (sodium, potassium, magnesium and calcium) as key aspects of abiotic environmental resources on North Uist (MacColl *et al.*, 2013). North Uist shows wide variations in pH, associated with differences in the concentrations of alkaline metals (Waterston *et al.*, 1979). The main objective of the present study was to investigate the zooplankton species that occur on North Uist and to describe their community structures across the environmental gradients that are present and between lochs with and without fish. This study provides the first baseline observation of the scale of zooplankton variation in this region.

2.3 Methods

2.3.1 Study areas

Fish samples and zooplankton were collected from lochs on the island of North Uist (57°35”N; 7°18”W) in the Western Isles of Scotland in April and May (2014 and 2015). North Uist is a small island with an area of 303 km^2 (Haswell-Smith, 2008). Because ice covered all of the Western Isles during the period of the Last Glacial Maximum around 25,000 years ago (Ballantyne & Hallam, 2001), freshwater organisms thus must have invaded the lochs on these islands after the ice had receded, approximately 10,000–15,000 years ago (Jordan *et al.*, 2010). The lochs, which are largely isolated from one another, are unusually variable in their basic water chemistry, as already described, which has contributed to an unusual ecological diversity for such a small area.

2.3.2 Sampling sites

Zooplankton were sampled in lochs both with and without fish. For the purposes of this project, 30 sites were selected (Fig. 2.1, Table 2.1) that spanned the full range of water chemistry and included lochs both with and without fish. Three loch types were defined in this study. Lochs without fish (N = 11) were all small and were confined to the eastern part of the island, where the freshwater is oligotrophic. Lochs with fish formed two groups: a set of small eastern lochs (N = 6) that closely matched the fishless lochs, and a wider set (N = 13) distributed across the island (Table 2.1) that covered a wide array of resource conditions, including naturally eutrophic machair lochs.

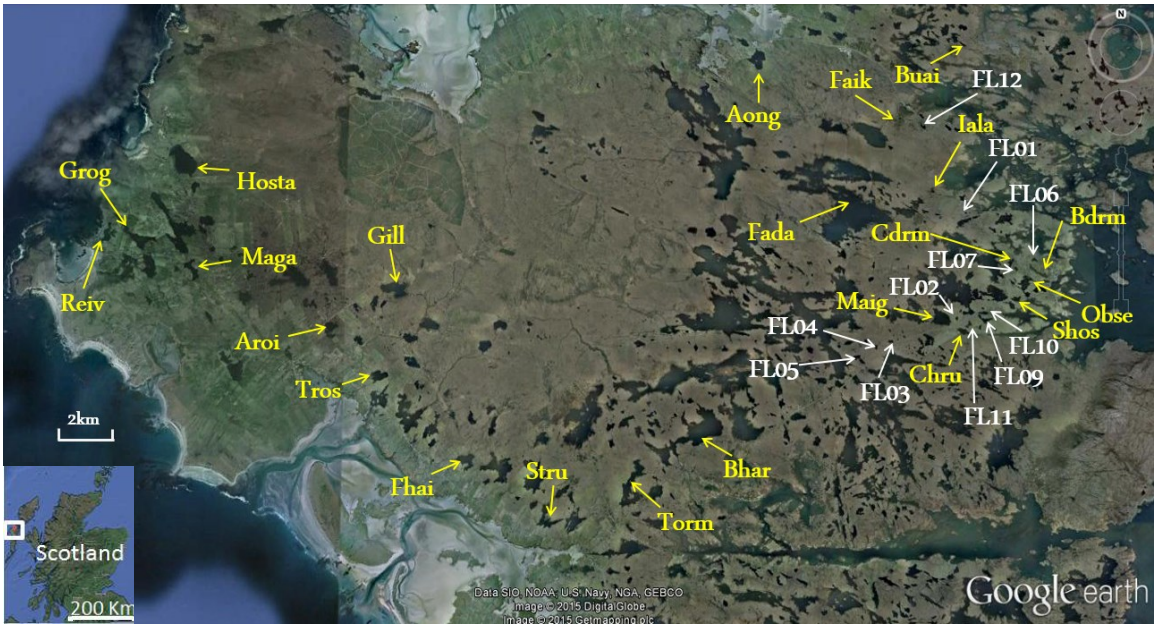


Figure 2.1. North Uist showing sampled zooplankton from 30 lochs in April and May (2014 and 2015). White arrows represent lochs without fish and yellow arrows represent lochs with fish.

2.3.3 Sampling and collecting of the environmental data

2.3.3.1 Abiotic factors

Sampling for estimating abiotic conditions was conducted on the day zooplankton were collected and minnow traps were set. An ultrasound depth finder (Plastimo Echotest II handheld depth sounder) was used to record the depth at several (20–180) points across each loch. The area of each loch was estimated from Google Earth using Web-based planimetric software http://www.freemaptools.com/area-calculator.htm) (MacColl *et al.*, 2013). pH and water conductivity were measured using a calibrated Multimeter (Multi 340i, WTW, Weilheim, Germany). The concentrations of alkaline metals (sodium, potassium, calcium, magnesium) were calculated by collecting filtered water samples in the field from each loch. These samples were frozen and returned to the University of Nottingham lab. Samples were analysed for metallic cation concentrations by inductively coupled plasma mass spectrometry (ICP-MS), anions using a Dionex DX500 ion chromatograph with an IonPac AS14A (4 x 250 mm) at the Division of Agriculture and Environmental Science in Nottingham. A geochemical speciation model WHAM VI (Windermere Humic Aqueous Model, version 6) was used to predict and calculate the fractions of alkaline metals ($mol.L^{-1}$).

Water samples for chlorophyll pigments analysis were filtered using (0.45 millipore) GF/C filter papers to eliminate suspended particulate matter. The filter papers were folded in half, blotted and packed in aluminium foil and kept frozen in the dark until testing time. The filters containing the algal sample were cut into 5 to 10 strips and then homogenized in 5 mL of a mixture of acetone, methanol and water (80:15:5) overnight at -4° C. Extracts were then filtered with a 0.22-lm PTFE filter, dried under N_2 gas

and re-dissolved in a 70:25:5 mixture of acetone, ion-pairing reagent (IPR) and methanol for injection into an Agilent 1200 series high-performance liquid chromatography (HPLC) unit with separation conditions modified from (Chen *et al.*, 2001). Depending on its relative retention times and the absorption spectra of characterized pigments chlorophyll a was calibrated and identified from HPLC peaks. The concentrations of pigments was determined and corrected to ng/L^{-1}. An average of two readings per loch was taken between April 2014 and May 2014.

For abiotic factors apart from lake morphometry (depth and area), concentrations of metallic cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and mean pH values were used. Principal component analysis (PCA) was used to summarise variations in alkaline metal concentrations. The concentration of chlorophyll a was used as an index of resource availability (Waterston *et al.*, 1979). These factors were chosen because they are known to vary greatly between North Uist lochs and are common measures of environmental variation in aquatic systems (MacColl *et al.*, 2013) where they also correlate with resource availability (Waterston *et al.*, 1979).

2.3.3.2 Fish collection

Stickleback presence and abundance were established by setting a mean of 18 wire mesh minnow traps (Gee traps, Dynamic Aqua, Vancouver, Canada) overnight in 0.3–3 m depth of water within 5 m of the shore along stretches of bank comprising at least 50% of a lake's perimeter or 100 m, whichever was greater. The total number of stickleback caught was divided by the number of traps to provide an estimate of catch per unit effort (CPUE). The absence of stickleback from fishless lochs was confirmed in a similar manner by setting at least six traps overnight on at least three separate

occasions over two years. The relative densities of nine-spined sticklebacks (*Pungitius pungitius*, hereafter *Pungitius*) – the only other common small fish species and the most obvious competitors of three-spined sticklebacks in these lochs – were also estimated from these catches as the percentage of 100 haphazardly selected fish. The density of stickleback (CPUE) and relative abundance of *Pungitius* were used as indicators of variation in the biotic environment.

Lochs that have been confirmed to have any fish were named (lochs with fish). This including (nine-spined sticklebacks, three-spined sticklebacks and trout fish). On the other hand lochs that have no fish or juvenile fish detected inside the traps were considered (lochs without fish) or (fishless lochs). Trout fish were not considered in the statistical in this chapter because trout fish have a large size and don't have effective predation on small zooplankton. Their main food is large crustacean and small fish (Jeppesen *et al.*, 1997; Herrera-Martínez *et al.*, 2017). Trout is also not available in all lochs with fish and I could not detect any juvenile trout in fish traps in all fishless lochs.

Table 2.1. Variation in environmental parameters for lochs on North Uist was investigated in this study. Names and abbreviations of lakes sampled. Sites beginning 'FL' do not have stickleback populations, Sites in bold refer to small eastern lochs with fish. Mean depth and area of lochs, pH and EC (electrical conductivity) are the means of measurements taken in April and May (2014 and 2015) Chlorophyll a is the mean of measurements in April and May 2014. CPUE is the fish density represented by catch-per-unit-effort, p.p. is the relative abundance of trapped nine-spined *Pungitius pungitius* in fish community. Conductivity was listed in this table because of its high correlation with PC1 alkaline (r=0.83, P <0·001). PC1 is the first principal component of alkaline metals concentrations.

Lake name	Loch code	Geographic Coordinates	Area (ha)	Depth (m)	pH	EC µS/c	CPUE	Chla (ng/L)	p.p. %	PC1
Aonghais	Aong	57°38'40.8"N 7°16'13.2"W	9.6	1.02	7.7	138.4	0.61	28.7	0	-1.4
Mhic A'Roin	Aroi	57°35'40.1"N 7°25'49.2"W	6.5	0.91	7.7	142.6	0.56	69.7	39	-0.8
-	**Bdrm**	57°36'14.9"N 7°09'53.2"W	2.4	1.06	7.3	204	6.67	47.5	0	1.2
a'Bharpa	Bhar	57°34'33.2"N 7°17'31.2"W	53.9	4.14	7.3	156.5	0.94	25.5	0	-1.7
na Buaile	**Buai**	57°38'50.2"N 7°11'35.6"W	1.7	0.84	7.1	140	11	17.7	0	-0.4
-	**Cdrm**	57°36'22.4"N 7°10'38.7"W	1.32	1.29	7.0	316	2.7	85.3	0	2.7
ChadhaRuaid	**Chru**	57°35'37.9"N 7°11'44.2"W	2.1	0.82	6.9	158.3	11.17	85.4	0	-1.9
Fada	Fada	57°36'57.9"N 7°14'15.0"W	11.3	0.9	6.7	118.3	3.11	15.5	0	-1.8
Fhaing	Fhai	57°34'12.2"N 7°22'45.3"W	8	0.96	7.4	144	5.71	18.8	44	-0.7
Gille-bhride	Gill	57°36'06.3"N 7°24'21.3"W	14	1.01	7.2	136.8	0.74	9.8	6	-0.7
Grogary	Grog	57°36'36.5"N 7°30'04.6"W	14.8	0.69	7.7	303	9.83	51.8	32	4.5
Host	Host	57°37'31.6"N 7°29'10.2"W	25.8	4.24	7.5	314.5	0.43	80.1	31	3.2
Iala	**Iala**	57°37'11.0"N 7°12'20.8"W	0.6	0.75	6.8	144	4.38	87.8	0	-1.3
nam	Maga	57°36'15.9"N 7°28'52.4"W	6.5	0.8	7.4	258.5	8.07	50.2	16	1.5
Maighdein	Maig	57°35'45.9"N 7°12'12.6"W	9.6	3.4	7.5	227.5	2.39	38.5	0	-1.1
na Reival	Reiv	57°36'39.8"N 7°30'49.5"W	6.1	0.74	8.1	441	7.08	327.0	49	5.1

Shior	**Shos**	57°35'58.8"N 7°10'32.1"W	1.5	3.43	6.7	165	0.17	20.2	0	-0.5
nan Struban	Stru	57°33'30.5"N 7°20'58.4"W	19.2	1.28	7.3	145	27.92	28.5	0	-0.8
Tormasad	Torm	57°33'53.5"N 7°19'14.2"W	21.1	1.89	7.6	159.9	2.28	40.8	0	-0.5
-	FL01	57°36'56.7"N 7°11'37.1"W	0.12	1.07	6.3	151.9	0	19.4	0	0.2
-	FL02	57°35'46.2"N 7°11'51.8"W	0.35	1.31	7.0	169.4	0	37.9	0	-0.1
-	FL03	57°35'30.9"N 7°13'20.7"W	0.53	1.82	6.0	125.5	0	3.6	0	-1.2
-	FL04	57°35'27.0"N 7°13'40.5"W	0.43	1.39	6.4	114.1	0	7.7	0	-0.9
-	FL05	57°35'17.3"N 7°13'59.3"W	0.69	0.6	6.4	119.5	0	4.3	0	-0.8
-	FL06	57°36'25.7"N 7°10'06.3"W	0.65	1.31	6.9	170	0	3.3	0	0.4
-	FL07	57°36'18.7"N 7°10'30.9"W	0.44	1.24	6.8	175	0	7.7	0	0.7
-	FL09	57°35'44.5"N 7°11'14.1"W	1.8	1.53	7.5	136	0	12.6	0	-0.8
-	FL10	57°35'49.4"N 7°11'14.9"W	0.92	1.27	7.6	213	0	31.0	0	0.5
-	FL11	57°35'41.7"N 7°11'35.7"W	0.85	0.93	7.6	126	0	101.0	0	-1.0
-	FL12	57°37'54.6"N 7°12'31.0"W	0.59	0.72	5.6	99	0	7.3	0	-1.6

2.3.3.3 Sampling and estimation of zooplankton

To investigate zooplankton, samples were collected by using a zooplankton net (diameter 25 cm; length 1 m; mesh 54 µm). In large lochs, replicated samples were taken by towing the net behind a boat for a specific distance (usually 100 m). A boat could not be used in small fishless lochs, however, so the net was repeatedly towed for a specific distance at three or four points around these lochs. A small bucket was occasionally used to collect samples, which were then filtered by a net. Multiple samples were taken in order to reach a sufficient volume that would cover the probability of detecting organisms in a small area. This kind of sampling, in which containers are used instead of a net, is generally recommended in shallow eutrophic small-size locations to reduce clogging by small particles in shallow areas, since such particles could reduce the efficiency of net filtering (De Bernardi, 1984). All sampled organisms were preserved for further analysis in methanol sucrose solution (1 sucrose solution (4%): 1 methanol solution). Samples were examined under a light microscope and organisms were identified to species whenever possible using appropriate keys (Edmondson, 1959; Scourfield & Harding, 1966; Harding & Smith, 1974; Pontin, 1978).

A Stempel pipette was used to remove an aliquot of each sample for counting purposes. The zooplankton density for each taxon was determined by counting the number of each taxon in three 1 ml subsamples using a Sedgwick Rafter slide and then taking the average. The result was multiplied by the volume of the zooplankton sample to yield the total number of each taxon in the sample. This number was then divided by the volume of the water column (m^3) filtered by the net (De Bernardi, 1984). The volume of the water filtered by the net was determined in an indirect

way (De Bernardi, 1984), assuming roughly that the column of water captured by the net can be calculated as:

$$V = \pi \times r^2 \times d$$

Where:

V= is the volume of water filtered by the plankton net

r=radius of the mouth of the net

d=Length of the water column crossed by the net.

The average zooplankton density in each litre in the entire water column, as well as the density of the main taxa, were then calculated in ind/m^3, according to (De Bernardi, 1984):

$$\text{No. of each Taxon} = (N \times Vz) / Vn$$

Where:

N = number of organisms in 1 ml

Vz = Volume of zooplankton sample (ml)

Vn = Volume of the water column collected by the net (m^3).

2.3.4 Biodiversity statistics and the community structure of zooplankton

2.3.4.1 Species richness

The index was calculated by counting the total number of species in each location (Colwell, 2009) omitting immature copepods from the estimation (Zokan & Drake, 2015).

2.3.4.2 Frequency (F)

Species frequency was calculated using the following formula (Omori & Ikeda, 1984):

$$\mathrm{F} = \mathrm{Ts}.100/\mathrm{Tos}$$

Where Ts is the number of samples in which the taxon is present and Tos is the total number of samples. The results were then presented as percentages (%).

2.3.4.3 Diversity metrics

Species diversity in ecological studies is commonly measured using three main indices: species richness, Shannon-Wiener and Simpson's diversity. Species richness reflects the number of species present (contingent on sampling effort), but gives no information on species abundances. A higher Shannon Wiener index value shows higher species number in more equal abundances, whereas a high Simpson's index shows a community low in species in which only a few species dominate the total community abundance. These metrics can be reflected in Hill numbers or so-called effective number of species (Chao *et al.*, 2014), which integrate species abundance and species richness into an overall diversity measure defined as:

$$D^q = \left(\sum_{i=1}^{s} pi^q\right)^{1/(1-q)}$$

In which S is the number of species in the community and (*p*i) is relative abundance. The parameter q can be interpreted in three ways, richness (q = 0) as the number of species, exponential of Shannon-Wiener's index (q=1) as the number of average species, and inverse Simpson index (q =

2) as the number of dominant species (Chao *et al.*, 2014). Hill numbers can be presented on a single graph as a function of q, and the interpretation of Hill numbers depends on the chosen value of "q". Values of Hill numbers do not have to be integers, and all have common units of species richness. The diversity curve is a decreasing function of q (Hill, 1973). For a typically even population, the diversity profile is a constant of species richness. On the other hand, the more uneven the distribution of relative abundances, the more steeply the curve declines (Jost, 2010; Magurran, 2013).

2.3.5 Statistical analysis

To obtain the best estimate of the density (ind/m^3) of the three main groups of zooplankton (Rotifera, Cladocera and Copepoda), the association between zooplankton group abundance and factors that might affect the density of each loch was investigated. Abundance data were modelled using generalised linear models (GLMs) in GenStat (17th edition, VSN International Ltd., Hemel Hempstead, UK). GLMs was checked and validated by visual assessment using residuals' graphs and Shapiro- Wilk normality test ($P > 0.05$) was used to confirm normality. Pearson correlations were used to evaluate the relationship between the density of cladocerans in 2014 and 2015 and 2014 and 2016, as discussed in chapter 4.

Loch, tow type (with boat or bucket), year and Julian date were included as fixed effects. Julian days were treated as a continuous variable, whereas tow type, year and loch were defined as categorical variables. Any terms that were not significant were dropped from the full model to achieve a minimum adequate model. Wald's F statistic was used to assess the significance of variables. Abundance data for Cladocera and Copepoda were square-root transformed to achieve normality and were modelled with

normal error distributions and identity link functions, while Rotifera abundance was modelled with Poisson errors and a logarithm link function.

A one way ANOVA with post hoc test was used to determine statistically significant differences between independent samples of total zooplankton means density. Non-normally distributed data sets were log transformed to achieve normality. Statistical tests were performed using 0.05 as the level of significance to examine the differences in total zooplankton density between the three loch groups: (1) large with fish, (2) small with fish and (3) lochs without fish.

GLMs were also used to analyse the relationship between zooplankton community measures (abundance and species richness as response variables) and environmental factors (lake morphometry, pH, chlorophyll a, principal component (PC) resource scores, stickleback CPUE and percentage of *Pungitius*) as explanatory variables. Models had normal error distributions and identity links. The significance of components was determined by Wald F-tests, and any non-significant terms were sequentially dropped to generate a minimum adequate model.

2.4. Results

2.4.1 Variation in environmental parameters for lochs on North Uist

2.4.1.1 Abiotic factors

Table 2.1 shows the variability of a set of environmental variables for different lochs. Overall, lochs without fish were smaller than all those with stickleback ($t = 2.792$, $df = 28$, $p = 0.009$), but no differences in depth were found between the two sets ($t = 1.026$, $df = 28$, $p = 0.313$). Water chemistry showed that lochs varied greatly, from acidic freshwater at the

eastern side of the island to alkaline at the western edge. The results showed that lochs with fish tended to be more alkaline (t= 3.124, df = 28, p = 0.004) and to have higher conductivity (t= 2.188, df = 28, p = 0.037) and higher chlorophyll a (t= 3.563, df = 28, p = 0.0013) than lochs without fish. Although lochs were not surveyed at random, these findings certainly presented a real and qualitative pattern. Fishless lochs also tended to be relatively common on the eastern side of North Uist (Fig. 2.1), but were rare in the west. When a set of comparably sized small eastern lochs with and without stickleback were compared, no significant differences were found in their pH or depth.

For alkaline metal measurements, the first principal component (PC1) in the analysis of such metals explained 80.4% of variation in the data. All the measured factors had positive loadings on PC1. The second principal component (PC2) in the alkaline metal analysis explained 15.9% of variation in the data (Table 2.2); PC1 was hence used as an overall index of alkaline metal concentrations.

Table 2.2. PC scores of alkaline metals of 30 freshwater zooplankton populations on North Uist in April and May (2014 and 2015).

Factors	PC1 alkaline metals	PC2 alkaline metals
K^+	0.50	0.08
Mg^{2+}	0.47	0.06
Na^+	0.51	-0.06
Ca^{2+}	0.52	0.04
Percentage of variation	80.4%	15.9%

2.4.1.2 Zooplankton species composition

A total of 39 species were identified in this study (Table 2.3), with some juvenile copepods impossible to identify beyond the genus level. Rotifera comprised the largest number in the zooplankton community on North Uist with 18 species, followed by Cladocera (16 species) and copepods (5 species). The highest number of species occurring in one loch was 19 in Fhai and the lowest was five in Aong (Table 2.3). Twenty-five species were shared by lochs with and without fish. Seven species occurred only in lochs with fish, and 7 species in lochs without fish.

Table 2.3. Species occurrence and total species richness of zooplankton communities on North Uist in April and May (2014 and 2015) where the dark shading highlights the presence of each species.

Zooplankton species	Aong	Aroi	Bdrm	Bhar	Buai	Cdrm	Chru	Fada	Fhai	Gill	Grog	Host	Iala	Maga	Maig	Reiv	Shos	Stru	Torm	FL01	FL02	FL03	FL04	FL05	FL06	FL07	FL09	FL10	FL11	FL12
Rotifera																														
Kellicottia longispina (Kellicott, 1879)					■	■		■	■	■	■	■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■
Keratella serrulata (Ehrenberg, 1838)		■	■			■	■	■	■		■							■		■		■	■	■	■	■	■	■		■
Asplanchna amphora (Hudson, 1889)			■		■	■	■		■		■				■		■	■	■	■	■	■	■	■	■	■		■	■	
Monostyla closterocerca (Schmarda,1895)							■		■			■	■	■										■		■	■	■		
Trichotria tetractis (Ehrenberg, 1830)						■	■		■																		■			
Brachionus angularis (Gosse, 1851)			■			■			■		■			■		■				■				■	■	■	■	■		■
Keratella cochlearis (Gosse, 1851)		■	■		■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Tripleuchlanis plicata (Levander, 1894)					■														■											
Gastropus minor (Rousselet, 1892)					■		■		■	■			■					■		■									■	■
Cephalodella gibba (Ehrenberg, 1830)					■		■			■			■			■	■	■		■										■
Polyarthra remata (Skorikov, 1896)						■										■	■			■	■		■	■	■		■	■	■	
Colurella adriatica (Ehrenberg, 1831)													■																	
Testudinella patina (Hermann, 1783)						■										■					■									
Rotaria neptunia (Ehrenberg, 1830)																				■										
Filinia longiseta (Ehrenberg, 1834)																					■							■		
Euchlanis dilatata (Ehrenberg, 1832)						■			■																	■			■	
Lepadella ovalis (Müller, 1786)						■										■														
Trichocerca porcellus (Gosse, 1851)						■																					■			
Cladocera																														
Bosmina coregoni obtusirostris (Sars,1861)	■	■	■	■		■	■	■	■	■	■	■			■		■	■	■	■		■		■	■		■	■	■	■
Daphnia hyalina galeata (Sars, 1864)	■	■		■				■	■	■	■	■		■	■		■	■	■									■		■
Daphnia longispina (O. F. Müller, 1776)			■																						■					
Alona rectangular (Sars 1862)						■	■	■	■		■		■			■				■		■		■		■	■		■	■

Chydorus sphaericus (O.F. Müller, 1776)																														
Diaphanosoma brachyurum (Liévin, 1848)																														
Polyphemus pediculus (Linnaeus, 1761)																														
Bythotrephes longimanus (Leydig, 1860)																														
Acroperus harpae (Baird, 1834)																														
Leptodora kindtii (Focke, 1844)																														
Ceriodaphnia reticulata (Jurine, 1820)																														
Alonopsis elongate (Sars 1862)																														
Eurycercus lamellatus (O.F. Müller, 1776)																														
Sida crystallina (O. F. Müller, 1776)																														
Holopedium gibberum (Zaddach, 1855)																														
Drepanothrix dentata (Eurèn 1861)																														
Copepoda																														
Bryocamptus zschokkei (Schmeil, 1893)																														
Cyclops leuckarti (Claus, 1857)																														
Cyclops strenuus(Fischer, 1851)																														
Cyclopoid copepodite																														
Nauplius cyclops																														
Mesocyclop sp																														
Diaptomus wierzejskii (Richard, 1888)																														
Diaptomus Copepodites																														
Nauplius *Diaptomus*																														
Species richness	**5**	**8**	**9**	**7**	**9**	**1**	**1**	**9**	**1**	**9**	**1**	**9**	**1**	**8**	**7**	**1**	**9**	**1**	**9**	**1**	**1**	**1**	**8**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

2.4.2 Dominance and abundance of zooplankton species

The results of multiple regression analysis for estimating the final zooplankton density of the three main loch groups (Table 2.4) showed that Rotifera, Cladocera and Copepoda abundance varied greatly between lochs as well as between years. Other factors were also significant predictors for estimating zooplankton density in each loch. Rotifera was significantly associated with tow type, year and Julian days. Year and Julian days were significantly correlated with Cladocera density, while Copepoda density was significantly related to tow type.

Table 2.4. GLMs results of the relationships between zooplankton density and potential explanatory variables affecting density. Abundance data for Cladocera and Copepoda were modelled with normal error distributions and identity link functions while Rotifera abundance was modelled with Poisson errors and a logarithm link function. Probability values which are < 0.05 are in bold type.

Response	Fitted model	Wald	df	*P*
Rotifera	Loch	1460606	29,79	**<0.001**
	Tow	29126	1,79	**<0.001**
	Year	7012	1,79	**<0.001**
	Julian day	0	1,78	0.656
Sqrt (Cladocera)	Loch	5.47	29,79	**<0.001**
	Year	6.71	1,79	**0.011**
	Julian day	14.73	1,79	**<0.001**
	Tow	0.00	1,78	0.945
Sqrt (Copepoda)	Loch	2.95	29	**<0.001**
	Tow	10.53	1,80	**0.002**
	Julian day	0.15	1,79	0.695
	Year	0.24	1,78	0.627

The total zooplankton density varied greatly, from a minimum of 6,050 ind/m^3 in Maig to a maximum of 107,003 ind/m^3 in Bdrm (Fig. 2.2). Rotifera was the most abundant group, followed by Copepoda (Fig. 2.3). These species' percentages in lochs without fish were both 51%, while in large and small lochs with fish the percentages were 30% and 50%, respectively. The three lochs groups (large with fish, small with fish and lochs without fish) were significantly different in terms of total zooplankton density ($F_{2, 27}$ = 2.98, P = 0.043, Fig. 2.3). In particular, small lochs with fish (n = 6) and lochs without fish (n = 11) showed significantly higher densities (p > 0.05) than large lochs with fish (n = 13).

The most frequent species of Rotifera (occurring in more than 50% of samples) included *Kellicottia longispina*, *Keratella cochlearis*, *Asplanchna amphora* and *Keratella serrulata (*Fig. 2.4, Appendix 2.2). In addition, *Kellicottia longispina*, *Keratella cochlearis* and *Asplanchna amphora* were key contributors to Rotifera density in all lochs, comprising 42%, 32.1% and 10.3%, respectively.

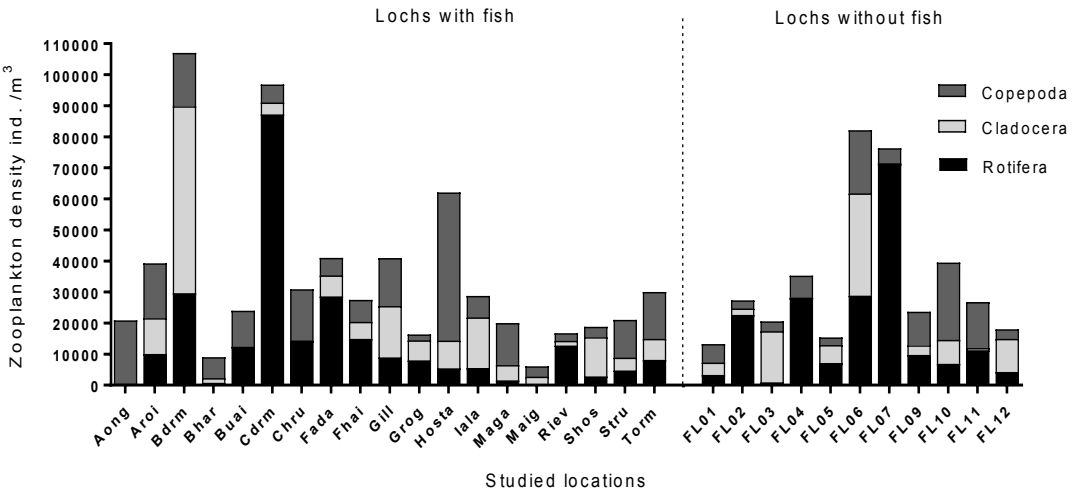


Figure 2.2. Total density and regional distribution of the main zooplankton groups in lochs with and without fish on North Uist during April and May (2014 and 2015).

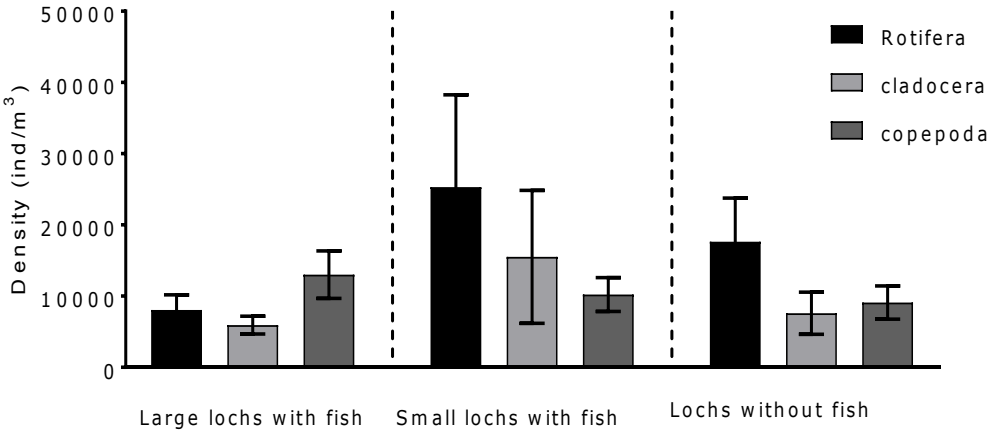


Figure 2.3. Differences in zooplankton group density from the three main classified groups of lochs (large with fish (n=13), small with fish (n=6) and lochs without fish (n=11) on North Uist. The values for the mean density are given with standard error (±SE). Small lochs with fish and lochs without fish showed a significant higher densities than Large lochs with fish (p > 0.05).

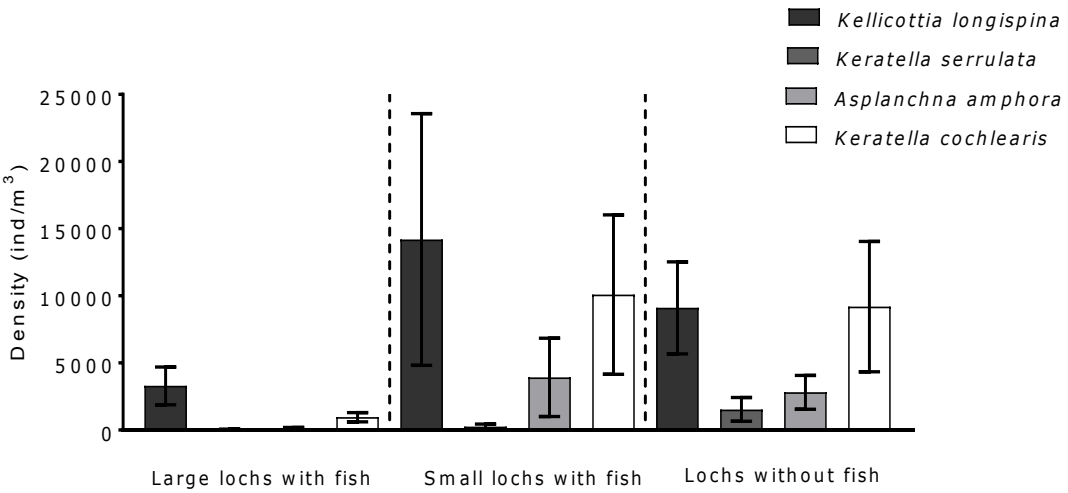


Figure 2.4. Mean density of dominant species of Rotifera from the three main classified groups of lochs (large with fish (n=13), small with fish (n=6) and lochs without fish (n=11) on North Uist. The values for the mean density are given with standard error (±SE).

Copepoda, the second-most widespread and abundant group of zooplankton on North Uist, comprised 27% of the total zooplankton in lochs without fish, while in large and small lochs with fish, they comprised 50% and 20%, respectively (Fig. 2.3). A remarkably high density of Copepoda was also

recorded in Host (47,737.8 ind/m^3), while the lowest density (1,936 ind/m^3) was recorded in Riev (Fig. 2.2). Two main species were recognised as being the most dominant: *Cyclops strenuus* (Fischer, 1851), followed by *Diaptomus wierzejskii* (Richard, 1888), which comprised 50.9% and 47.5% of the total number of copepods found in this study, respectively. *Cyclops strenuous*, which was the most frequently found species in this study, appeared in 100% of the lochs on North Uist, while *Diaptomus wierzejskii* appeared in 76.7% of the lochs on North Uist (Appendix 2.2).

Nauplii, the smallest developmental stage of Copepoda, comprised a high percentage of the total density of the developmental stages of Copepoda (nauplii, copepodites and adults). The percentages were as follows: large lochs with fish (39%), small lochs with fish (52%) and lochs without fish (56%).

The last major group of the zooplankton community found in this study, Cladocera, comprised 20% of species in large lochs with fish, and compared with 22% in lochs without fish. In comparison with other crustacean plankton (Copepoda) examined in this study, Cladocera density varied greatly between lochs (Fig. 2.2). A remarkably high density (68,361 ind/m^3) was recorded in Bdrm, while no Cladocera species were found in FL04.

Two species of Cladocera were found to be especially common: *Bosmina coregoni obtusirostris* (Sars, 1861) and *Daphnia hyalina galeata* (Sars, 1864). Those two species, especially *Bosmina*, are also considered to be the most important plankton food in the fish diet of North Uist (more details are available in chapter 5), although their relative abundance varied greatly between populations (Fig. 2.5). These two species were also very common among other Cladocera species in all locations that were examined on North

Uist (Fig. 2.6). Different patterns of consistency in Cladocera density were found when comparing the density of *Daphnia* and *Bosmina* species in 2014 and 2015 and other density values that were obtained when collecting samples of those two species in May 2016 (see chapter 4). The density of *Bosmina* populations in May 2016 showed a strong positive correlation (Pearson correlation) with the log mean density found in April and May 2014 and 2015, while no significant correlation was found with the log mean density of *Daphnia* populations during the same periods (r = 0.61, p = 0.008 for *Bosmina*; r = 0.28, p = 0.17 for *Daphnia*).

Alona rectangular and *Chydorus sphaericus* were also relatively common across populations, while numerous other species of Cladocera occurred sporadically (Table 3.2). Many of these species were characterised by a large body size, including *Diaphanosoma brachyurum*, *Polyphemus pediculus*, *Bythotrephes longimanus*, *Acroperus harpae*, *Leptodora kindtii*, *Ceriodaphnia reticulata*, *Alonopsis elongata*, *Eurycercus lamellatus*, *Sida crystallina*, *Holopedium gibberum* and *Drepanothrix dentata*.

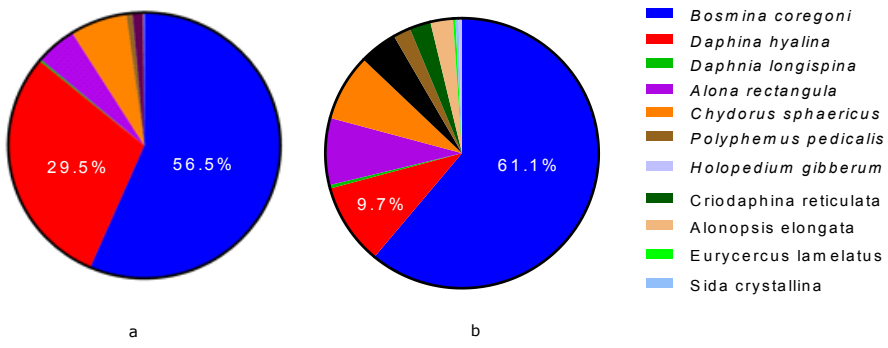


Figure 2.5. Relative abundance of cladoceran species in lochs with fish (a) and without fish (b) on North Uist in April and May in 2014 and 2015. *Bosmina* and *Daphnia* populations were the dominant among other Cladocera species in all studied locations.

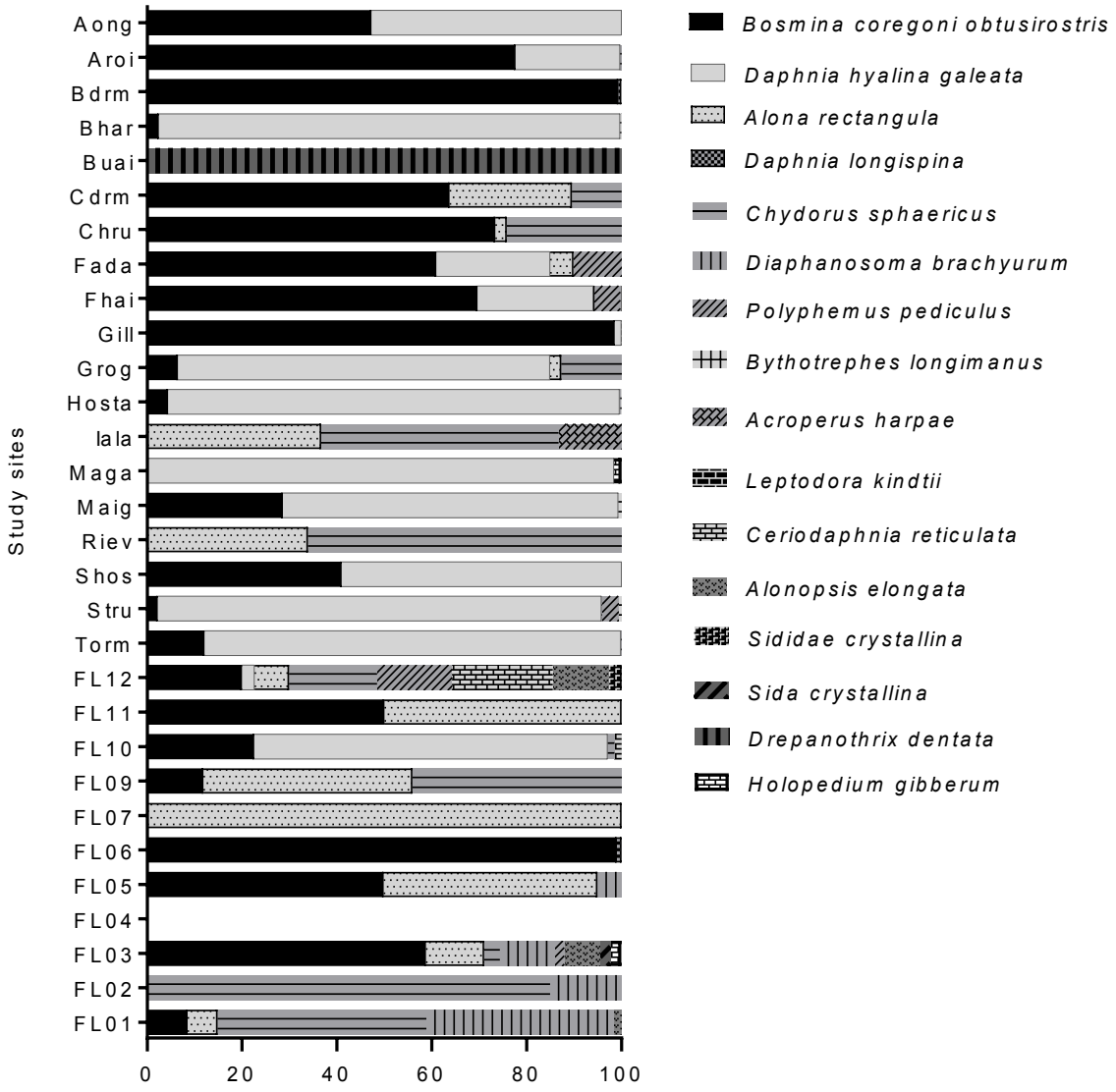


Figure 2.6. Frequency of Cladocera species in the 30 lochs on North Uist explaining how many times in percentage (%) each species appeared. No cladocerans were recorded in loch FL04. *Bosmina coregoni obtusirostris, Daphnia hyalina galeata, Alona rectangular, Chydorus sphaericus* were common across populations. Numerous other Cladocera species occurred sporadically.

2.4.3 Zooplankton diversity on North Uist

Lochs on North Uist have different numbers of zooplankton species (Table 3.2, Fig. 2.7). Differences were also observed when comparing groups of lochs. The three lochs groups (large with fish, small with fish and lochs without fish) were significantly different in the total number of species ($F_{2, 27}$ = 3.53, P = 0.039). Lochs with no fish and small lochs with fish were found to have a higher number of species than large lochs with fish ($p < 0.05$; Fig. 2.7 and 2.8); differences were also significant when comparing groups of large lochs with fish with lochs without fish ($p < 0.05$; Fig. 2.7 and 2.8).

Overall, the Hill diversity profile curves showed that relatively small lochs (with or without fish) showed more uneven distributions of relative abundance, as described by the steeper curves. In contrast, species assemblages in large lochs with fish were signified by curves that were relatively constant at the level of species richness (implying a high level of evenness), with the exception of Fhai and Stru, which showed high and steep curves (Fig. 2.8).

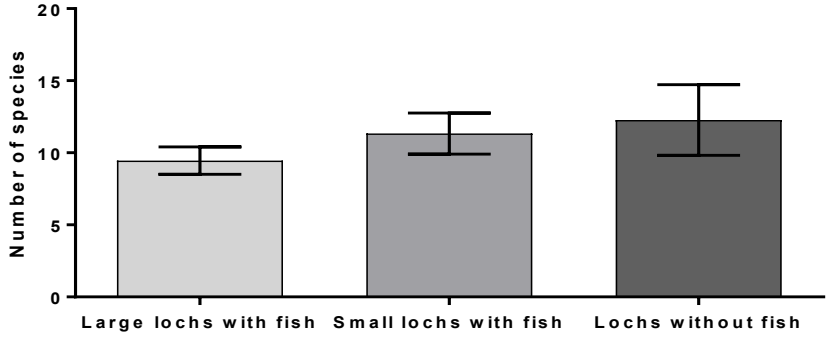


Figure 2.7. Zooplankton species richness differences between classified groups of lochs on North Uist. Error bars indicate standard error (± SE). Lochs with no fish (n = 11) and small lochs with fish (n = 6) showed significantly higher number of species ($p < 0.05$) than large lochs with fish (n = 13); the differences were also significant ($p < 0.05$) when the two groups of large lochs with fish were compared with lochs without fish.

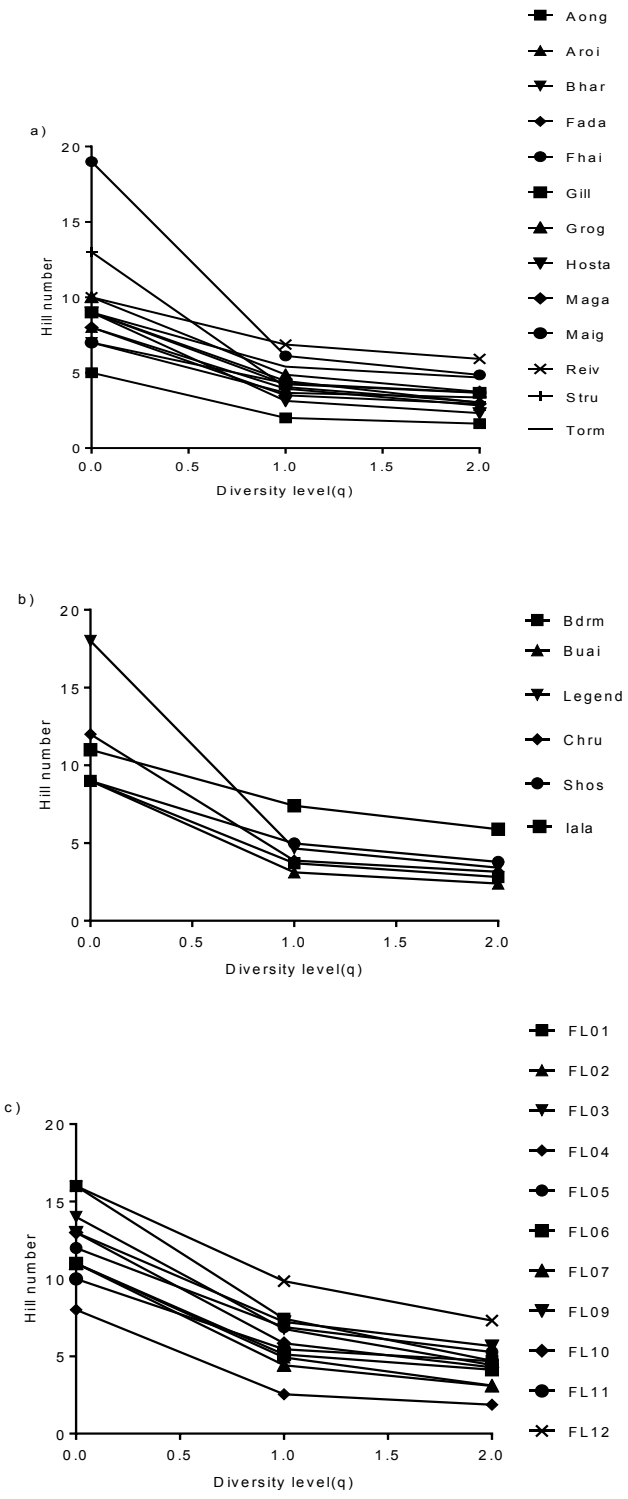


Figure 2.8. Hill numbers at three diversity levels, species richness (q = 0), estimated exponential Shannon's index (q= 1) and estimated inverse Simpson's index (q= 2) for a: Large lochs with fish, b: small lochs with fish and c: small lochs without fish.

2.4.4 Zooplankton and environmental factors

In general, environmental factors indicative of resource variability and fish density showed few associations with zooplankton abundance (Table 2.5). In terms of loch morphometric factors, loch depth was negatively associated with Rotifera density across North Uist but was unaffected by loch area. No significant association was found between Cladocera or Copepoda density in either loch area or loch depth in this study.

No association was found between species richness and abundance of zooplankton with alkaline metals. In contrast, pH was negatively associated with total zooplankton species richness on North Uist, and a positive correlation between Copepoda density and pH was found. Chlorophyll a, a measure of algal biomass, was found to be unrelated to zooplankton species richness and abundance on North Uist.

Finally, fish presence was not significantly associated with the abundance of zooplankton groups or species richness, although in the subset of lochs containing fish, a negative relationship was found between Cladocera abundance and stickleback density (Wald $F_{1,17} = 5.384$; $p < 0.035$).

Table 2.5. GLMs results for estimating the relationship between ecological factors and zooplankton abundance and species richness in 30 freshwater lochs on North Uist. Models were fitted using identity link functions and normal errors. Probability values < 0.05 are in bold type.

Response	Fitted model	df	Wald	*P*	estimate±SE
Log (Rotifera)	Mean loch depth	1,28	5.77	**0.02**	-0.58±0.24
	PC1 alkaline metals	1,27	2.41	0.13	
	Loch area	1,26	0.72	0.40	
	Chl a	1,25	0.29	0.59	
	Pungitius	1,24	0.14	0.71	
	CPUE	1,23	0.08	0.78	
	pH	1,22	0.06	0.814	
Log (Cladocera)	*Pungitius*	1,28	0.58	0.45	
	Chl a	1,27	0.88	0.36	
	PC1 alkaline metals	1,26	0.59	0.45	
	CPUE	1,25	0.56	0.46	
	pH	1,24	0.29	0.6	
	Mean loch depth	1,23	0.2	0.66	
	Loch area	1,22	0.04	0.85	
Log (Copepoda)	pH	1,28	4.80	**0.04**	0.55±0.25
	Chl a	1,27	3.49	0.07	-0.01±0.003
	PC1 alkaline metals	1,26	0.85	0.36	
	Pungitius	1,25	0.3	0.59	
	Loch area	1,24	0.04	0.85	
	Mean loch depth	1,23	0.01	0.92	
	CPUE	1,22	0	0.99	
Log (species richness)	pH	1,28	5.95	**0.02**	-0.22±0.09
	Pungitius	1,27	2.66	0.11	
	Loch area	1,26	2.21	0.15	
	CPUE	1,25	1.78	0.19	
	PC1 alkaline metals	1,24	0.52	0.48	
	Chl a	1,23	0.09	0.76	
	Mean loch depth	1,22	0.09	0.77	

2.5 Discussion

Few previous studies of temperate zooplankton have contrasted community structure across so many water bodies within such a small area or with such varied environmental conditions as those examined in the present study, which found that the lochs on North Uist support diverse and abundant of zooplankton communities.

In terms of both species richness and abundance, Rotifera was generally the most important group, although Copepoda was more abundant in the large lochs. Both species richness and abundance varied greatly between lochs; these differences were difficult to explain.

In terms of the morphometry of the lochs, zooplankton abundance and species richness were higher in small lochs, and Rotifera was more abundant in shallow lochs. In general, fish occurrence had a weak association with the diversity and composition of zooplankton groups, but with only a subset of lochs containing fish. The only other environmental factor to predict elements of the zooplankton community was pH: species richness was higher and Copepod abundance was lower in higher-acid lochs.

A widespread dominance of Rotifera in species richness and abundance among other types of zooplankton has been found in many freshwater bodies, such as lakes, ponds, rivers and streams (Florescu & Moldoveanu, 2017). Rotifera species have also been found to exhibit high density levels in the Northern Hemisphere (Segers, 2008), which may be attributable to their short life span, high parthenogenetic reproductive rate and generalised feeding (Wallace, 2002; Moreira *et al.*, 2016).

The results of this study have shown that the dominant species of Rotifera showed remarkably high density patterns in shallow lochs (most of which

were fishless lochs) compared with lochs with fish. This situation may be due to competition for food with Cladocera in lochs without fish as well as predation from other invertebrates and fish larvae, both of which may regulate the population dynamics of Rotifera (Williamson, 1983; Grundström, 1987). Neill (1984) reported on the regulation of Rotifera densities by crustacean zooplankton, while Castro *et al.* (2005) demonstrated the importance of depth in shaping Rotifera assemblages.

The dominance of the small-bodied cladoceran *Bosmina* found in this study may be explained by the fact that predation is highly selective among large-bodied Cladocera (Brooks & Dodson, 1965; Skoglund *et al.*, 2013). Most large Cladocera species have low occurrence on North Uist. These species have been reported by another study (on Loch Lomond) as isolated rare individuals that preferred littoral or deep habitats (Chapman, 1965). More generally, large-bodied Cladocera species are known to be found only sporadically in these habitats (Gillooly & Dodson, 2000; Meerhoff *et al.*, 2007; Dejenie *et al.*, 2012).

The greater abundance of cyclopoid than calanoid copepods found in this study agrees with the results from other researchers, who have suggested that this greater abundance occurs as a result of the small size of cyclopoids compared with calanoids, which may enable cyclopoids to avoid planktivorous fish. Compared with herbivorous calanoids, cyclopoids may also benefit from being omnivorous organisms that feed on both algae and small prey (Xie & Yang, 2000; Peacor *et al.*, 2012). Although Copepoda do not exhibit any parthenogenetic reproduction, they can compete with other groups of zooplankton in terms of rapid reproduction. Female Copepoda can store sperm from any previous mating (Bayly, 1986; Tundisi & Tundisi,

2012) and use them in later reproduction or can form resting eggs that may survive for more than 300 years (Hairston, 1995).

Copepoda are generally considered to be one of the most successful groups in those freshwater habitats that have originated from marine ecosystems (Boxshall & Defaye, 2007). Different distributions of the relative abundance of zooplankton assemblages were observed in this study. Uneven or patchy distribution was observed in lochs without fish and in small lochs with fish. In comparison with large lochs with fish, these lochs more often had a clearly dominant species. Other studies have reported that uneven distributions may be caused by several factors, including fish predation and variation in the environment (Choat, 1982; Rutherford *et al.*, 1999). Some studies, however, have reported that predators maintain a density-dependent regulation of diversity by reducing competitive exclusion between species (Thorp, 1986).

The lower levels of evenness found in small lochs with fish may also be due to these lochs' small surface area, which may lead to more competition for resources and more intensive predation. These traits may selectively affect species that have less tolerance under variable environments (Leibold, 1990) and can cause community fluctuations in the population dynamics of zooplankton communities, where the abundance of certain species increases to compensate for decreases of other species (Estlander *et al.*, 2017).

The role of predation evenness was not significantly correlated with species diversity in this study, but it might have had an indirect impact. The pressure of predation is generally believed to control diversity when the individuals of the most abundant species are removed selectively, which in turn reduces the competition found within communities in order to take up

limited resources and increase the evenness of zooplankton (Thorp, 1986; Xie & Yang, 2000; Donald *et al.*, 2001).

This study did not find strong evidence that the occurrence of fish in lochs affects the abundance of zooplankton. This situation may be because any overall long-term differences among lochs are overshadowed by short-term variations in density within lochs. The pattern of sampling used in this study, which concentrated on spatial replication at the cost of temporal replicates within lochs, may have failed to catch all lochs at the same points in their annual cycles, which are known to be quite cyclical in terms of their zooplankton abundance. This sampling method could have overestimated the importance of exceptional events, or missed them altogether (d'Alcalà *et al.*, 2004).

The results of the present study revealed an unexpected negative relationship between pH and species richness, meaning that more species were found in oligotrophic than eutrophic lochs. This result contrasts with the findings of Wetzel (2001) who found decreased species richness in dystrophic and acid ecosystems.

At the same time, this study's hypothesis was that not all variations in the zooplankton community would be a response from direct and indirect impacts of fish occurrence; some changes in the zooplankton community may likely be indicative of changes of resource availability (Pothoven *et al.*, 2012). The structure and composition of the Copepoda group are generally known to show remarkable variations in response to seasonal, annual and spatial scales, which together may also be related to the ambient environment (Paffenhöfer, 1993; Peterson & Keister, 2003). Particularly varied species composition has been found in the littoral zone of standing

water bodies with slightly acidic water under oligo- to mesotrophic conditions. The present study's results showed a positive relationship between copepod abundance and pH (Table 2.5). This finding agrees with Hessen & Nilssen (1983) that found that the copepod group favoured living in eutrophic, high-pH systems.

Finally, uncertainties still remain about the actual factors that shape the structure of zooplankton communities (Martinez-Sanz *et al.*, 2012). Although the present study found variations in biodiversity between lochs with and without fish on North Uist, the role of predation was not the only factor to appear to affect the abundance and distribution of zooplankton species across locations on North Uist. The results have highlighted the dominance of certain species over other species, which may indicate that ecological factors on North Uist are critical: only highly tolerant species can survive in the long term.

Chapter 3. Variation in cladoceran life history across lakes is more dependent on top-down than bottom-up control

3.1 Abstract

Top-down, predator driven control of variation in cladoceran life history strategies is widespread and has been well documented, but to what extent, this depends on variation in resource driven, bottom-up effect has been less well explored. Here, I explore variation in the life history strategies of cladocerans in a set of lochs across a natural gradient in resource availability. In this system there is also substantial variation in the presence and density of three-spined stickleback, which is a major predator of cladocerans. While there was little consistent variation in cladoceran density between lakes in relation to stickleback density, variation in stickleback density was an excellent predictor of variation in cladoceran life history parameters (probability of being gravid, size and number of eggs in the brood chamber and minimum size at reproduction). Egg and ephippia production varied between lochs with and without fish which may be due to selection for the most effective timing of reproduction under different predation regimes. Patterns of association between cladoceran life history and resource availability tended to be weaker. Consistent with previous findings, our results suggest that top-down effects have a major impact on cladoceran life histories, with higher predation favouring faster life histories and higher reproductive investment. This pattern is little affected by variation in resource conditions.

3.2 Introduction

Ecologists have repeatedly demonstrated the role of bottom-up (resources) and top-down (predation) factors in structuring ecological communities (Hunter & Price, 1992). There has been a long debate on how these factors interact. Herbivorous zooplankton communities provide good examples of structuring by both factors (Nicolle *et al.*, 2011), but studies investigating their interaction are less common (Hülsmann, 2011). The potential for structuring to be driven by the demographic consequences of life history responses to either type of factor are also little investigated, despite the fact that this might set the stage for eco-evolutionary feedbacks between predators and prey (Pelletier *et al.*, 2009).

Predators have been observed as having a major structuring role in many systems. Their effects on lower trophic levels, such as zooplankton, have perhaps been best studied in freshwater systems (Brett & Goldman, 1996; Hulot *et al.*, 2014). These are often relatively closed natural systems with defined boundaries, which makes them well suited to the study of the population dynamics and community structure of animals. In these systems, fish are frequently considered to be the top predators, and their absence in fishless habitats is a crucial characteristic (Pintar & Resetarits, 2017).

The concept of cascading trophic interactions explains differences in productivity among lakes. A low density of planktivorous fish increases herbivorous zooplankton and decreases phytoplankton biomass (Carpenter *et al.*, 1985). Conversely, high productivity and energy flow through the food web at all trophic levels occurs where predation intensities are intermediate (Power, 1992).

The influence of planktivorous fish on lower trophic levels in freshwater lakes, can be seen in different patterns. Species composition, size frequency, morphology and the life histories of zooplankton together with the development of vertical migration are known to be affected by fish predation (Brooks & Dodson, 1965; Palkovacs & Post, 2008; Palkovacs & Post, 2009). Some of these factors are a direct result of predation itself but others are plastic or evolved responses on the part of zooplankton to deal with predation and may be induced by kairomones For example, in the presence of predation, zooplankton may mature earlier, at a smaller size and increase their fecundity (Fisk *et al.*, 2007; Walsh *et al.*, 2012). Such patterns may be driven by size selective predation on larger prey (Walsh & Post, 2012).

The strong effects of alewife fish (*Alosa pseudoharengus*) on zooplankton has driven the evolution of the life history traits of the *Daphnia* species (Walsh & Post, 2012; Huss *et al.*, 2014). The intense predation of alewife fish also engenders strong selection on large–bodied zooplankton prey in which in turn increases the reproductive effort of small size-classes (Walsh & Post, 2011). Furthermore, under intense alewife predation, *Daphnia* have a faster growth rate, earlier maturation, large clutch sizes and shorter interclutch intervals. Several marked changes in the life history of *Daphnia* were also observed when tilapia (Cichlidae) and mosquitofish (*Gambusia holbrooki*) and pumpkinseed fish (*Lepomis gibbosus*) were introduced to aquatic ecosystems (Castro *et al.*, 2007; Tolardo *et al.*, 2016). The effects were summarised as reducing the age and size at primipara and increasing the rate of offspring production. Fisk *et al.* (2007) also noticed that *Daphnia* from fishless lakes produce large offspring, reproduce at a larger

body size, and were larger at birth than *Daphnia* co-existing with Salmonid fish.

However, the response of Cladocera to predation pressure is not isolated from the variable environmental factors in water bodies. Resource-driven effects have been reported in many places. Studies have revealed that changes in the ambient environment, such as food quality, may introduce a shift in life history traits (Guisande & Gliwicz, 1992).

Food limitation is considered to be a key factor in determining the life history of Cladocera: age at maturity is expected to be delayed and size at maturity is reduced at low chlorophyll a levels (Vanni & Lampert, 1992).

In general, when food is limited, *Daphnia* produce fewer and larger offspring (Guinnee *et al.*, 2007). It has been predicted that variation in the availability of resources to individuals may lead to variation in clutch size (McGinley *et al.*, 1987; Einum & Fleming, 2000), and the number of offspring produced is often equivalent to the amount of resources a mother invests in producing offspring (Smith & Fretwell, 1974).

A study on the relationship between the quality and quantity of phytoplankton (food quality) and the growth, birth-rate and longevity of *Daphnia hyalina* Leydig revealed that low food quality led to low growth and birth rates (Vijverberg, 1976). However, water chemistry also influences the life history of Cladocera. In low levels of hardness and alkalinity, *Daphnia* have been observed to delay reproduction, reduce fertility at first brood and increase body length at first reproduction, compared to *Daphnia* raised at high hardness and alkalinity (Jesus *et al.*, 2014). Furthermore, low resources may increase allocation for reproduction and therefore reduce the

availability of resources for maintenance, which may affect the survival of the organism (Snell & King, 1977).

Another strategy used by Cladocera to avoid stressful conditions is the production of sexually diapausing (resting) eggs that hatch when conditions are better (De Meester *et al.*, 2006). The appearance of resting eggs in the population is an indicator of the end of parthenogenetic reproduction and the beginning of gametogenic reproduction. This transition usually starts before or at the time when the population reaches its maximum density (Kuris, 1990). The formation of resting eggs is considered as a response to two main sources of stress, scarcity of food and predation risk (Pijanowska & Stolpe, 1996; Gliwicz *et al.*, 2001). Although both reproduction types are found together, selecting for the most effective achievable one through proper timing of reproduction stage is required (Gliwicz *et al.*, 2001).

Research regarding top-down and bottom-up factors is dominated by empirical studies. What is needed more is information on how interaction between bottom-up and top-down factors shape life history responses in the field. There is also a need for more studies that consider additional factors that may influence the strength of eco-evolutionary dynamics.

As a member of the crustacean group, Cladocera provides a good opportunity to examine reproductive capacity and monitor biological responses under the variation of environmental factors over short time scales (George & Edwards, 1974). The Cladoceran species, and the *Daphnia* and *Bosmina* species in particular, are good models for studying population dynamics (Masundire, 1992; Lampert, 2006). In general, most demographic, empirical and field studies have focused on the *Daphnia* species, while the *Bosmina* species has been less well studied (DeMott &

Kerfoot, 1982; Balseiro *et al.*, 1992). Both species belong to the group of animals that predominantly produce their offspring through parthenogenic reproduction, which is a form of asexual reproduction, and their eggs can easily be collected and quantified (Hebert, 1978).

The main question in this study is how Cladoceran species react to top-down (fish predation) and bottom-up (environmental) factors with regard to their reproductive strategies and life history parameters. In North Uist, different small aquatic systems offer natural experimental systems in which there is considerable variability in the relative strength of trophic status (bottom-up) and physical-chemical factors. In addition, the biotic factors of fish populations (top-down) show great spatial variations in aquatic systems. In general, ecological factors varied greatly between an archipelago of lochs (lakes) on the Scottish island of North Uist, and great diversity in the zooplankton community was also found (see chapter 2). There is a strong variation in pH, which is associated with variations in the concentrations of alkaline metals (Waterston *et al.*, 1979). The variations in pH, alkaline metals (sodium, potassium, magnesium and calcium) and chlorophyll a were used as key aspects of abiotic environmental resources on North Uist (see chapter 2).

3.3 Methods

3.3.1 Study site

I studied cladocerans in lakes spanning a broad array of resource conditions on the Scottish island of North Uist (57°36' N 7°20' W) (Waterston *et al.*, 1979; MacColl *et al.*, 2013; Magalhaes *et al.*, 2016). I sampled cladocerans in lochs both with and without fish. In all locations fish were trapped and density estimated as fish caught per trap per night ('catch per unit effort',

CPUE) (see chapter 2 for more details). The analysis was based on one set of locations (with and without fish); this is unlike the analysis in chapter 2, as *Bosmina* and *Daphnia* do not exist in all lochs, and separating them into three groups may result in a small sample size and make it difficult to compare groups. However, other differences in size and depth were controlled in the analysis.

Cladocerans were caught using a plankton net as part of a wider sampling of the zooplankton community (see chapter 2 for more details). Samples were concentrated to a minimum sample size and preserved in a methanol–sucrose solution (Haney & Hall, 1973). Sucrose was added to prevent damage to the cladoceran carapace and reduce the loss of eggs from brood chambers due to osmotic ballooning after death in the cladoceran species (Haney & Hall, 1973). Species were identified based on their morphological characteristics according to (Edmondson, 1959; Scourfield & Harding, 1966). The densities of *Bosmina* and *Daphnia* were obtained in each loch based on data from chapter 2. Samples were collected from 30 sites from two occasions in April and May in 2014 and 2015 (Appendices 3.1 and 3.2).

3.3.2 Fecundity parameters

I selected the two most common cladoceran species in our samples for quantification of life history parameters: *Bosmina coregoni obtusirostris* (Sars 1861) and *Daphnia hyalina galeata* (Sars, 1864). Data for another species, *Daphnia longspina* e included in one part of the analysis.

The proportion of gravid females was determined as the number of egg-bearing females among a haphazardly selected sample of 50. Ten gravid females of each species were selected haphazardly from each population to estimate clutch size, by counting the number of eggs or small embryos in

the brood chamber of each individual. The major and the minor axis of one haphazardly chosen egg in each clutch were also recorded to calculate the size of the egg (Lagergren *et al.*, 2002). Body size of *Bosmina* and *Daphnia* was measured from the anterior eye (rather than the anterior carapace, which may be affected by helmet development during cyclomorphosis) to the base spine (Hart & Bychek, 2010). Moreover, the minimum size of mature females (gravid females) of each population was arbitrarily determined as the length of the smallest female in 50 haphazardly selected gravid females (Ślusarczyk, 1997). The number of females carrying ephippia in a cubic meter of water in each population was counted as well.

3.3.3 Variation of environmental factors on North Uist

The following ecological factors were quantified and used from chapter 2: alkaline metals, pH, Chlorophyll a, stickleback mean density (CPUE) and relative abundance of *Pungitius*. Alkaline metals (Na^+, K^+, Ca^{2+}, Mg^{2+}) were closely correlated with each other, and their variation was summarised by using principal component analysis and taking the first principal component (chapter 2).

3.3.4 Data analysis

A two-tailed Pearson correlations were used to quantify correlations between *Daphnia* and *Bosmina* body size and life history parameters.

Generalised linear models (GLMs) with binomial error distributions and logit link functions were used to analyse the variation in the proportion of gravid females between populations. Population was included as a factor. Associations between the proportion of gravid females, and measures of the biotic and abiotic environmental were also examined in this model.

Generalised linear models (GLMs) with normal distributions and identity links functions were used to analyse the variation in the life history parameters between populations. Population was included as a factor.

Unpaired t-test was used to examine the difference in *Bosmina* and *Daphnia* body size life history parameters between lochs with and without fish. Unpaired t-test was also used to examine the difference between lochs with and without fish in the production of eggs and ephippia.

GLMs was checked and validated by visual assessment of residuals' graphs and Shapiro- Wilk normality test ($P > 0.05$) was used to confirm normality. Non-normally distributed data sets were log or square root transformed to achieve normality.

3.3.5 Comparison of top-down and bottom-up controls on life history

I used generalised linear models (GLMs) with normal errors and identity link functions to evaluate the association between the mean cladoceran life history parameters (proportion of gravid females, clutch size and egg size) of different lakes and ecological factors related to top-down and bottom-up

effects, while also controlling for lake size and depth. Non-significant terms were dropped sequentially from the full model until a minimum adequate model was reached. The significance of components was determined by Wald F-tests and non-significant terms were sequentially dropped to generate a minimum adequate model. Simple linear regression was then used to examine the significant terms obtained from Generalised linear models. All statistical analyses were conducted using GenStat 15th edition (VSN International, 2011).

3.4 Results

The results showed that density of *Bosmina* and *Daphnia* populations was not affected by the abundance of stickleback. Across all lochs with fish, the log density of cladocerans was not related to stickleback density (*Bosmina*, r^2= 0.066, $F_{1,21}$=1.51, p =0.233; *Daphnia*, r^2=0.21, $F_{1,15}$=3.94, P =0.066; Fig. 3.1).

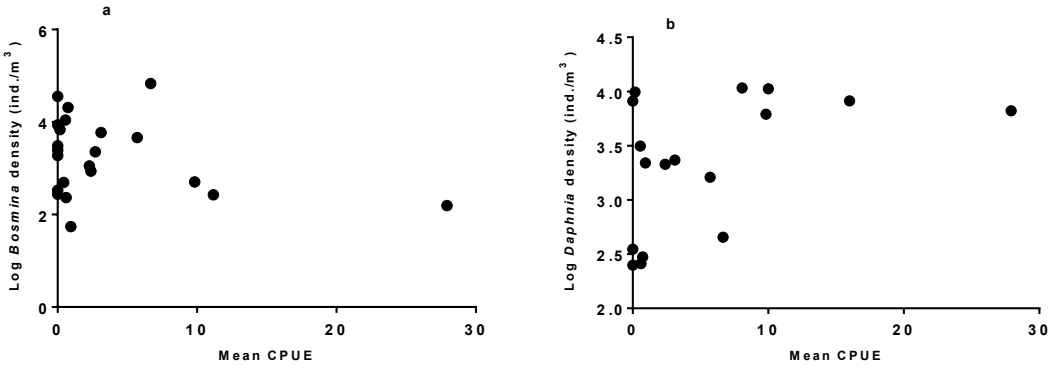


Figure 3.1. The relationship between the mean stickleback density (catch per unit effort: CPUE) and the mean density of (a) 23 *Bosmina* populations and (b) 17 *Daphnia* populations

3.4.1 Fecundity parameters

There was substantial variation among populations in the proportion of gravid females (for *Bosmina*, Wald X^2_{22}=207.7, p <0.001; for *Daphnia*, Wald X^2_{16}=379.1, p <0.001). In general the proportion was more than 50% in lochs with fish, but less in lochs without fish (Appendix 3.1). There was also significant difference in clutch size and egg size between *Bosmina* populations (Wald $F_{22,207}$=10.4 , p <0.001 for clutch size; Wald $F_{22,207}$=15.05 , p <0.001 for egg size) and *Daphnia* populations (Wald $F_{16,153}$=19.8 , p <0.001 for clutch size; Wald $F_{16,153}$ = 58.55 , p <0.001 for egg size).

The size of *Bosmina* and *Daphnia* females showed significant differences between populations on North Uist (Fig.3.2), (Wald $F_{22,207}$ = 5.42, p <0.001, for the size of *Bosmina*; Wald $F_{16,153}$ =26.86, p <0.001 for *Daphnia*).

There was significant correlation between individual body size of *Bosmina* and individual life history parameters (log transformed clutch size, Pearson correlation, r = -0.133, n=230, p =0.043; Square root of egg size, r = 0.245, n =230, p <0.01). For *Daphnia* there was no significant correlation between individual body size and log transformed clutch size (Pearson correlation, r=0.066, n=170, p = 0.3895), while a significant correlation between individual body size and egg size was found, r= 0.2758, n=170, p <0.01). For the association between mean body size and the minimum size of gravid females measured at the population level, there was no pattern for *Bosmina* (Pearson correlation, r=0.3, n=23, P =0.165), but there was a significant negative correlation for *Daphnia* (Pearson correlation, r =-0.63, n=17, P <0.01).

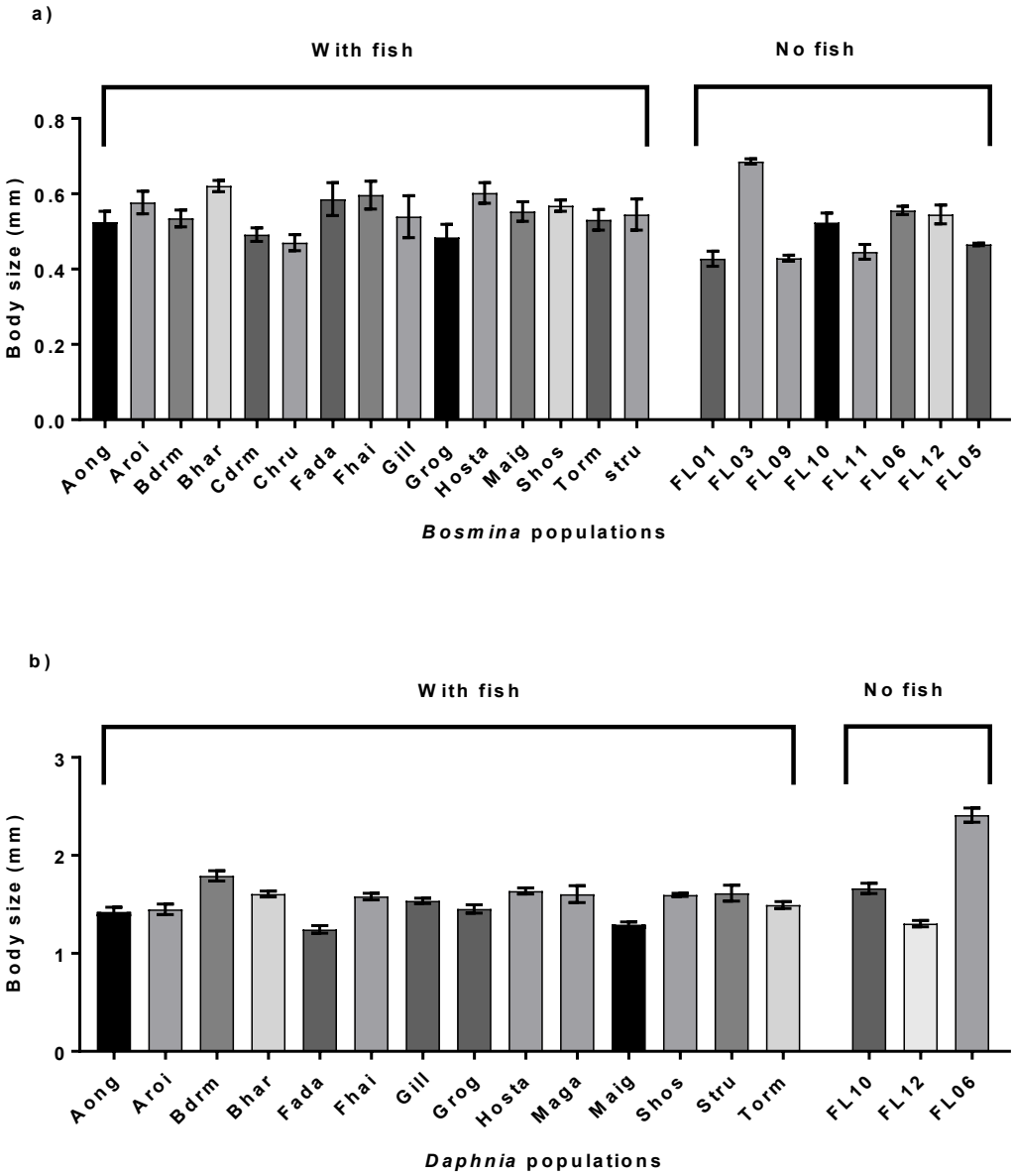


Figure 3.2. *Bosmina* (a) and *Daphnia* (b) mean body size in lochs with and without fish on North Uist. The size of *Bosmina* (n=23) and *Daphnia* (n=17) females showed significant differences between populations on North Uist. Error bars indicate standard error (±SE).

3.4.2 Comparison of top-down and bottom-up constraints on *Bosmina* and *Daphnia*

Stickleback density was the ecological variable that was most consistently associated with variation in cladoceran life history (Table 1 and 2). The proportion of gravid females of both *Daphnia* and *Bosmina* increased with the density of fish (Table 3.1; Fig. 3.3 a, b). There was also a significant negative relationship between the minimum size of mature females and fish density (Fig. 3.3 c, d).

The mean number of eggs in the brood chamber of gravid females (clutch size) increased with stickleback density for both *Bosmina* and *Daphnia* (Table 3.2; Fig. 3.4 and 3.5). Egg size of both *Daphnia* and *Bosmina* also increased with fish density (Table 3.2; Fig. 3.4 and 3.5).

On the other hand, environmental factors indicative of resource variability showed fewer associations with life history parameters. In terms of loch morphometric factors, the reproduction rate of *Daphnia* was higher in large lochs, and females tended to produce small eggs (Table 3.2; Fig. 3.6 a). Positive associations were also detected between depth and the number of gravid females and the egg size of *Daphnia*. However, these relationships were mainly affected by the deepest four lochs among the study locations (Bhar, Host, Miag and Shos). Excluding them from the analysis rendered these two factors insignificant. However, *Daphnia* egg size was more strongly related to the combination of mean CPUE and loch area (Wald $F_{1,14=}4.64$, p =0.04, parameter estimate ±SE, -0.00004±0.00001) than the effect of the loch depth (Wald $F_{1,14}=1.59$, p =0.288, estimated parameter ±SE= 0.002±0.002).

Higher loading values for PC1 resource factors and chlorophyll a appeared to have a positive effect on the number of eggs in the *Bosmina* population (Fig. 3.6 g, h). Conversely, higher pH values were more closely correlated with the percentage of gravid females than with *Bosmina* clutch size (Fig. 3.6 e,f). No single factors had a significant correlation with *Bosmina* egg size and *Daphnia* clutch size. The percentage of *Pungitius* also had no effect on any of life history parameters (Table 3.2).

Table 3.1. Results from the GLMs regarding the association between the proportion of gravid *Bosmina* and *Daphnia* females and other environmental factors. The response variable, number of gravid females was modelled with a binomial distribution and a logit link function. Probability values which are < 0.05 are in bold type.

Response	Fitted model	χ^2	df	*P*	Estimate ± SE
Bosmina gravid females	CPUE	67.11	1	**<0.001**	0.13± 0.016
	Loch area	3.74	1	0.053	-0.01± 0.005
	Pungitius	3.01	1	0.083	0.008± 0.005
	Chl a	1.14	1	0.286	
	Mean loch depth	0.719	1	0.397	
	PC1 alkaline metals	0.65	1	0.419	
	pH	0.65	1	0.419	
Daphnia gravid females	Mean loch depth	55.92	1	**<0.001**	0.64 ± 0.16
	CPUE	16.54	1	**<0.001**	0.05 ± 0.012
	pH	15.11	1	**<0.001**	0.64 ± 0.16
	Chl a	3.38	1	0.066	0.009 ± 0.005
	PC1 alkaline metals	2.93	1	0.087	
	Loch area	2.35	1	0.126	
	Pungitius	0.861	1	0.353	

Table 3.2. The results of GLMs regarding the relationships between *Bosmina* and *Daphnia* life history traits (clutch size and egg size) and four putative environmental explanatory variables (density of sticklebacks, relative abundance of *Pungitius*, pH and loch area and loch depth). The models were fitted with normal errors and identity link functions. Probability values that are < 0.05 are shown in bold type.

Response	Fitted model	Wald F	df	*P*	Estimate ±SE
Bosmina clutch size	CPUE	73.37	1,18	**<0.001**	0.21 ± 0.02
	Chl a	7.63	1,18	**0.013**	0.016± 0.006
	pH	6.64	1,18	**0.019**	0.75 ± 0.28
	PC1 alkaline metals	6.31	1,18	**0.022**	0.25 ± 0.09
	Mean loch depth	2.22	1,17	0.155	
	Loch area	1.29	1,16	0.272	
	Pungitius	0.02	1,15	0.878	
Bosmina egg size	CPUE	6.115	1,21	**0.022**	-0.001 ± 0.001
	Chl a	3.929	1,20	0.062	-0.0004 ± 0.002
	Mean loch depth	3.795	1,19	0.067	0.001 ± 0.004
	PC1 alkaline metals	1.680	1,18	0.210	
	pH	1.551	1,17	0.230	
	Pungitius	0.231	1,16	0.637	
	Loch area	0.113	1,15	0.742	
Daphnia clutch size	CPUE	19.08	1,15	**<0.001**	0.35 ± 0.08
	PC1 alkaline metals	1.031	1,14	0.327	
	Loch area	0.798	1,13	0.389	
	Pungitius	0.408	1,12	0.536	
	Mean loch depth	0.313	1,11	0.585	
	Chl a	0.005	1,10	0.945	
	pH	0.002	1,9	0.964	
Daphnia egg size	CPUE	9.338	1,13	**0.009**	-0.001± 0.0004
	Mean loch depth	8.551	1,13	**0.012**	0.01±0.004
	Loch area	3.494	1,13	**0.044**	-0.001±0.0006
	pH	1.788	1,12	0.206	
	PC1 alkaline metals	0.197	1,11	0.666	
	Pungitius	0.018	1,10	0.896	
	Chl a	0.001	1,9	0.978	

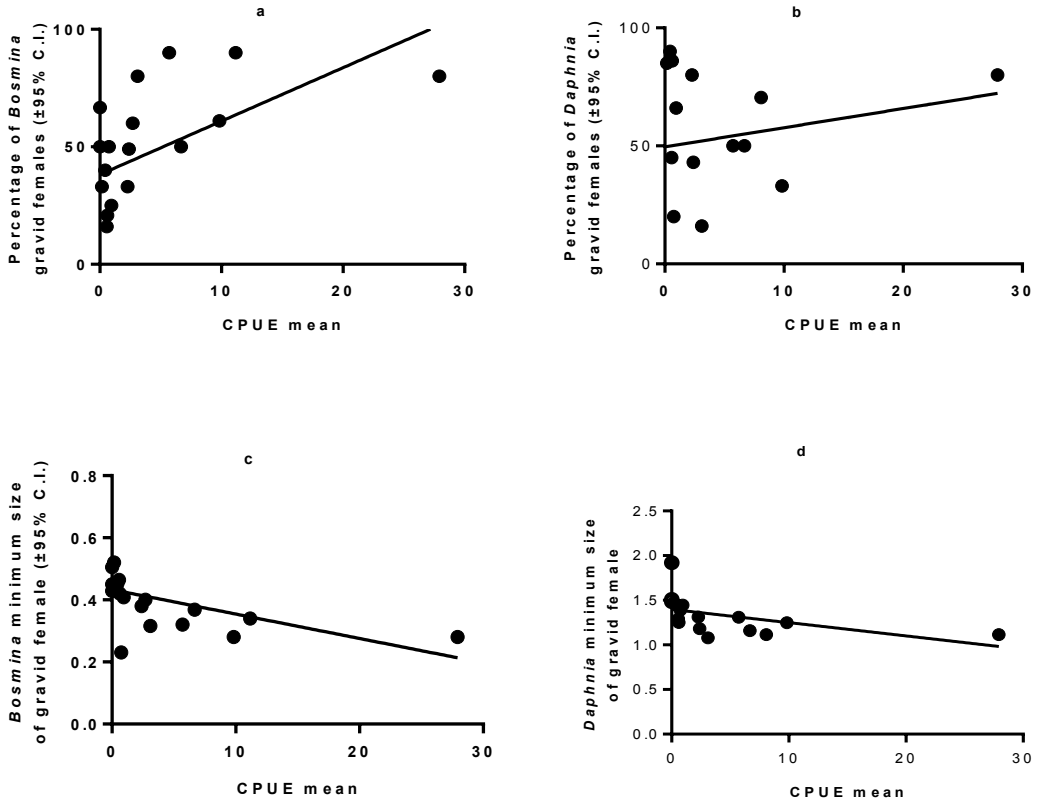


Figure 3.3. The effect of stickleback density on the proportion of gravid females (±95% confidence interval (C.I.) and the size of the smallest gravid female across 17 populations of *Daphnia* and 23 populations of *Bosmina*. Solid lines are the fitted regression lines.

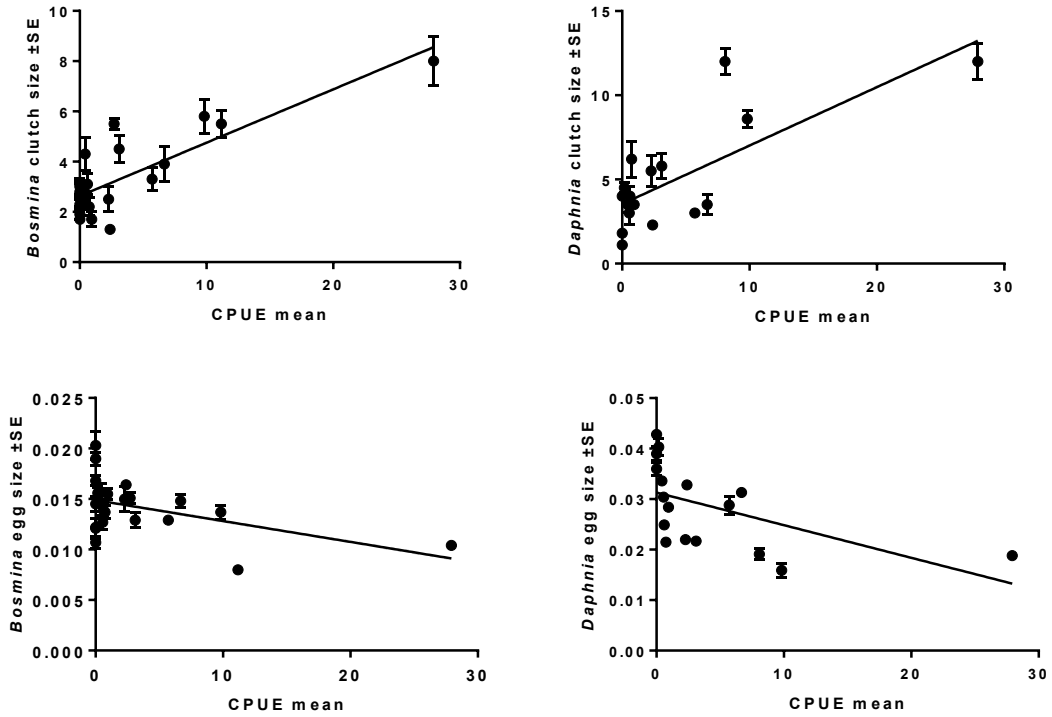


Figure 3.4. The effect of stickleback abundance on clutch size and egg size among 17 populations of *Daphnia* species and 23 populations of *Bosmina.* The values for clutch size and egg size are given with ±SE. Solid lines are the fitted regression lines.

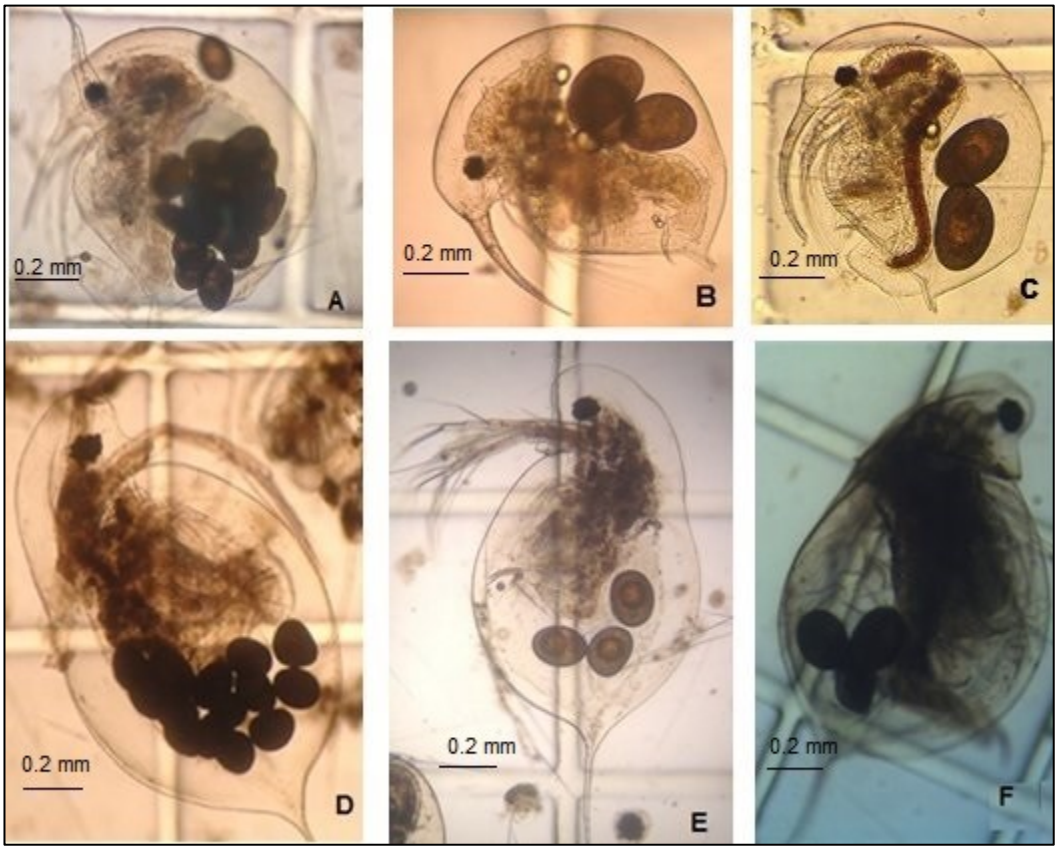


Figure 3.5. *Bosmina* (A) and *Daphnia* (D) with a high number of small size eggs in the brood chamber in lochs with a high density of stickleback (Stru). Large size and small number of eggs for *Bosmina* (B) and *Daphnia* (E) were found in lochs where fish were in very low density (Shos) and (C, F) where fish were absent (FL10).

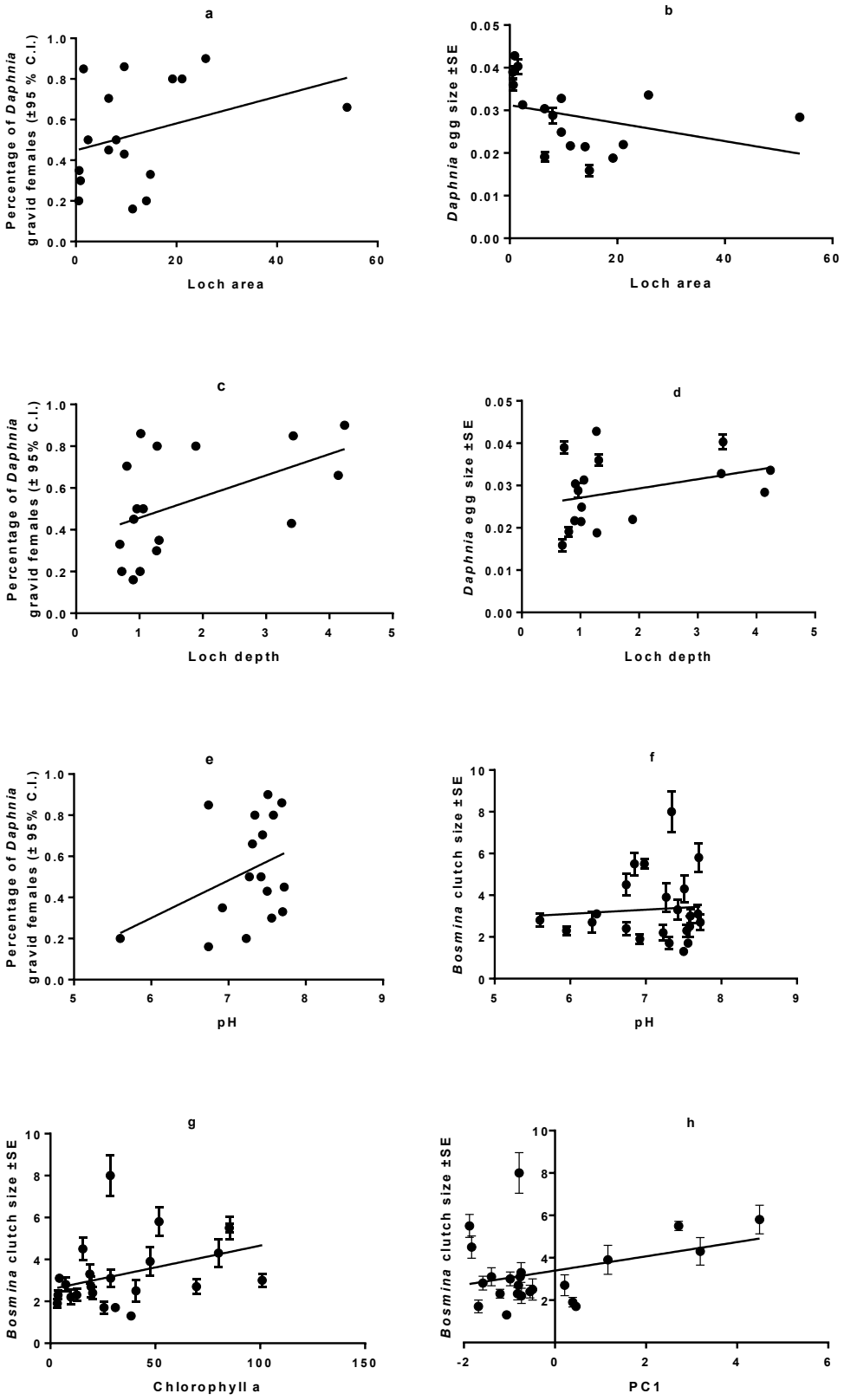


Figure 3.6. Relation between environmental factors and life history parameters among 17 populations of *Daphnia* species and 23 populations of *Bosmina.* The values for clutch size and egg size are given with ±SE. Solid lines are the fitted regression lines.

3.4.3 Cladocera diapause response in the presence and absence of fish on North Uist

Significant differences were observed between lochs with and without fish regarding the production of eggs and ephippia by *Bosmina* and *Daphnia* adult females in April and May 2014 and 2015 (Fig. 3.7 and 3.8).

Daphnia and *Bosmina* populations in lochs without fish were less likely to carry ephippia in the early and late spring. Instead, all gravid females carried eggs in the early spring (Fig. 3.7).

In lochs with fish *Bosmina* females with ephippia were found in significantly higher numbers in late spring than in early spring ($t=2.073$, $df=24$, $p=0.04$; Fig. 3.7 a, b). On the other hand, no *Bosmina* females with ephippium were found in fishless lochs in April, and only 20% of gravid females were found with ephippia in May.

Similarly, in *Daphnia* populations, no ephippium were seen in late spring in lochs without fish, while 18% of gravid females were found in lochs with fish (Fig. 3.7 c, d). However, in comparison with the number of *Bosmina* females with ephippia, no significant difference in *Daphnia* females with ephippia was found between early spring and late spring ($t=0.344$, $df=26$, $p=0.733$).

Finally, it seems quite likely that *Daphnia* and *Bosmina* populations tend to have a short life cycle that ends with the production of resting eggs in large quantities in early spring and increasing in later spring. Conversely, populations in the presence of fish presence continue to produce offspring, suggesting a long life cycle with least signs to produce resting eggs in late spring.

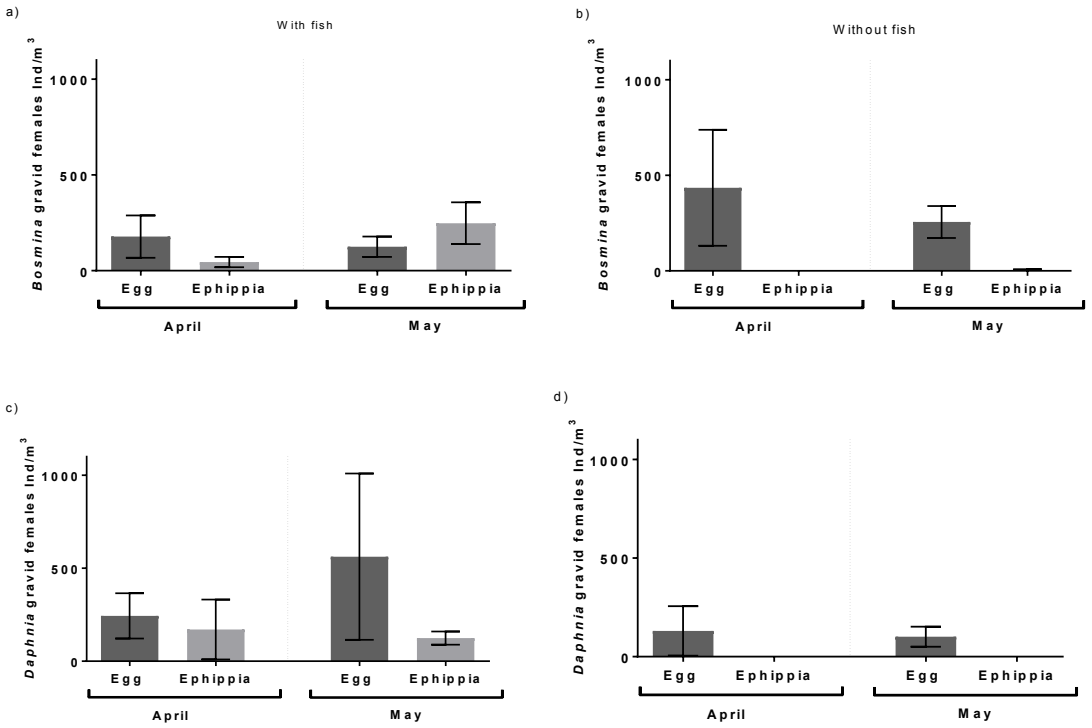


Figure 3.7. A comparison between the mean density SE± of *Bosmina* (a, b) and *Daphnia* (c, d) carrying eggs or ephippia in lochs with fish (a, c) and lochs without fish (b, d) in two times periods, April and May 2014, 2015 on North Uist.

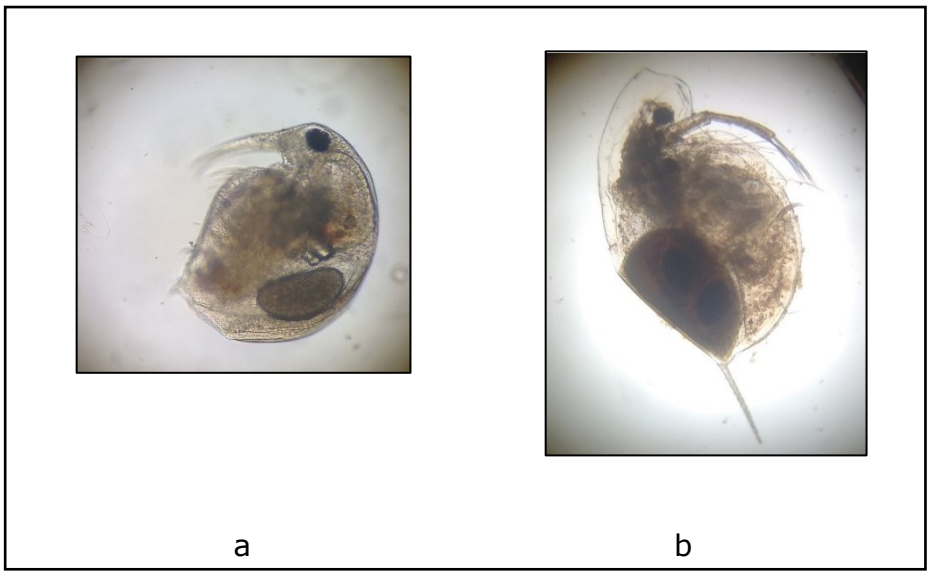


Figure 3.8. Ephippia of *Bosmina* (a) and *Daphnia* (b) species on North Uist during April and May (2014 and 2015).

3.5 Discussion

Variation in reproductive strategies of cladocerans on North Uist was clearly more strongly associated with variation in predation, than with variation in resources, suggesting strong top-down control of life histories. In spite of no direct effect of stickleback presence on cladoceran density in the study, a strong impact of fish presence was observed on cladoceran reproductive parameters on North Uist. Our results agree with what is predicted by life history theory that under predation, populations tend to increase the production of eggs to have more offspring and females tend to mature early and achieve a short life cycle which may increase the survival rate (Stearns, 1976). The increase in mean clutch size in lochs with fish indicates more effort being invested in producing small eggs, which may give the chance to small juveniles to avoid predators.

Although reproductive variation in cladocerans was strongly associated with indices of predation risk, it was not completely independent of other environmental variation, including geomorphological variation such as depth, suggesting a role for other forms of control on cladoceran life histories.

It has been well established that fish have a strong effect on the reproduction of zooplankton, and this may cause feedbacks to enhance their reproductive rate (Ślusarczyk, 1997; Riessen, 1999). Despite predators favoured selection to large females bearing eggs (Maszczyk & Gliwicz, 2014), the proportion of gravid females of *Bosmina* and *Daphnia* increased remarkably in the presence of fish. Simultaneously, increasing the production of eggs in the presence of fish was probably an adaptive response to substitute removing of large females, by potentially bearing more eggs.

It may be that the immediate demographic effect of predation on density is offset by the increased reproductive rate in populations that experience predation (Ślusarczyk, 1997), leading to no net change in population size. Indeed changes in reproductive rates in predated populations could have happened as a response to the extended reduction in population density under predation, rather than to the direct threat of predation (George & Edwards, 1974; Ślusarczyk, 1997).

No strong evidence that the average size of the cladocerans on North Uist was associated with presence and abundance of fish. This disagree with studies suggested that fish exploit size-selective predation (Brooks & Dodson, 1965; Galbraith Jr, 1967) on large cladocerans. However, the positive correlation between size and number of eggs may suggest that the tendency to enlarge the size and hence volume of the brood chamber to hold more eggs is favoured under intense predation (Lagergren *et al.*, 2002). A unique case of large *Daphnia magna* was found to survive under variable fish predation and was assumed to a rapid phenotypic shift in size, which decreases at the time of the presumed increase in predatory fish (Lampert, 1993).

The appearance and development of successive generations from egg to adult could be followed by changing numbers in arbitrarily defined size classes (Marroni *et al.*, 2016; Martin, 2017). Our observation of minimum size of gravid females in current study is consistent with the theoretical expectation that predation often causes a reduction in the age and the size at first reproduction (Castro *et al.*, 2007; Tolardo *et al.*, 2016). The results agree with Taticchi *et al.* (1992) who suggested that *Daphnia galeata* and *Bosmina longirostris* cycles may begin with small individuals and that large size individuals only appear when the population is established.

Our results agree with Gliwicz & Boavida (1996) who revealed the variability in maturation size of cladoceran species in lakes stocked with fish compared with fishless lakes. On the other hand, our results disagree with a study by (Reissig *et al.*, 2006) which hypothesised that fish may cause a homogenised cladoceran community in size as a consequence of removing large species. The strategy of reproduction at smaller size may increase the chance of released offspring completing their life cycle and producing offspring of their own before being eaten by predators (Mikulski, 2005).

Predation was also associated with the egg size of planktonic cladocerans. Reduction in egg size is a common phenomenon when fish predation is high (Lampert, 1993). One of the interpretations of this strategy is that small eggs may hatch into small neonates which may be less vulnerable to predation (Goulden *et al.*, 1987). Conversely, Cladocera in fishless lochs tend to have few large eggs and produce large offspring where high risk to be preyed by dominant large invertebrates in the absence of fish (Lüning, 1992).

Most of the formation of ephippia (resting eggs) in this study was in late spring. It is most likely that ephippia production is an evolved response to very low survival probability of parthenogenetic cladoceran females at this time of the year, perhaps in response to chemical cues released by fish, high temperature and/or long-day photoperiod (Pijanowska & Stolpe, 1996). However, a more plausible explanation is that predation pressure increases remarkably in late spring and summer when high density of planktonic cladocerans occurs in this time of the year (Townsend *et al.*, 1986; Velthuis *et al.*, 2017)). This may be associated with remarkable blooming of phytoplankton in this period, which is a major food source of zooplankton (Velthuis *et al.*, 2017). This in turn increases mortality of

zooplankton (Lampert *et al.*, 2014), and may initiate the diapause stage (Lees, 2016).

Finally, besides the strong evidence of the effect of predation pressure on the reproductive capacity of cladocerans in our study, there were some indications that other environmental factors have effects on fecundity parameters. Despite little discernible effect of pH on reproductive capacity (Zervoudaki *et al.*, 2013), it was related to water chemistry and food content. *Bosmina* reproduction was more sensitive to chlorophyll a content which is indirectly affected by variation in nutrients and minerals concentration of water needed for phytoplankton growth (Zervoudaki *et al.*, 2013). Previous investigations have shown a significant increase in egg production and egg characteristics coincident with the chlorophyll a peak in water particularly in spring (Guisande & Gliwicz, 1992). However this association between reproductive parameters and food limitation was not clear for all populations. In Stru, where the highest *Bosmina* clutch size was recorded, there was a low value of chlorophyll a compared with other lochs (chapter 2). Clutch size and egg size of *Daphnia* in Stru were also the highest among other lochs. The highest fish density was also recorded in this loch, and this may suggest an interaction between biotic and abiotic factors in determining reproduction. However it seems that life history responses to fish are not displayed under severe resources limitation (Hülsmann *et al.*, 2004; Gliwicz & Maszczyk, 2007).

On the other hand, lochs morphometry (depth and area) seems also to affect *Daphnia* fecundity. My results agree with a study by (Bernatowicz & Pijanowska, 2011) which suggested that *Daphnia* was more sensitive to avoid predators in water layers even when there are plenty of food available to their trophic levels that they exist in. Cladoceran species can swim

actively and cover large distances enabling them to select better habitat with sufficient oxygen and food concentrations, where they can maximize their fitness and minimize predation risk (Taticchi *et al.*, 1992).

In conclusion, our results suggest that top-down effects have a major impact on cladoceran life histories, with higher predation favouring faster life histories and higher reproductive investment. This pattern is little affected by variation in resource conditions. Overall this is consistent with the 'life-dinner principle'. It is well known that endangering life is different from endangering hunger. Maciej Gliwicz Z. wrote, "The risk of becoming subject to predation may become fatal within seconds, while the risk of starvation may persist for days or weeks with future compensation always being possible" (Maciej Gliwicz, 2002, p 2298).

Chapter 4. Transgenerational life history variations in cladocerans on North Uist

4.1 Abstract

Laboratory life-table experiments were conducted to quantify if differences in key life history features of the freshwater cladoceran crustaceans *Bosmina coregoni obtusirostris* (Sars 1861), *Daphnia hyalina galeata* (Sars, 1864) and *Daphnia longispina* (O. F. Müller, 1776) in lochs with and without fish will remain consistent after three generations.

Differences were substantially similar to those measured in wild-caught cladocerans. *Daphnia* and *Bosmina* from lochs with fish tended to release their offspring faster and at a smaller size than did those from fishless lochs. Females showed a significant reduction in age and size at first reproduction in *Daphnia* and *Bosmina* populations in lochs with fish when compared with fishless lochs. Moreover, both *Daphnia* and *Bosmina* offspring from lochs with fish grew faster in their first 10 days. In contrast to previous studies, size of offspring was not related to size of females, but size at birth was strongly related to growth rate, and the age and size at first reproduction. Differences in several life history parameters between populations remained consistent after three generations in common garden conditions, strongly suggesting that they have an evolved, heritable basis.

4.2 Introduction

Evolution in prey is driven mainly by the presence and absence of predators (Reznick *et al.*, 1990; Walsh & Reznick, 2008). Many species respond to the

presence of predators by altering their life history parameters (Abrantes *et al.;* Macháček, 1993; Abrams & Rowe, 1996; Caramujo *et al.*, 1997; Walsh & Post, 2011; Packer & Walsh, 2017). However, it is seldom clear whether these responses in life history are phenotypic plasticity (in which phenotypic expression of one genotype produces more than one phenotype depending on environmental context) or evolved from genetic differences (Walsh *et al.*, 2015; Packer & Walsh, 2017).

Here I examine variation in life history parameters between cladocerans from evolutionarily young lochs with and without fish on the Scottish island of North Uist. Cladocerans provide a classic example of plastic responses to predation, but to what extent, responses are genetically hard-wired or 'canalised' is seldom clear. It is important to know this, partly just to understand the nature of adaptation (can it be achieved only by plasticity?), but also because the extent of evolutionary responses define the potential for predator-prey coevolution and eco-evolutionary feedbacks (Palkovacs & Post, 2008; Becks *et al.*, 2010; Bolnick *et al.*, 2011). There has been a great of interest in the latter in the past decade, especially in the context of predator-prey interactions. Evolutionary variation on contemporary timescales has been predicted as an essential driver for ecological change (Koch *et al.*, 2014). Various pronounced impacts on prey communities may be seen under predation, in terms of rapid evolution (microevolution) (Pijanowska *et al.*, 1993; Yoshida *et al.*, 2003). It is now recognised that evolutionary change can affect species interactions within a few generations, and that ecological and evolutionary change may interact reciprocally (Koch *et al.*, 2014). It seems likely that the potential for such feedbacks will be greater in circumstances where prey exhibit permanent, fixed differences in response to predators than plastic, contextual ones.

Previous work has shown that predation pressure by fish in late spring and summer has an important impact on cladocerans and may cause high mortality (Townsend *et al.*, 1986; Stibor, 1992; Romare *et al.*, 1999). Responses by cladocerans include morphological responses such as the development of spines, as well as changes in life history. Cladocerans in lakes with fish tend to exhibit fast population growth, maturing early and producing large clutches (Fay *et al.*, 2016). In the absence of fish, cladocerans may be exposed to predation by large invertebrates, which can result in large size at birth and delaying maturity (Lüning, 1992). In general life history theory suggests that increased rates of mortality of adults will favour the evolution of faster life histories, with more rapid growth, and younger age and smaller size at maturity (Jones, 2011). The variability in ontogenesis of species may result in shaping the duration of embryonic development (Kotov & Boikova, 2001). Variation in life-history parameters, both within and between populations, can be attributed mainly to size at birth and the number of offspring (Everett, 1991).

Studies have revealed that changes in life history parameters such as greater investment in fecundity are a direct plastic response to fish kairomones (Walsh *et al.*, 2016; Mikulski & Pijanowska, 2017). Thus, it is clear that phenotypic plasticity is an important component of the evolutionary response by cladocerans to predation, but it is less well known whether (evolved) differences between populations experiencing different levels of predation persist in cladocerans raised in common conditions without experiencing stimuli such as kairomones. Intermediate effects mediated by maternal effects are also possible, if the effect of predation associated stimuli are transmitted to subsequent generations in mothers' pouches by the alteration of the maternal epigenome (Harris *et al.*, 2012),

but these effects should decay rapidly from generation to generation in the absence of ongoing stimuli.

However, many experiments on life history have reported a correspondence between models explaining the life history tables of species, even when the species show differences in size and fecundity (Svensson & Gorokhova, 2007).

In the previous chapter, I presented field evidence that *Daphnia* and *Bosmina* exhibit a shift in their life history parameters (probability of being gravid, size and number of eggs in the brood chamber, minimum size at reproduction) as a main response to the risk of fish predation. However, these changes were not tested experimentally, and could be plastic, evolved or a mixture of both. Furthermore, important life history parameters related to development, such as duration of embryonic duration, size at birth, age and size at first reproduction are not easy to quantify from field data. Here I avoided these difficulties by capturing cladoceran females in the wild, and rearing them through three generations in the lab.

4.3 Methods

Female *Bosmina coregoni obtusirostris*, *Daphnia hyalina galeata* and *Daphnia longispina* were collected from ten lochs on North Uist in May 2016. Data for both species of *Daphnia* were included in one part of the analysis. Samples were collected using a 52-µm plankton net equipped with a bottle-end towed for a specific distance. Five lochs had fish while other five had no fish (Table 4.1).

Live cladocerans were transported to a laboratory at the University of Nottingham. To establish a first lab generation, ten live females of *Bosmina* and *Daphnia* carrying eggs (in embryo stage zero) were selected from each

population. Eggs in embryo stage zero have no distinctive features of embryos, have large central fat droplets and are slightly ellipsoidal to spherical (Giardini *et al.*, 2015). Females from the wild were kept first individually in small 150 ml vials filled approximately three-quarters full with water brought from the loch from which the sample was originally collected. The water was filtered and well aerated before use with air sources in separate tanks. The water was also replaced daily to maintain its quality. The vials were maintained under controlled temperature and photoperiod similar to those of their original locations.

A concentrated prepared mix of combined 5 different concentrated species of live algae: *Nannochloropsis, Tetraselmis, Isochrysis, Pavlova, Thalassiosira weissflogii* (Reefphyto Ltd) served as a food resource. The food concentration was adjusted to 10^5 to 10^6 cells/ml which is the normal optimal algal density for cladoceran culture (Lavens & Sorgeloos, 1996). The number of eggs in each female from the wild was counted. The duration of embryo development, was calculated as the time elapsed between the appearance of parthenogenetic eggs in the brood pouch until the time when the embryos were released (Mikulski & Pijanowska, 2017). After releasing offspring of the first generation of *Daphnia* and *Bosmina* from the wild, 10 haphazardly chosen offspring from each population were moved to a single vial, each neonate representing a signal individual belonging to each mother in each population. In most of fishless populations there was only one neonate produced by each mother. The life history variation in subsequent generations of *Bosmina* was investigated while *Daphnia* did not survive well enough to be included in these measurements. I examined characteristics of the third generation of *Bosmina* that originated from the third clutch of the second generation of the females brought from the wild (Fig. 4.1). The

second and third-generation were reared in the lab in 150 ml vials filled with dechlorinated Nottingham tap water (approx. pH 7.5) that was stored in tanks with a biological filter (Fluval, Askoll, Italy) and an air source. Newly arrived individuals were acclimated slowly by replacing water in the vials from the new source during the first week. All specimens were housed in a room with controlled temperature (13.5 ± 1℃), a photoperiod of 16 hr light/8 hr dark and food quantities mimicking the natural habitat in North Uist. The specimens were monitored twice daily. The main purpose for rearing three generations is to let enough time for animals to acclimate under standard conditions and to ensure that each individuals is independent (Ebert *et al.*, 1998).

For both *Daphnia* and *Bosmina*, the life-history traits investigated were the number of eggs and the duration of embryonic development in the first clutch size of the first generation, the size of neonates at birth and the length increment of the instars (growth rate). The latter was calculated by measuring the difference in body length after the first ten days between the released juvenile instars and instars reaching the stage of adolescence (Streit *et al.*, 2013). Age and size of instars when they first reproduce and carry their first clutch of eggs were also measured. Body size of cladoceran adults from the wild was also measured. Body size of adults and instars of *Bosmina* and *Daphnia* was measured from the anterior eye (rather than the anterior carapace, which may be affected by helmet development during cyclomorphosis) to the base spine (Hart&Bychek, 2010). All the measures of offspring were done by picking each individual carefully by fine pipette to a separate Petri dish under a dissecting microscope to take photos for measuring the body size using ImageJ 1.48v software (National Institute of Health, USA).

4.3.1 Statistical analysis

Pearson correlations were used to evaluate the clutch size of cladoceran (*Bosmina* and *Daphnia*) between populations from the wild in 2016 and those collected in (2014 and 2015, chapter 3). Shapiro- Wilk normality test ($P > 0.05$) was used to confirm normality. Non-normally distributed data sets were log transformed to achieve normality. Unpaired t-test was used to examine the difference in *Bosmina* and *Daphnia* life history parameters between lochs with and without fish in the first generation produced directly from populations collected from the wild. Generalised Linear Models (GLMs) with normal errors and identity links were also used to evaluate the relationship of life history traits in the first generation produced directly from *Bosmina* populations collected from the wild and those reared in the lab. This was to explore the relationship between generations to quantify transgenerational responses in these species. GLMs was checked and validated by visual assessment for residuals' graphs. Finally, life history traits in this experiments depend on sampled collected in 2016. To compare the outcome of this experiment with other measurement of life history trait in chapter 3, I correlated clutch size measurements in this chapter with those measured in chapter 3. The reason for that is to see if the reproductive status of cladocera in 2016 has changed or if it is still in the same approach.

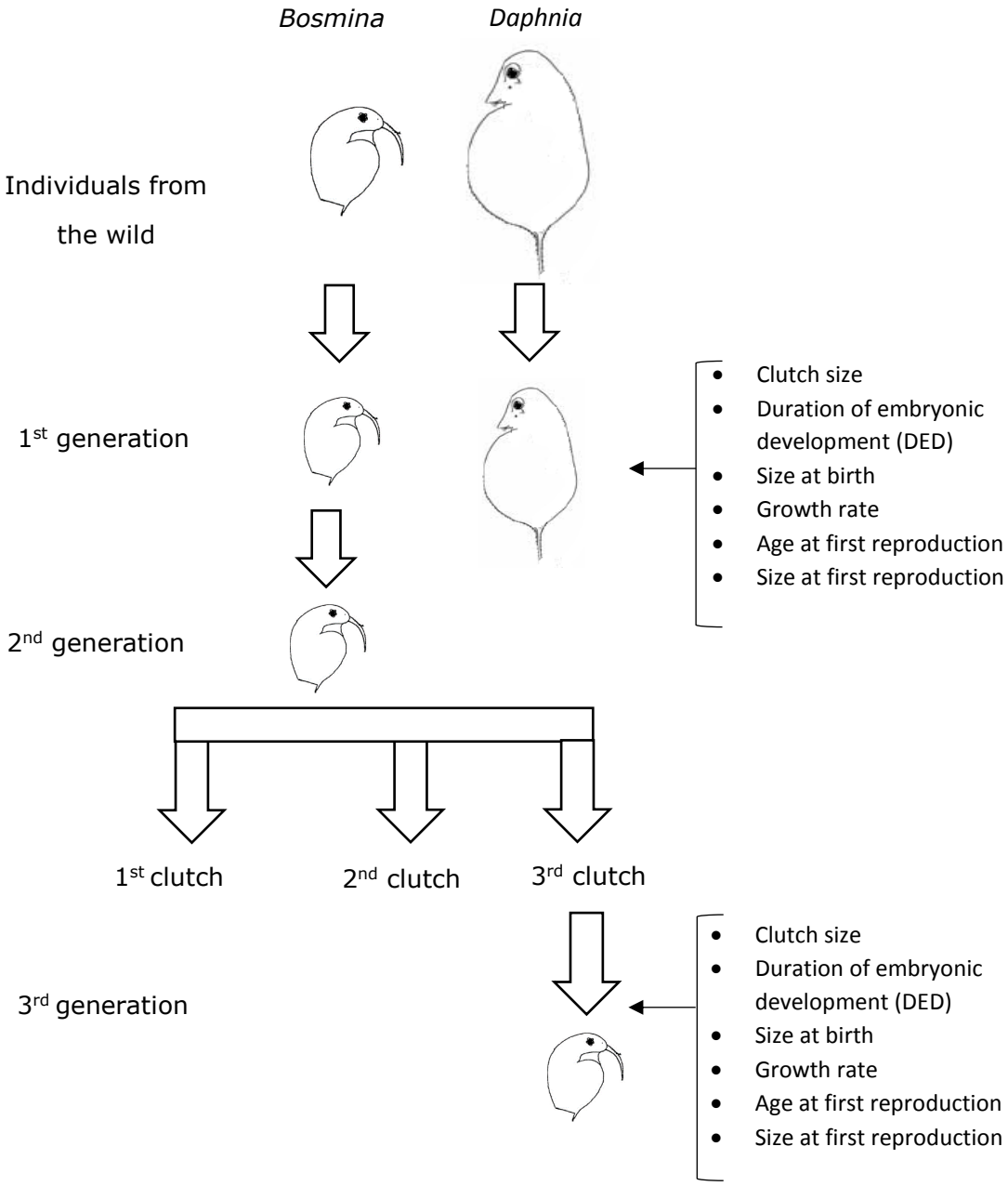


Figure 4.1. Experimental design shows one generation of *Daphnia* and three successive generations of *Bosmina* individuals from North Uist. The first set of investigations was on the (first generation) offspring originated directly from wild gravid females. The second generation was used to produce three clutches of parthenogenetic eggs. The second set of life-history investigations was in the third generation, which originated from the third clutch of the second generation.

4.4 Results

4.4.1 Clutch size variation and interannual correlations of *Bosmina* and *Daphnia* on North Uist

Correlations (Pearson correlation) of the clutch size of *Bosmina* and *Daphnia* individuals from the wild in May 2016 (Table 4.1) and those recorded in (May 2014 and 2015, Chapter 3) have more constant patterns (Fig. 4.1 a, b) ($r = 0.8$, $n = 10$, $p < 0.001$ for *Bosmina*; $r = 0.763$, $n = 6$, $p = 0.005$ for *Daphnia*).

Table 4.1. Cladoceran clutch size ±SE collected from five lochs with fish (in bold) and five lochs without fish in May 2016 on North Uist.

Loch	Loch type	*Bosmina coregoni*	*Daphnia hyalina*	*Daphnia longispina*
Bdrm	With fish	3.3±0.45	-	4.5±0.62
Fhai	With fish	2.8±0.29	4.5±0.17	-
Grog	With fish	3.4±0.45	5.6±0.50	-
Stru	With fish	8.2±0.33	7.7±0.21	-
Torm	With fish	3±0.21	3±0.15	-
FL01	No fish	1.6±0.16	-	-
FL03	No fish	1.1±0.10	-	-
FL06	No fish	2.6±0.27	-	2.3±0.15
FL10	No fish	1.1±0.10	1±0.00	-
FL12	No fish	2.5±0.22	1.3±0.15	-

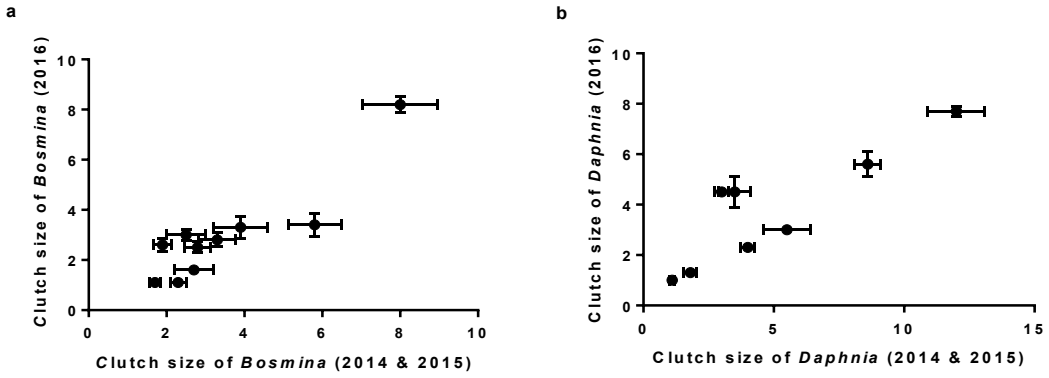


Figure 4.2. Correlation between the clutch size (±SE) of *Bosmina* (a) and *Daphnia* (b) species during April and may in 2014 and 2015 (Chapter 3) and those collected from the wild in May 2016, among five lochs with fish and five without for *Bosmina*, and five lochs with fish and three without for *Daphnia*.

4.4.2 Life history comparisons among *Daphnia* and *Bosmina* populations from North Uist

Life history traits of the first generation in *Bosmina* and *Daphnia* females collected from the wild showed significant differences between lochs with and without fish. All measured traits had higher values in lochs without fish, except growth rate and clutch size, which were lower (Table 4.2; Fig. 4.3 and 4.4).

Table 4.2. t-test results comparing life history traits between lochs with and without fish for the first generation of *Bosmina* and *Daphnia* collected from the wild.

	Bosmina			*Daphnia*		
Life history trait	t	d.f	*p*	t	d.f	*p*
Clutch size	3.77	8	<0.01	2.9	6	<0.025
Duration of embryonic development	4.36	8	<0.002	2.83	6	<0.03
size at birth	2.19	8	<0.03	3.51	6	<0.01
Growth rate	2.12	8	<0.03	4.22	6	<0.01
Age at first reproduction	9.87	8	<0.001	3.63	6	<0.01
Size at first reproduction	4.29	8	<0.003	3.44	6	<0.01

4.4.3 Life history traits associations for *Bosmina* and *Daphnia* on North Uist

There was no significant correlation between the mean size of mothers in a population and the size of newborn offspring (Wald $F_{1,8}$= 0.22, p = 0.65 for *Bosmina*; Wald $F_{1,6}$= 0.39, p =0.56 for *Daphnia*). In *Daphnia*, age and size at first reproduction and growth rate were influenced positively by the size at birth (Wald $F_{1,6}$ = 21.96, p = 0.003 for age at first reproduction; Wald $F_{1,6}$ = 8.08, p = 0.03 for size at first reproduction; Wald $F_{1,6}$ = 33.35, p =0.001 for growth rate).

No association was found between the growth rate of *Bosmina* and size at birth (Wald $F_{1,8}$ = 0.82, p = 0.392), although there was a significant positive correlation between the size at birth and age and size of first reproduction of *Bosmina* (Wald $F_{1,8}$ = 7.5, p = 0.025; Wald $F_{1,8}$ = 9.87, p = 0.014) respectively.

There was also a significant positive correlation between age of first reproduction and size at first reproduction in both *Bosmina* (Wald $F_{1,8}$ = 28.73, p < 0.001) and *Daphnia* (Wald $F_{1,6}$ = 306.4, p < 0.001), while individuals with higher growth rate mature and reproduce earlier (Wald $F_{1,8}$ = 5.12 , p = 0.045 for *Bosmina*; Wald $F_{1,6}$ =172.6 , p < 0.001 for *Daphnia*).

4.4.4 The relationship between life history traits in the wild and in the lab experiment

For some life-history parameters of *Bosmina* populations, values measured on lab-reared individuals from the third generation were significantly correlated with values measured on individuals taken from the wild (number of eggs in the first clutch size: Wald $F_{1,8} = 19.5$, $p = 0.002$; duration of embryonic development: Wald $F_{1,8} = 17.17$, $p = 0.003$; size at birth: Wald $F_{1,8}=19.5$, $p = 0.002$) (Fig. 4.5 a-c). However, less association was observed for other parameters (growth rate: Wald $F_{1,8} = 3.8$, $p = 0.087$; age at first reproduction: Wald $F_{1,8} = 1.92$, $p = 0.203$; size at first reproduction: Wald $F_{1,8} = 4.75$, $p = 0.061$) (Fig. 4.5 c–e).

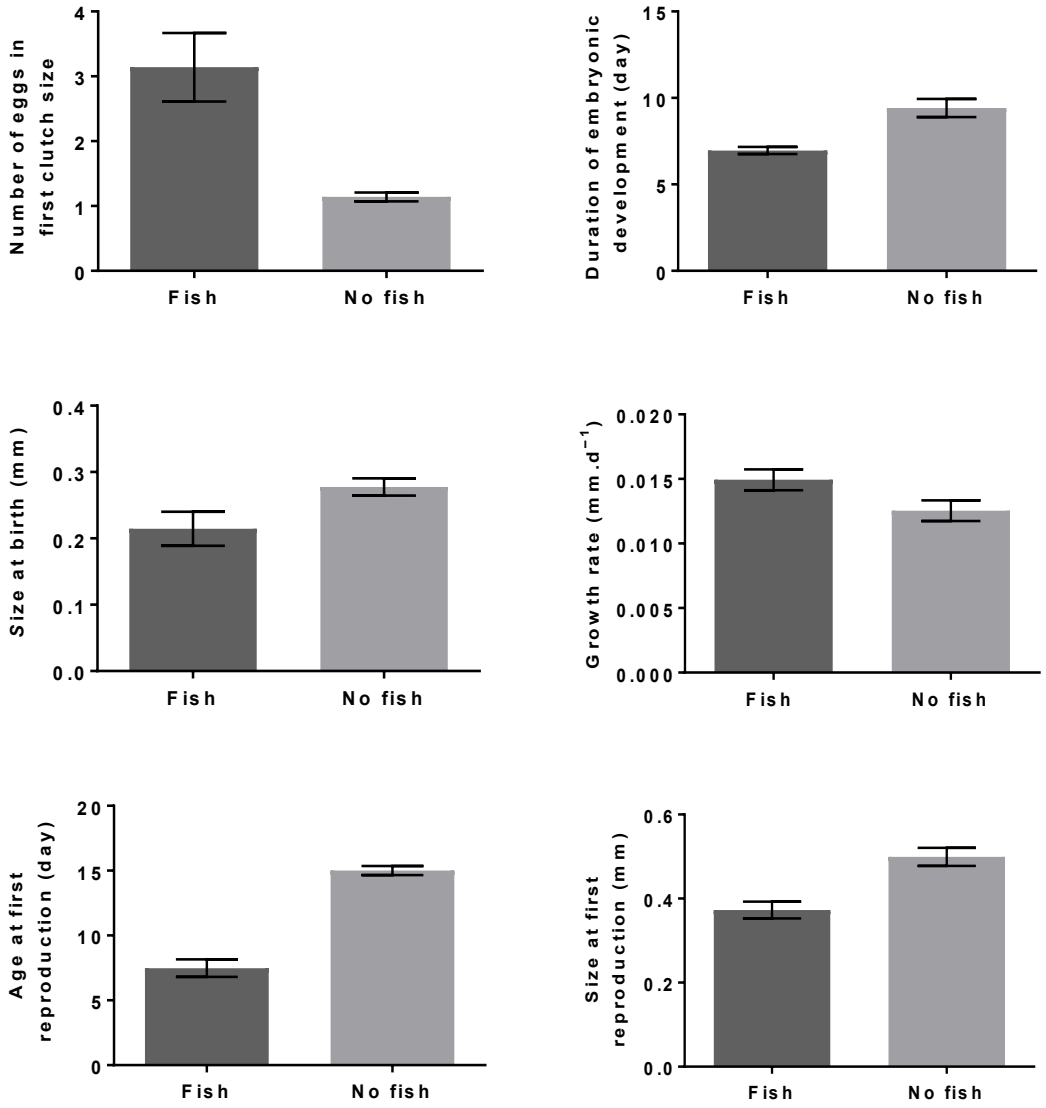


Figure 4.3. Life-history traits of the experimental *Bosmina* populations of the first generation among lochs with and without fish on North Uist. Error bars indicate standard error (±SE).

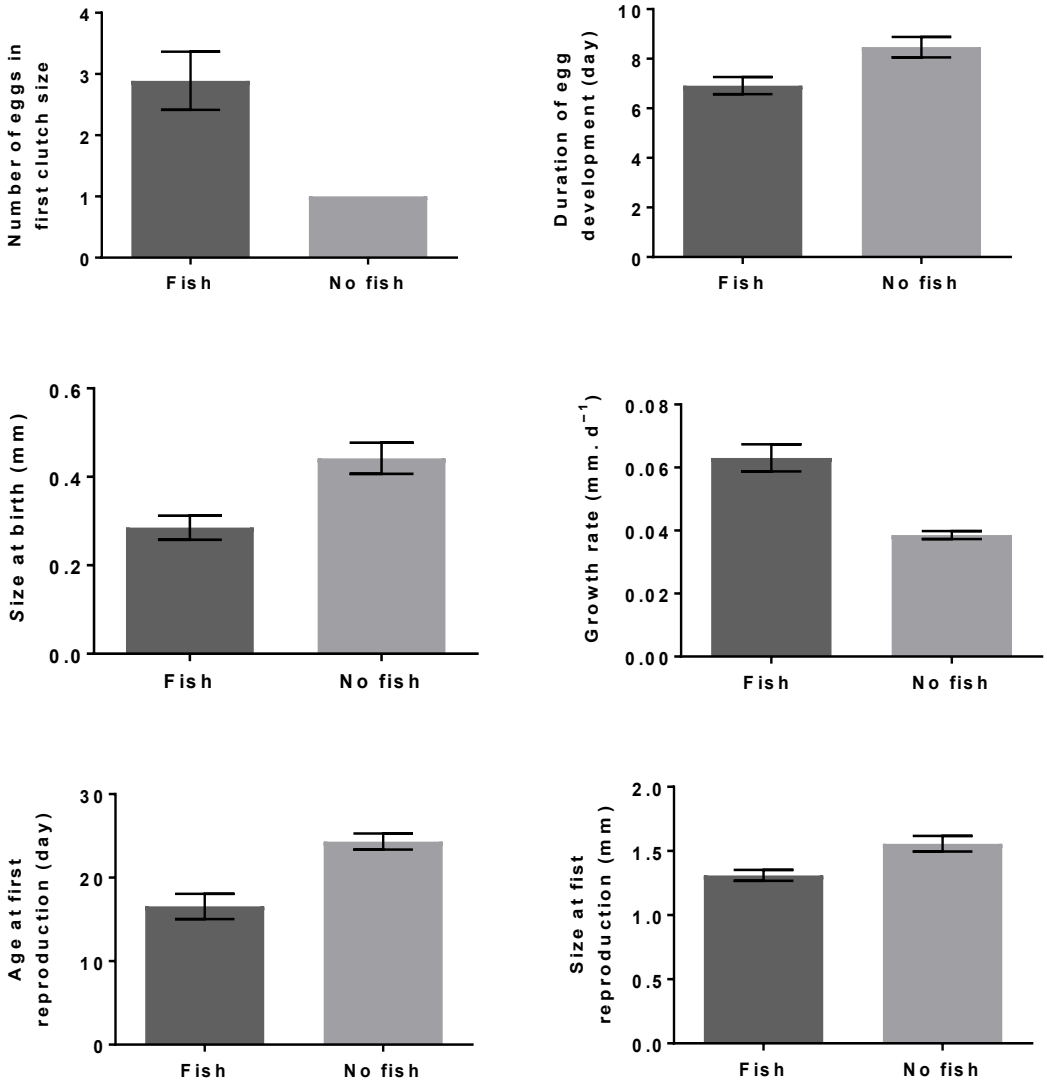


Figure 4.4. Life history traits of the experimental *Daphnia* populations of the first generation among lochs with and without fish on North Uist. Error bars indicate standard error (±SE).

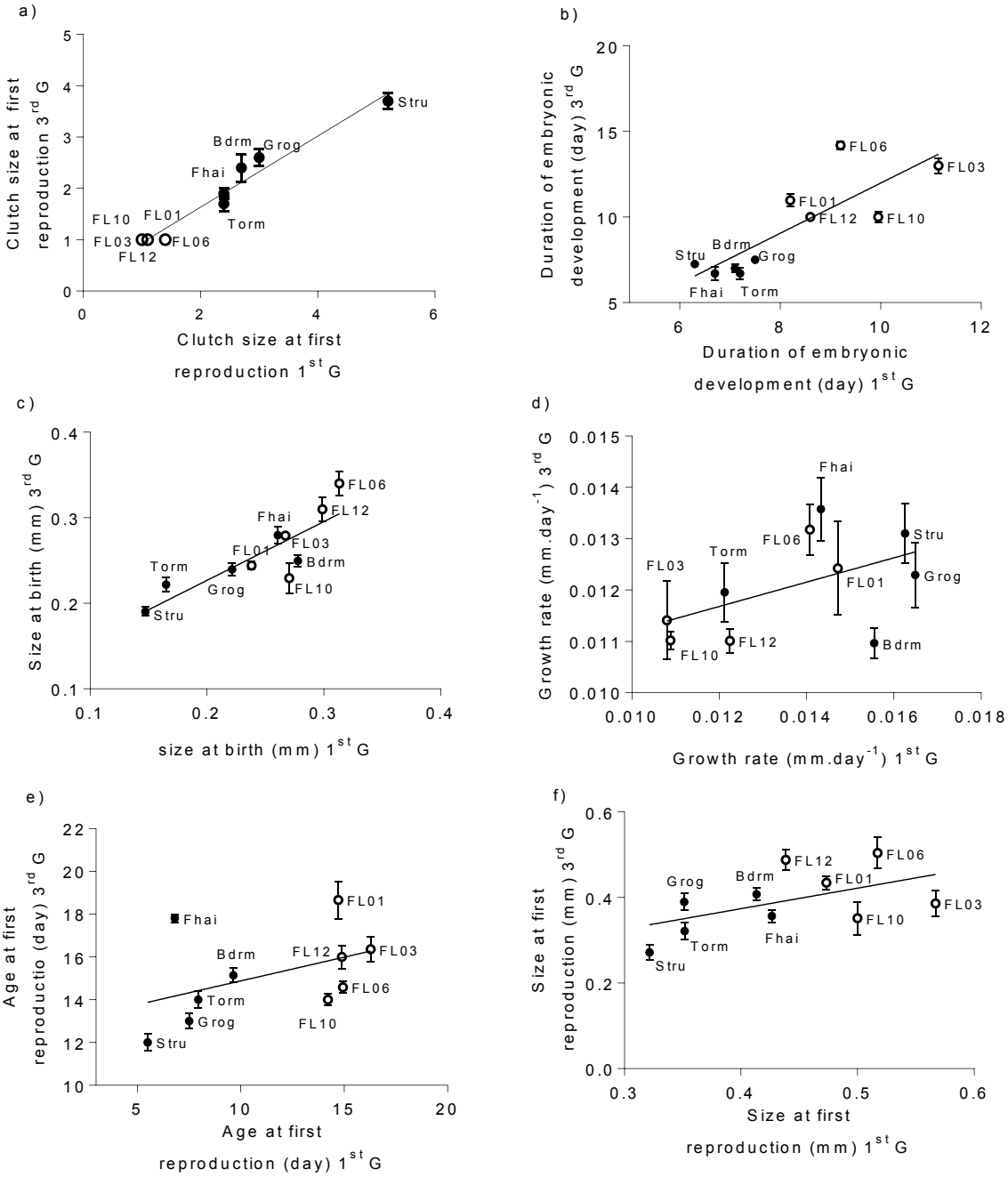


Figure 4.5. Relations of life history-traits between the first generation of wild collected populations (1st G) and the third generation of lab-reared populations (3rd G) of *Bosmina* from lochs with fish (filled circles) and lochs without fish (open circles) on North Uist. Solid lines are the fitted regression lines. Error bars indicate standard error (±SE).

4.5 Discussion

Cladocerans from lochs with fish exhibited life-history traits suggestive of faster lifestyles: faster growth, and maturity at a younger age and smaller size. This is consistent with results of previous experiments (Lynch, 1980; Dorazio & Lehman, 1983; Stibor & Lüning, 1994; Mikulski & Pijanowska, 2017). The clutch size was also higher in the presence of fish. Several of the traits of third-generation offspring reared in common garden conditions in the lab correlated with those of their parents, suggesting evolved differences between populations. This was especially true of traits early in development, including clutch size, duration of embryonic development and size at birth. Correlations with traits exhibited later in development (growth rate and age and size at first reproduction) were weaker or not significant. This makes sense, given the greater opportunity for the environment to affect these traits.

The zooplankton samples collected from the wild for this chapter provide additional information about the community structure and life-history patterns of zooplankton on North Uist. Interannual patterns among cladocerans from lochs with and without fish in 2014 and 2015 were highly comparable to those in our samples in 2016 in terms of clutch size. This provides strong evidence of consistent variation in strategies of cladocera to survive under the influence of different trophic and predation conditions through generations. Clutch size represents a fundamental life-history trait of major importance for individual reproductive success (Ebert, 1994; Rizo *et al.*, 2017). The significant correlation between clutch size in 2014 and 2015 and those measured 2016 allows us to consider the outcome of this chapter as complementary to the investigation of life-history parameters in Chapter 3.

The direct observation of clutch size and embryonic development in the brood pouches of females in our study showed a high number and rapid development of eggs in locations where cladocera are more vulnerable to attack by predators. Successful establishment and rapid increase of a population at risk of predation may be achieved by having large clutch size and decreasing the duration of embryonic development (Ślusarczyk, 1997; Svensson & Gorokhova, 2007). With embryos inside the brood chamber, the female becomes slow and more vulnerable to a predator. The reduction in the duration of embryonic development time of most cladoceran embryos would be favourable for female survival under threat of predation (Valentin & Marazzo, 2004; Svensson & Gorokhova, 2007).

Selection based on size is a crucial cause of evolution in cladocera. In fishless lochs, where there is no risk to large invertebrates from fish (Lovell *et al.*, 2017), small-sized cladoceran offspring are vulnerable to invertebrates (Lynch, 1980), whereas large-sized offspring cannot be devoured by other invertebrates (Lüning, 1992). Animals that live under a high risk of being eaten by fish decrease their size at birth, but grow continuously to give birth for as long they can (Dodson, 1974; Lynch, 1980). This variance in size is not without cost, because decreasing size to avoid fish predation in early lifetime means simultaneously increasing exposure to invertebrates (Lynch, 1980). Subsequently, under this overlap, it is expected that mortality in this environment will be high (Hülsmann & Voigt, 2002). Under these circumstances, minimizing this pressure will be attained by producing more offspring and reproducing early (Walsh & Post, 2012). Our data showed that the evolution of earlier reproduction may stem from the strong association with a faster growth rate, which is well

documented when animals experience a high density of predators (Packer & Walsh, 2017).

The results of the present study showed considerable differences in growth rate between the two types of lochs in *Daphnia* compared with *Bosmina.* This may arise from the fact that high *Bosmina* density in lochs with fish may limit their growth rate. This was consistent with Declerck *et al.* (2003) who found that the increase of intraspecific competition among high densities of small cladocera may result in limiting the growth rate of individuals. Size of the neonates appeared to depend only on the risk of fish predation in the population of origin, and was not related to maternal size. This was contrary to previous findings (Stibor, 1992; Sakwińska, 2002) which have shown small adults give birth to small neonates and that large size at birth results in large size at first reproduction (Sakwińska, 2002). Cladocera adjust their size at first reproduction in two ways: in response to chemical kairomones that are released from fish (Doksæter & Vijverberg, 2001) and by hatching small neonates from small eggs, which then mature to a smaller size (Lampert, 1993; Hanazato, 2001). The early age of first reproduction of *Daphnia* and *Bosmina* in our experimental work contrasts with other studies, which indicated that large size neonates should have a higher probability of early reproduction (Sakwińska, 2002; Streit *et al.*, 2013). The correlation between the small size of offspring after birth and early age and small size at first reproduction in this study may indicate the contribution of another factor which induced the animals to mature early.

Our results suggest that whether variation in traits is hard wired (evolved) or strongly dependent on the environment (plastic) may vary between groups of traits. Traits early in development, including clutch size, duration

of embryonic development and size of the newly birthed offspring, have a stronger transgenerational signal, suggesting that they are evolved. However, the growth rate of neonates and the age and size of first reproduction showed only a weak transgenerational signal, suggesting that they may be largely environmentally determined.

Our results agree with Carvajal-Salamanca *et al.* (2008) who revealed the importance of environmental effects in shaping the life-history traits and phenotypic responses of offspring in subsequent generations of Daphnia.

This correlation in life history traits (embryonic development time and size of the newly offspring, clutch size) between generations may provide evidence for the evolution of postfertilization maternal provisioning in a stable environment (controlled experimental conditions) with high resources (Harris *et al.*, 2012). It has traditionally been assumed that evolution does not occur fast enough to influence contemporary ecological processes. However, many studies now demonstrate that evolution in populations with considerable genetic variation in traits can have a substantial impact on population dynamics (Hendry, 2013). Furthermore, the direction and strength of selection can be modified in turn within a few generations (Yoshida *et al.*, 2003; Becks *et al.*, 2010; Becks *et al.*, 2012). Although the isolated loch ecosystems on North Uist were only established when species invaded new freshwater environments after the last glaciation approximately 10,000-15,000 years ago (Dynesius & Jansson, 2000; Jordan *et al.*, 2010), and this is not very long for evolution to take place, it has clearly been more than enough for zooplankton to adapt and diverge genetically. The fact that cladocera show these evolved patterns in their life histories, in the absence of predator associated stimuli, increases the

potential for co-evolutionary or eco-evolutionary feedbacks, whose existence is predicated on such genetic responses in populations (Becks *et al.*, 2012).

In conclusion, I found strong evidence for increased fecundity rates of cladocera in lochs with fish, and these changes in life history were highly heritable through generations on North Uist. This sets the scene for the co-evolutionary or eco-evolutionary feedbacks between cladocera and their stickleback predators.

Chapter 5. Stickleback feeding performance: linking morphology to feeding specialisation

5.1 Abstract

Variation in ecological factors plays an essential role in shaping foraging traits and allows us to understand to what extent feeding performance within species, determines the efficiency of exploiting different prey items. We examined functional trophic divergence among 22 stickleback populations on North Uist that differed in the proportion of benthic and planktonic prey they included in their diets. At the individual level, morphological traits were only weakly associated with stomach contents. However, anadromous sticklebacks exhibited mechanical properties associated with feeding on plankton while freshwater populations seem to be more specialised to feeding on benthic prey. Stickleback populations that feed on benthic prey had reduced jaw protrusion relative to other populations.

Across fish populations there was a trade-off between force and velocity in their feeding mechanisms. Stickleback populations that consumed high proportions of benthic invertebrates in their diet applied high mechanical advantage in their jaw apparatus in both simple opening lever and opercular four-bar system. In contrast, high-velocity transmission was characteristic of those populations that fed on planktonic and evasive species. Variation in trophic traits between populations was associated also with loch depth, area and trout presence. Finally, I suggest that feeding on zooplankton may drive an eco-evolutionary feedback loop under increased prey communities, which may further shape fish trophic morphology.

5.2 Introduction

Adaptive radiation is the rapid evolution of diverse populations, ecotypes and species from a single common ancestor, and may be the origin of much extant biodiversity. Diversification is generally accepted to be the evolutionary result of natural selection engendered by the occupation of new and varied niches (Schluter, 2000; Grant & Grant, 2011). Such evolution often results in specialisation to the requirements of the occupied niche (Bolnick *et al.*, 2002; Eklöv & Svanbäck, 2005; Gavrilets & Losos, 2009), and specific environments or ecosystems may increase or enhance morphological diversification (Simpson, 1944; Simpson, 1955). However specialisation also gives rise to a hallmark of adaptive radiation, a trade-off in performance between niches: traits well adapted to one specialised niche are often less good in another (Schluter, 1995). The adaptive radiation of foraging traits has been particularly well studied, the divergence of beak size and shape of Darwin's finches (*Geospiza spp.*) is a famous example (Arbogast *et al.*, 2006), yet for most such traits the functional significance of their variation remains obscure (Jamniczky *et al.*, 2015).

Adaptive radiation may be especially common when organisms disperse to isolated environments with high ecological opportunity, such as newly formed islands (Baldwin, 2007; Givnish *et al.*, 2009) or lakes formed by glacial retreat (Ólafsdóttir *et al.*, 2007). Northern temperate fish provide good examples of the latter. Primary divergence during their speciation commonly involves three processes: transitions between freshwater and marine habitats, allopatric differentiation without separate or distinct niche shifts and ecological transformations within various ecosystems or habitats (Seehausen & Wagner, 2014). These lead to repeated divergence in

morphology (Schluter & Nagel, 1995; Schluter, 2000) along major resource dimensions of habitat, food size and food type (Schluter, 2000). Northern temperate fish exhibit a wealth of trophic trait divergence for such young taxa (Taylor & McPhail, 1999; McGee *et al.*, 2013), but the functional significance is poorly understood, although it likely leads to strong trade-offs in ability to utilise different diets (Schluter, 1993; Schluter, 1995).
We know about trophic trait divergence in the adaptive radiation of northern temperate fish, and we added to this by looking at different other examples. For instance, zebrafish are obligate suction feeders that have thin elongate mandibles, with which they feed on small aquatic crustaceans, insects and plankton (Hernández *et al.*, 2002). A group of East African cichlid also evolved short jaws for biting and stripping algae attached to rocks, whereas others feed on pelagic prey and have evolved elongated, gracile jaws exploiting suction feeding (Albertson & Kocher, 2006). Other fish species including the American sunfish and several families of coral reef fish may evolve along this pattern of functional direction (Motta, 1988; Wainwright & Shaw, 1999; Wainwright *et al.*, 2004). This shows the direct role played by the trophic apparatus in dietary divergence (Muschick *et al.*, 2011).

Sticklebacks are an important evolutionary model in this respect. Their unusual ability to exploit new environments containing different resources and to undergo extensive evolutionary modifications make them an ideal species to study the factors that underlie morphological diversity (Magalhaes *et al.*, 2016). It has been shown that there is significant variation in trophic apparatus among stickleback species in some aquatic ecosystems (Lavin & McPhail, 1986). It seems reasonable to believe that this variation in trophic apparatus might be associated with higher prey capture success (Lavin & McPhail, 1986). The observed variation in trophic

morphology probably results in variation in capture kinematics, from rapid but weak prey capture of small, fast-moving pelagic organisms (Wintzer, 2004) to strong but slow effort needed to handle large prey or to search for food at the bottom of water bodies (McGee *et al.*, 2013). Furthermore, different species of three-spined sticklebacks exhibit divergence in mouth size that is probably connected with suction feeding (McGee *et al.*, 2013). It is well known that a larger mouth results in an expansion of the fish's buccal cavity size and increases the suction pressure available to capture prey (Holzman & Wainwright, 2009). It has been assumed that limnetic sticklebacks, which feed on rapid zooplankton like copepods, have a functional system adapted to utilise greater speed in capturing prey items (Holzman *et al.*, 2012; McGee *et al.*, 2013). The morphological potential to produce suction pressure during feeding can be recognised from the relative size of a series of functional craniofacial bones and muscles that act as sets of complex levers and linkages that expand the buccal cavity (Carroll *et al.*, 2004).

These dietary - morphological relationships are consistent with a tenet of the eco-morphological hypotheses which states that food use of organisms drives the evolution of their trophic morphology (Norton *et al.*, 1995), which may in turn feedback between functionally diverse trophic levels (Tirok *et al.*, 2011). The resulting morphological variation is very important in enabling the ecological segregation of co-existing fish species (Xie *et al.*, 2001; Holzman & Wainwright, 2009). For example, co-existing ecotypes of fish often diverge along a benthic-limnetic axis (McPhail, 1992). Feeding and efficiency of exploiting resources result in selection on trophic traits that differ between resource environments (Arnold, 1983). The resulting

evolution (and change in resource use) may in turn cause a chain of ecological effects through a food web.

5.2.1 Mechanical indices of jaw lever systems

An essential divergence occurs between fish species that feed on different prey items. This functional complexity can result in morphological and ecological diversification because of the inherent performance trade-off between feeding mechanisms (Lynch & Conery, 2003; Alfaro *et al.*, 2005; Wainwright, 2007).

Stickleback, like other fish exhibit substantial divergence foraging morphology. In general, functional divergence is often known to be associated by the evolution of jaw morphology, which mirrors the mechanical properties of the feeding apparatus. It has been hypothesised that diversification in resource use may induce divergent natural selection between populations and may play an important role in generating adaptive radiation (Schluter & McPhail, 1993; Lynch & Conery, 2003).

To determine the morphological basis of fish feeding structures, a series of biomechanical models have been used. In these models, sets of simple and complex levers and linkages represented craniofacial bones and muscles in the jaw system were used (Westneat, 2003). The simple lever model (Fig. 5.1) is one of the models that evaluate the magnitude of individual's lever ratio (length of inlever–outlever) in opening and closing jaw systems. Simple opening and closing jaw systems, explain how much force or velocity for benthic and planktonic fish feeders is needed to handle their prey (Westneat, 2003).

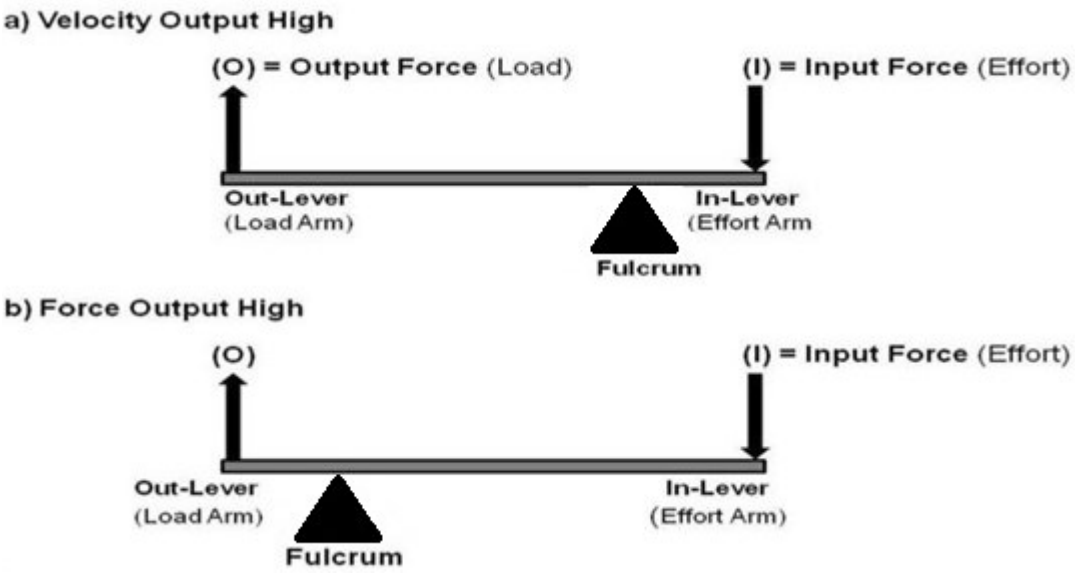


Figure 5.1. Two different positions of the components of simple lever of two fish groups (planktonic and benthic feeders) with different feeding performance. Simple lever system of fish on the top (a) with high jaw velocity (planktonic feeders). Simple lever system of fish on the bottom (b) with high force jaw efficiency (benthic feeders). This model is applied to both mechanical advantage of jaw opening and closing.

Although the morphology of the fish jaw is more complex than the simple lever description above, it has been found that fish exhibit two essential properties in their attack depending on the ratio of inlever to out-lever length. The first one produces force and is described as the mechanical advantage of the jaw opening. The mechanical advantage of the lower jaw in vertebrates has been widely used in the study of the structure and function of mammalian feeding systems (Hiiemae & Houston, 1971) but only Wainwright & Richard (1995) and Barel (1982) have applied this calculation to fishes.

The second, produces also force and is described as the mechanical advantage of jaw closing (Westneat, 2003). These ratios both measure the force transmission such that as the value approaches 1.0, the output force transmitted at the tip of the jaw approaches equivalence with the input

force applied by the adductor mandibular muscle. Greater mechanical advantage translates to greater force during jaw rotation, whereas lower mechanical advantage translates to weaker but more rapid jaw rotation. Furthermore, the inverse of the mechanical advantage is the displacement advantage, a ratio that measures the transmission of motion. Thus, there is a trade-off between powerful and rapid jaw movement (Westneat, 2003). The four-bar linkage model is more complex. This is based on closed chain linkage, and it is used to predict the amount of rotation in a set of levers in fish jaw (Hartenberg & Denavit, 1964; Westneat, 1994). In both models, fish that feed on evasive prey, like calanoid copepods, need more jaw velocity to handle prey that often escape (Kiørboe *et al.*, 1999; Wainwright & Shaw, 1999). On the other hand, benthic fish feeders exploit and handle buried macroinvertebrates forcefully (Westneat, 1994; Wainwright & Shaw, 1999).

Four-bar linkage systems are mechanically more complex than simple levers. The input to the opercular four-bar linkage is introduced by contraction of the levator operculai muscles that rotate the opercle. The system has a fixed link that extends from the quadrate-articular jaw joint to the opercular joint and is bordered by the pterygoids. Opercular rotation transfers a posteriorly directed force to the interopercle that functions as the coupler link in this four-bar mechanism. The posterior portion of the lower jaw is the output of this system and is depressed as the opercle rotates. The output component is therefore the same as the inlever in the simple lever system (Hulsey *et al.*, 2005).

Here, I study the functional consequences of the evolution of craniofacial diversity of stickleback in lochs on the Scottish island of North Uist, which

were colonised by stickleback after the last glaciation 10,000-15,000 years ago. Previous work has shown that sticklebacks in different lochs have diverged greatly from each other in response to local environments and resources (MacColl *et al.*, 2013; Magalhaes *et al.*, 2016). I show that they have also diversified greatly in functional foraging traits that determine the efficiency of capturing different prey items. In addition, it is known that predators vary in trophic traits for exploiting prey, but the question is whether the evolution of fish traits affects prey properties (Fussmann *et al.*, 2007; Kinnison *et al.*, 2008; Ferriere & Legendre, 2013). Here, I investigated whether there was any additional effect of evolved variation of fish traits on a Cladocera community.

5.3 Methods

5.3.1 Study area and Data collection

We captured three-spined stickleback from 19 freshwater (resident) and three anadromous (migratory marine) populations (Table 5.1) in April and May 2014. Some of extra samples were used from samples collected in April and May (2015). Fish were caught in unbaited minnow traps (see more details chapter 2). Traps were emptied into buckets and fish counted before a haphazard sample was collected.

Sampled fish were killed by destruction of the brain with a fine needle after being euthanised by an overdose of MS222 (Tricaine Methane Sulphonate) following UK Home Office requirements. Finally fish stored in 70% ethanol.

In all locations stickleback mean density was estimated as fish caught per trap per night (stickleback CPUE, catch per unit effort) and the following ecological factors were also quantified: pH, depth, area, and relative

abundance of *Pungitius* (see more details in chapter 2). For each loch, the presence of trout was also recorded based on data measured by the number of trout caught per angler per hour during formal competitions run by the North Uist Angling Club between 1956 and 2006 (MacColl & Aucott, 2014). Ten haphazardly selected fish per population for were used the analysis of trophic traits and dietary variation. The body length (standard length) of each fish was measured from the tip of the snout to end of caudal peduncle.

Table 5.1. Names, abbreviations and locations of lochs sampled on North Uist, Scotland. Fish type (resident or anadromous) was identified in this study. Body length is the mean of measurements of anadromous and resident three-spined stickleback caught from each location.

Loch name	Loch code	Geographic coordinates	Fish type	Fish mean body length (cm±SE)
Aonghais	Aong	57°38'40.8"N 7°16'13.2"W	Resident	3.17±0.08
Mhic A'Roin	Aroi	57°35'40.1"N 7°25'49.2"W	Resident	4.77±0.25
-	Bdrm	57°36'14.9"N 7°09'53.2"W	Resident	4.62±0.22
a'Bharpa	Bhar	57°34'33.2"N 7°17'31.2"W	Resident	3.25±0.05
na Buaile	Buai	57°38'50.2"N 7°11'35.6"W	Resident	3.78±0.21
-	Cdrm	57°36'22.4"N 7°10'38.7"W	Resident	4.36±0.17
ChadhaRuaidh	Chru	57°35'37.9"N 7°11'44.2"W	Resident	3.99±0.17
Fada	Fada	57°36'57.9"N 7°14'15.0"W	Resident	3.19±0.12
Fhaing Buidhe	Fhai	57°34'12.2"N 7°22'45.3"W	Resident	3.37±0.10
Gille-bhride	Gill	57°36'06.3"N 7°24'21.3"W	Resident	4.58±0.23
Grogary GROG	Grog	57°36'36.5"N 7°30'04.6"W	Resident	4.11±0.20
Host	Host	57°37'31.6"N 7°29'10.2"W	Resident	3.95±0.23
Iala	Iala	57°37'11.0"N 7°12'20.8"W	Resident	3.54±0.20
nam Magarlan	Maga	57°36'15.9"N 7°28'52.4"W	Resident	3.80±0.15
Maighdein	Maig	57°35'45.9"N 7°12'12.6"W	Resident	3.34±0.12
na Reival	Reiv	57°36'39.8"N 7°30'49.5"W	Resident	5.44±0.29
Shior Thomais	Shos	57°35'58.8"N 7°10'32.1"W	Resident	4.90±0.10
nan Struban	Stru	57°33'30.5"N 7°20'58.4"W	Resident	3.30±0.11
Tormasad	Torm	57°33'53.5"N 7°19'14.2"W	Resident	3.55±0.11
Fairy Knoll	Faik	57°38'06.3"N 7°12'59.3"W	Anadromous	7.09±0.10
Ob nan	Obse	57°36'10.1"N 7°10'20.1"W	Anadromous	6.76±0.19
Trosabhat	Tros	57°35'07.5"N 7°24'43.6"W	Anadromous	6.38±0.11

5.3.2 Dietary analysis

Whole intestinal tracts, from the oesophagus to the anus, were extracted from fish and transferred to 70% ethanol for storage. Contents were dissected into a glass petri dish. All prey items were identified to the lowest possible taxonomic level using An Olympus SZ dissection microscope. For smaller prey items a higher resolution Olympus BH-2 microscope was used.

To calculate the frequency of occurrence of different prey items, the number of stomachs in which each item occurred was recorded and divided by the total number of stomachs examined. I calculated a dietary index designed to reflect the relative proportion of zooplankton (copepods and cladocerans) and benthic prey in the diet of each fish. Benthic prey are generally larger than zooplankton, making simple counts unsuitable as measures of representation. I therefore, multiplied counts of each prey type by its average length to obtain an index of the contribution of that prey type to the diet. These contributions were summed and the proportions of zooplankton were calculated also for all prey types for each fish.

5.3.3 Preparation of specimens

A generalised clearing and staining protocol was applied to fish preserved in ethanol (Wassersug, 1976; Dingerkus & Uhler, 1977; Hanken & Wassersug, 1981; Song & Parenti, 1995). A series of steps were used in this protocol, summarised below (some steps were modified as needed):

1. Rehydrate the specimens through a graded series of water baths of decreasing concentration of ethanol (70%, 50%, 30%) and finally into 100% water.
2. Preserving the specimens in a fixative solution (10% buffered neutral formalin, pH 7) for one to seven days depending on the size of the fish.

3. Washing the specimens in several changes of water to remove formalin.
4. Maceration in which the specimens incubated in 1% KOH solution for 1 hour.
5. Bleaching the specimens in a solution 33% H_2O_2 overnight.
6. Clearing the specimens by first washing them two times in 2% sodium borax and then partially digesting them with a trypsin solution 1% until fish become translucent. In the previous step, I found time required for completed clearing varied between populations of stickleback. Individuals with large size and full armour need about 25 days to be entirely translucent, while for small individuals 10- 15 days was required. The trypsin digest restored the natural range of motion to various muscles and ligaments, thus permitting the manipulation of the head and jaws, and also clear tissue making the determination of the internal components easier to be identified.
7. Staining with Alizarin red stock solution until it is deep purple.
8. Finally, all specimens were transferred through a graded series of 0.5% KOH/glycerine solutions (70% : 30%, 40% : 60%) and finally into pure glycerine. Time again depended on the size of the fish.

The mouth of each fish was opened by using forceps to squeeze the fish's epaxial and hypaxial muscles as is usually the case while the fish is feeding. After opening the mouth, a small fine plastic pipette was placed into the buccal cavity so that it was dorsally pressed against the ventral surface of the neurocranium. The jaw was opened to the extent where the mouth gives its maximum gape and the jaw formed an opening angle of 90º (Rowlands *et al.*, 2006). A high-quality digital camera (Nikon D90 Digital SLR) was used to take the photos of the fishes at various positions and 1 mm graph paper was used with each photo for scale.

5.3.4 Trophic trait variation

To measure trophic trait variation, a series of landmarks were recorded from each of ten haphazardly selected fish per population. These landmarks were allocated based on previous studies on the trophic morphology of stickleback and other species of fish (Anker, 1974; Westneat, 1990; Westneat, 1994; Westneat, 2003; Carroll *et al.*, 2004; Westneat, 2004; Westneat, 2005; McGee *et al*., 2013; Arnegard *et al.,* 2014).

Most landmarks (Table 5.2) were digitised from photographs and analysed by using the program Klonk Image Measurement version 15.1.2.1, from which the linear distance between any pair of coordinates could be calculated.

5.3.5 Estimation of mechanical properties and suction index

Distances between morphological landmarks were used to estimate mechanical properties of different aspects of stickleback trophic morphology putatively associated with prey capture (suction index, jaw protrusion, gape and the dynamic properties of the simple lever and opercular four-bar systems). I standardised all traits measurements to fish body size by estimating residuals from linear regressions of each trait on standard length (SL), then calculated each trait for a common SL of 45 mm.

Table 5.2. Morphometric landmarks to evaluate morphological components and functional feeding systems of three-spined sticklebacks.

Functional feeding system	Abbreviations of morphometric Codes	Morphometric landmarks (Fig. 5.2)
Suction Index	SI	
Buccal length	BCL	1,6
Neurocranium outlever	NEO	1,3
Epaxial height	EPH	3,4
Epaxial width	EPW	3,5
Gape	G	1,2
Jaw protrusion	JPR	1,7
Mechanical and displacement advantage of jaw opening and closing	MA. And Disp. adv., jaw opening and closing	
Jaw opening outlever	OOL	2,8
Jaw opening inlever	OIL	8,9
Jaw closing inlever	CIL	2,12
Force and kinematic transmission of opercular four-bar	FT. and KT. opercular four-bar	
Coupler link	CL	9,11
Fixed link	FL	8,10
Input link	IL	10,11
Output link	OL	8,9

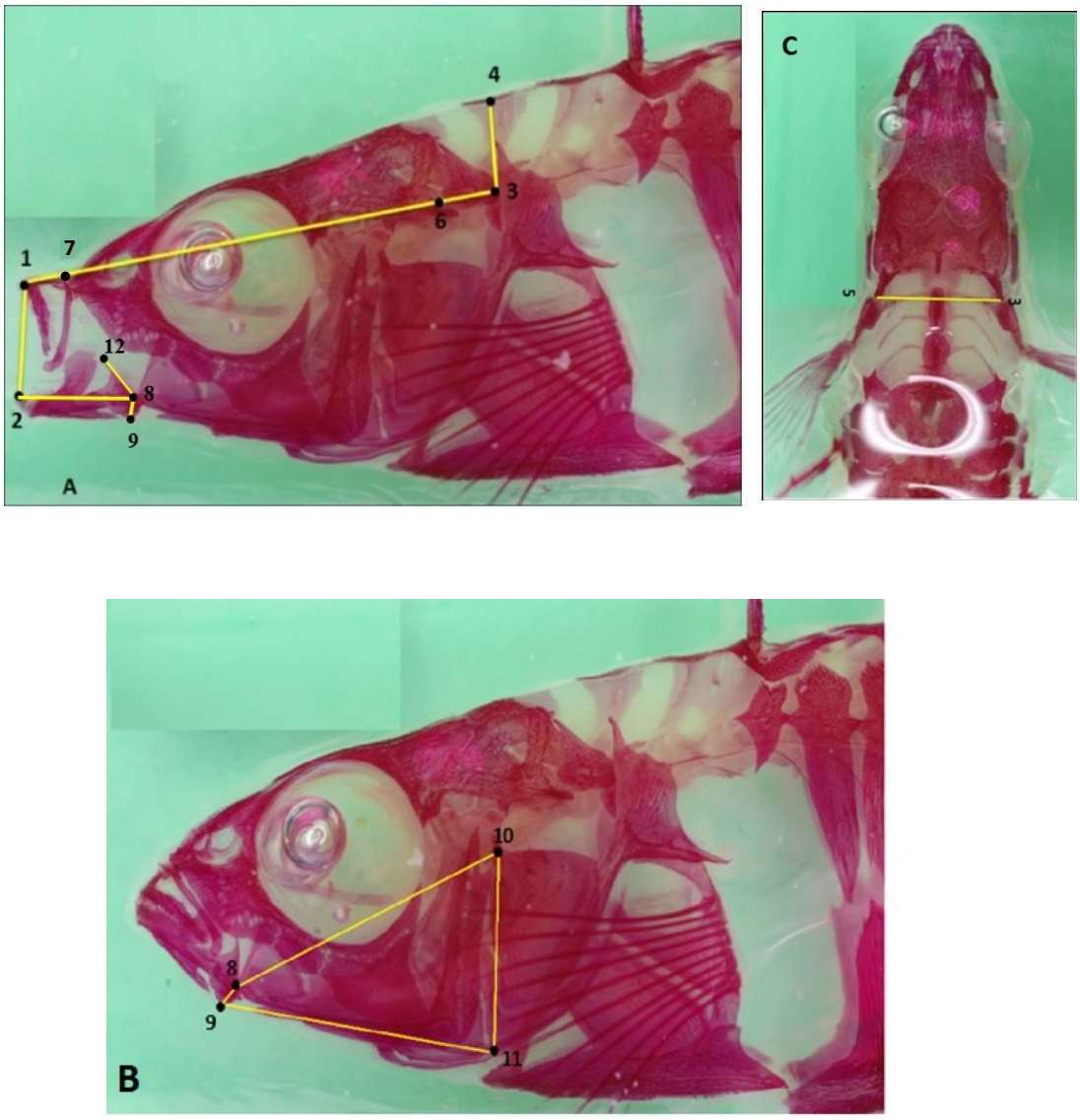


Figure 5.2. Functional components: A, C For suction index, Buccal length (1,6), Neurocranium outlever (1,3), Epaxial height (3,4), Epaxial width (3,5), Gape (1,2), Jaw protrusion (1,7); For MA. and Disp. adv., jaw opening and closing, Jaw opening outlever (2,8); Jaw opening inlever (8,9); Jaw closing inlever (2,12). B: FT. and KT. Opercular four-bar, Coupler link (9,11), Fixed link (8,10), Input link (10,11), Output link (8,9).

5.3.5.1 Suction Index

I measured five component traits contributing to the suction feeding index (Table 5.2, Fig 5.2). From dorsal photographs, the epaxial width (EW) was measured from the point of articulation between the supracleithrum and post-temporal to the same point on the opposite side of the fish. From lateral photographs of fish with opened mouths three components were measured:

1. Epaxial height (EH) was measured from the point of articulation between the supracleithrum and post-temporal to the dorsal-most extent of the epaxial.

2. Buccal length (BCL) from anterior-most extent of premaxilla to posterior-most extent of buccal cavity.

3. Gape (G) from anterior-most extent of premaxilla to anteriormost extent of dentary.

4. Neurocranium outlever (NOL) from anterior-most extent of premaxilla to point of articulation between the supracleithrum and post-temporal.

According to Arnegard *et al.* (2014) suction index was calculated as :

$$\mathrm{SI} = \frac{\mathrm{EW}.\,\mathrm{EH}^2}{(3\mathrm{BL}\,.\,\mathrm{G}.\,(\mathrm{NOL} - 1/2\ \mathrm{BL}))}$$

5.3.5.2 Mechanical advantage of jaw lever systems

The geometry of the teleost fish jaw can be described as a first class lever and decomposed into three main vectors (Table 5.2, Fig 5.2) :

1. Opening inlever (the distance between the quadrate-articular joint and the insertion of the interoperculo-mandibular ligament).
2. Closing inlever (distance between the quadrate-articular joint and

insertion point of adductor mandibulae onto the coronoid process).

3. Outlever (the distance between the quadrate-articular joint and the tip of the mandible).

The opening jaw inlever alone was measured under a microscope using an ocular micrometer because it is not in the same plane as the lateral body/head image and measuring it directly from photographs would have resulted in parallax error. Mechanical advantage of the jaw opening is calculated as the ratio between the opening inlever and the outlever. mechanical advantage of jaw closing and is calculated as the ratio between the closing in-lever and the outlever (Westneat, 2003). Furthermore, the inverse of the mechanical advantage is the displacement advantage, a ratio that measures jaw velocity.

5.3.5.3 Opercular four-bar model of lower jaw

If the shape of the linkage (and all necessary angles) is known for each snapshot of time, the force and kinematic transmission for each snapshot of time can be calculated. Force transmission in Opercular four-bar systems is calculated by the ratio of input rotation to output rotation (Anker, 1974). The inverse of force transmission in this system reveals the magnitude of kinematic transmission. Since the calculation of these two measures involves dynamic properties of the system, I used Matlab R2016a codes to calculate the force and kinematic transmission of this model.

5.3.6 Statistical analysis

The coefficient of variation was used to show the degree of variation of food items in stickleback stomachs in a ratio from the contribution of zooplankton and benthic prey items. Shapiro- Wilk normality test ($P > 0.05$) was used to confirm normality in all data sets. Non-normally distributed data sets were log transformed to achieve normality.

Unpaired t-test was used to examine the difference in trophic morphology between freshwater resident and anadromous populations on North Uist.

To summarise trophic trait variation across individuals, I performed a principal component analysis (PCA) using all trophic measurements (Table 5.3). Principal component axes that explained more than 10% of the total variance of the measured phenotypic traits were retained for further analysis. The relationship between principal components and mechanical indices was quantified with Pearson correlations. To measure the differentiation within and between populations I used generalised linear models (GLMs) to assess at which level the variance in measured variables occurred. The relationship between individual fish trophic morphology and the contribution of planktonic and benthic sources to the stomach contents was tested with generalised linear models (GLMs). The models were fitted by using identity link functions with normal errors. GLMs was checked and validated by visual assessment for residuals' graphs. Full models were the same for all response variables and included interaction of trophic morphology × population. This interaction assessed whether slops of the association between trophic traits and diets was consistent across populations.

5.3.6.1 Relationship between trophic morphology and environment

I also used Generalised linear models (GLMs) to examine associations between population means of trophic traits (suction index, mechanical and displacement advantage of opening and closing inlever, force and Kinematic transmission of opercular four-bar), and measures of the biotic and abiotic environmental variation between populations, including population mean benthic and planktonic contributions to stomach contents. Simple linear regression was then used to examine the significant terms obtained from Generalised linear models. Anadromous fish were excluded from this analysis because the habitats in which they are caught (breeding grounds) are not those within which they spend most of their lives. GLMs was checked and validated by visual assessment for residuals' graphs. Shapiro-Wilk normality test ($P > 0.05$) was used to confirm normality in all data sets. Non-normally distributed data sets were log transformed to achieve normality.

5.4 Results

5.4.1 Dietary variation

In total, thirty three food categories were identified from the 22 lochs (Fig 5.3, Appendix 5.1). In general the diets of sticklebacks were comprised of a number of dominant categories: chironomid larvae and pupae, Copepoda and Cladocera comprised on average 82.34% of the all stomach contents. Larvae and pupae of chironomid midges were observed in almost all populations and comprised 66.9% of total mean density of benthic items in examined stomachs followed by fish eggs with 8.1 % and *Gammarus duebeni* with 5.9%. *Bosmima coregoni* comprised 81.4 % of total mean density of plankton items followed by *Cyclops spp* with 12.2% and *Daphnia*

sp. with 1.78%. Although most populations showed a variety of food items in their stomachs, variation of contribution of zooplankton prey items (Coefficient of variation = 175.94 %) was more than the contribution of benthic prey items (Coefficient of variation = 94.35%). The frequency of all prey items is shown in (Appendix 5.2). *Bosmina coregoni* was the most frequent prey item overall (Fig. 5.3). In several populations individual stickleback had over one thousand of *Bosmina* in their stomach. Chironomid larvae were the most frequent benthic prey item, and the only one to be consumed across almost all lochs (Appendix 5.2, Fig 5.3). Zooplankton were dominant in the diet of two of the three anadromous populations. Benthic prey were more common in freshwater lochs, but some still specialised on plankton (Appendix 5.2 and 5.3, Fig 5.4). Some prey items were seen only in a single population. Examples include: water mite, Crab zoea larvae, and some cladoceran: species *Drepanothrix dentata*, *Bythotrephes longimanus*, *Simocephalus sp*, and *Evadne nordmanni.*

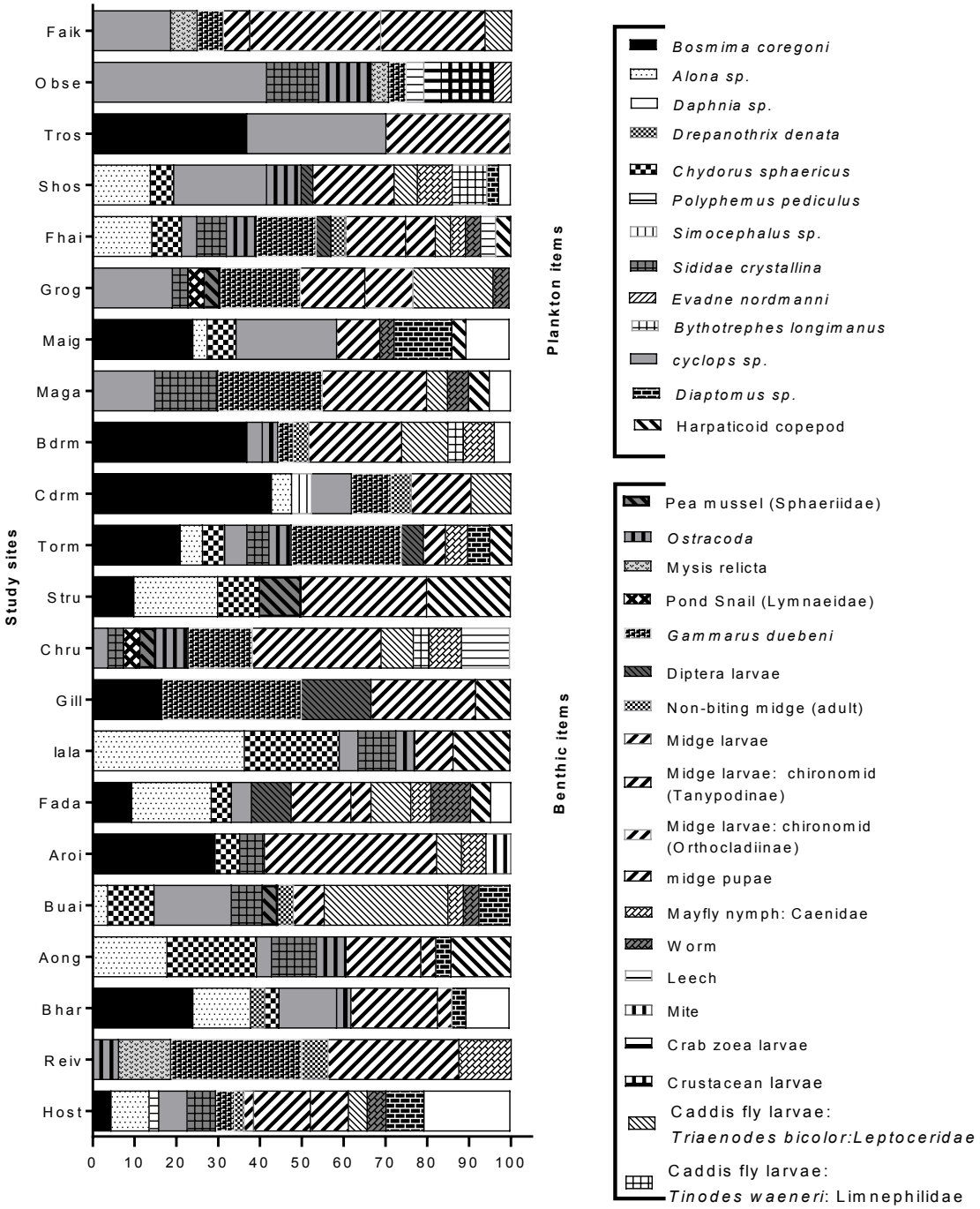


Figure 5.3. Percentage relative frequency of occurrence of prey items in stickleback diets in 22 populations on North Uist, showing two main groups of prey items in diets (benthos and plankton).

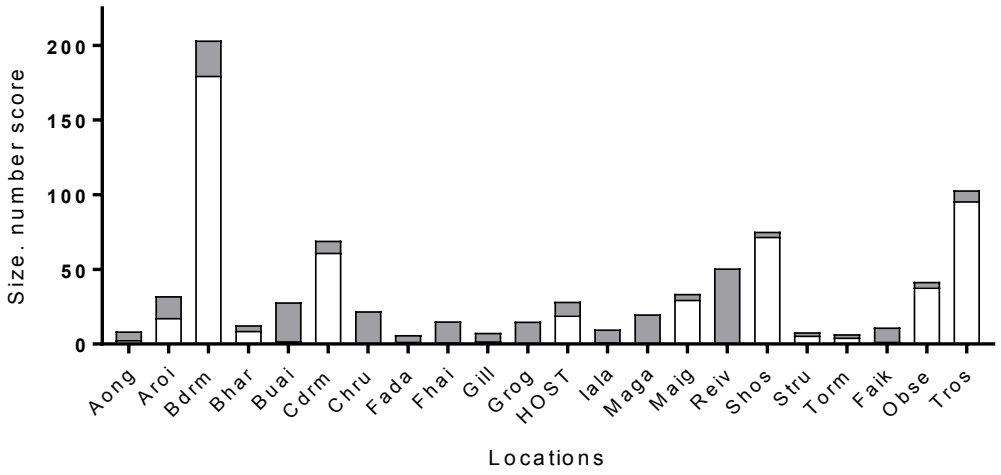


Figure 5.4. Contributions of zooplankton (open) and benthic (filled) prey to diet of three-spined stickleback in 22 populations on North Uist.

5.4.2 Trophic trait variation

In the PCA of trophic traits, only the first two axes were retained for further analysis. PC1 explained 48.4% of the variation. Weightings on this axis were all positive (Table 5.3, Fig. 5.5), suggesting that it described overall variation in (standardised) size of trophic traits between individuals. The second principal component explained 11.1% of the variation in the original data. Weightings on this axis were both positive and negative, suggesting that this axis described important variation in overall 'shape' of the trophic morphology. Jaw opening inlever which has a large influence on force transmission in the jaw system had a heavy negative loading on PC2, while jaw protrusion and epaxial height had heavy positive loading on PC2. In general most of the components that enhance kinematic transmission and suction feeding showed a positive loading on PC2, while components that have an influence on force transmission in the jaw system had a negative loading on PC2. Across all individuals, PC2 scores were significantly correlated with overall values of force of jaw opening and closing (Fig. 5.6;

MA. adv., jaw opening, r^2=-0.32, n=220, $p < 0.001$; MA. adv., jaw closing r^2=0.15, n=220, $p < 0.001$ and FT. opercular four-bar, r^2=-0.15, n=220, $p < 0.001$). Furthermore, there was also a significant positive correlation (r^2=0.13, n=220, $p < 0.001$) between PC2 and suction index (Fig. 5.6), but not strong as the previous correlations.

Table 5.3. Loadings of trophic trait variables on the first two PCs and the percentage of variance explained by each axis.

Trophic trait	PC1	PC2
Buccal length	0.386	0.042
Neurocranium outlever	0.379	0.012
Epaxial height	0.186	0.439
Epaxial width	0.265	-0.178
Gape	0.318	0.196
Jaw protrusion	0.253	0.482
Jaw opening outlever	0.309	-0.069
Jaw opening inlever	0.238	-0.545
Jaw closing inlever	0.280	0.250
Coupler link	0.304	-0.318
Fixed link	0.334	-0.190
Input link	0.057	0.042
Percentage of variation	72.1%	14.89%

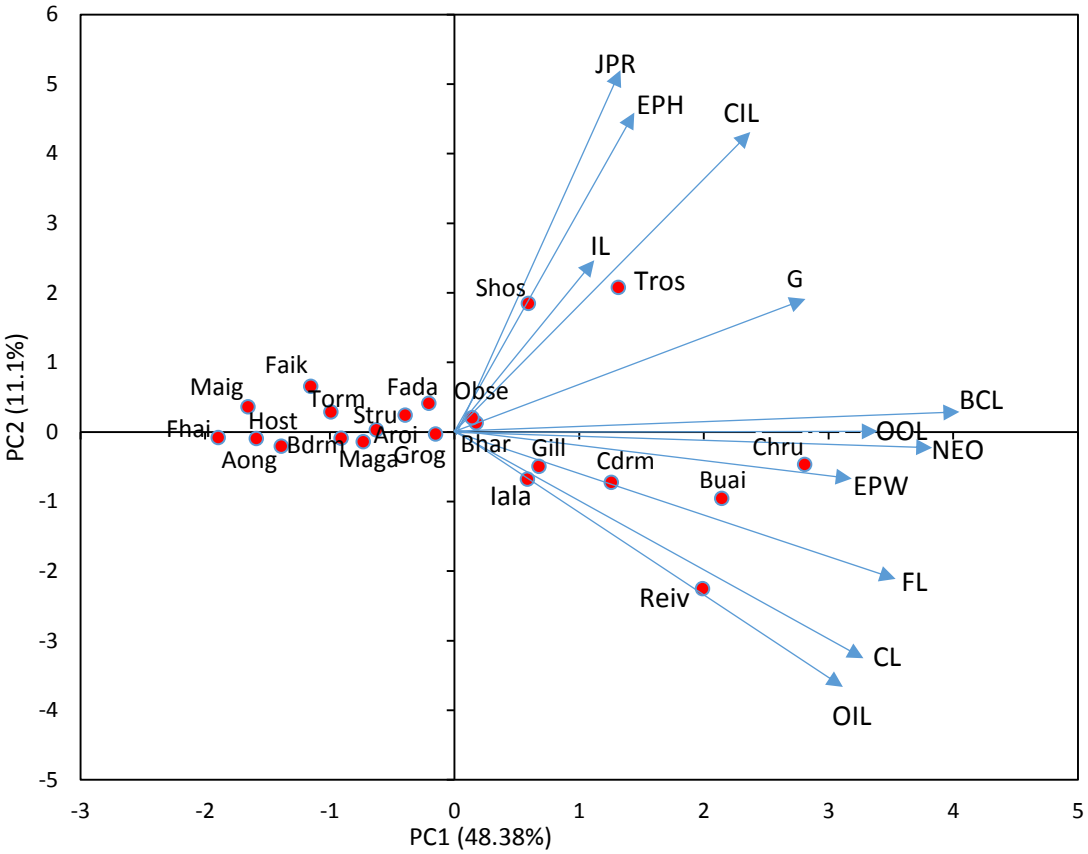


Figure 5.5. Population mean values of principal components axes PC1 and PC2 representing 59.9% of the variation in trophic traits of individual sticklebacks. Buccal length, BCL; neurocranium outlever NEO; epaxial height, EPH; epaxial width EPW; gape G; Jaw protrusion JPR; jaw opening outlever OOL; jaw opening inlever OIL; jaw closing inlever CIL; coupler link CL; fixed link FL; input link IL.

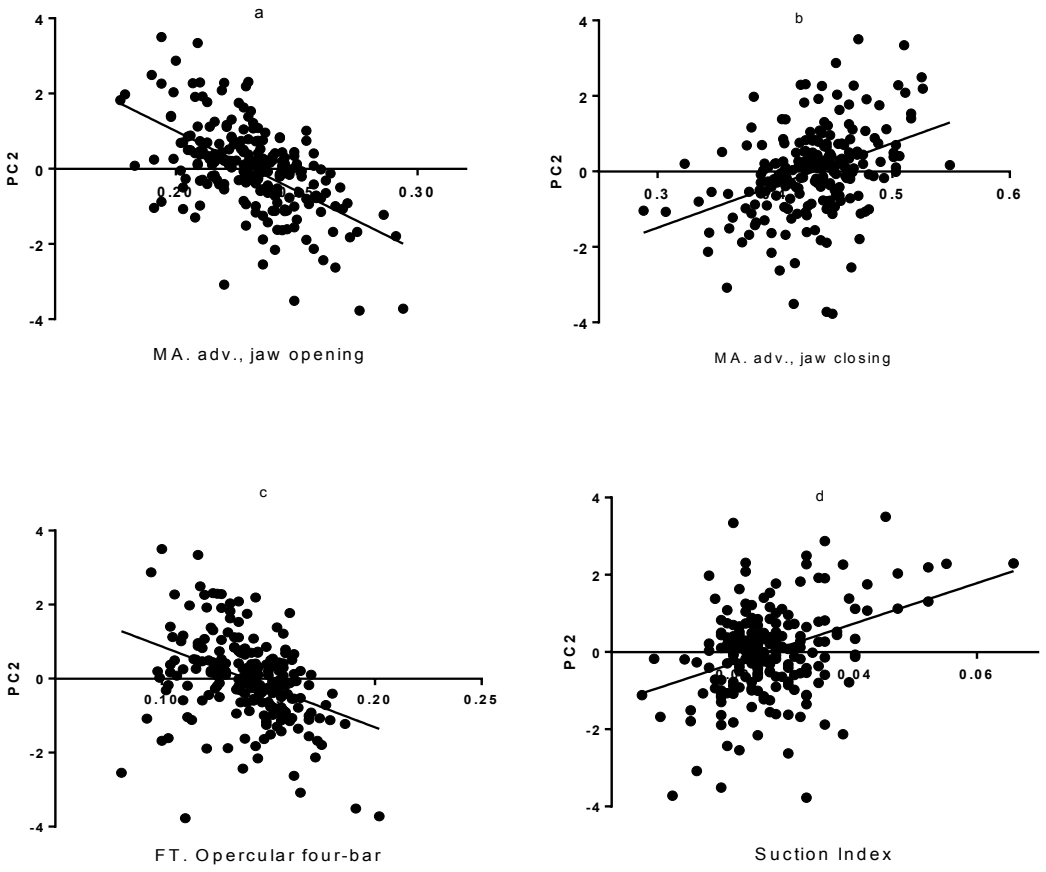


Figure 5.6. Relation between functional feeding factors (Mechanical advantage of jaw opening (a) and closing(b); force transmission of opercular four-bar (c), suction index (d) and second principal component explained variation of 12 trophic traits, buccal length, BCL; neurocranium outlever NEO; epaxial height, EPH; epaxial width EPW; gape G; Jaw protrusion JPR; jaw opening outlever OOL; jaw opening inlever OIL; jaw closing inlever CIL; coupler link CL; fixed link FL; input link IL of three-spined stickleback in 22 populations.

5.4.3 Estimation of mechanical properties

The results revealed that there were a significant difference among lochs for all trophic traits and derived mechanical properties (Table 5.4, Appendix 5.4, 5.5).

Table 5.4. Analysis of interpopulation variation in trophic traits of 22 populations of stickleback on North Uist. The GLMs with normal errors and identity link was fitted, with 'population' as a factor.

Response variables	Wald F	df	*P*
Suction index	6.21	21,198	<0.001
Epaxial height	4.25	21,198	<0.001
Epaxial width	5.03	21,198	<0.001
Neurocranium outlever	3.59	21,198	<0.001
Buccal length	3.32	21,198	<0.001
Gape	3.18	21,198	<0.001
Jaw protrusion	2.76	21,198	<0.001
Mechanical advantage of jaw opening	5.05	21,198	<0.001
Mechanical advantage of jaw closing	4.81	21,198	<0.001
Jaw opening outlever	2.33	21,198	<0.001
Jaw opening inlever	12.06	21,198	<0.001
Jaw closing inlever	4.43	21,198	<0.001
Force transmission of opercular four-bar	6.88	21,198	<0.001
Opercular four bar	6.88	21,198	<0.001
Coupler link	4.01	21,198	<0.001
Fixed link	3.64	21,198	<0.001
Input link	67.52	21,198	<0.001

5.4.3.1 Suction index, jaw protrusion and fish gape

At the level of individual fish, no association was recorded between the planktonic contribution in fish stomachs and suction index, jaw protrusion or gape (Table 5.5). On the other hand, fish with high benthic contributions in their diet showed a significantly lower suction index. This relationship was constant across populations (no interaction with population).

5.4.3.2 Mechanical and displacement advantage of jaw lever systems

There were significant negative relationships recorded between mechanical advantage of jaw closing and benthic prey contribution in fish stomachs (Table 5.5). No association was found between Opercular four-bar system and stomach contents in this study. These significant relationships did not vary among populations. On the other hand, there were no significant relationships between functional properties of trophic traits of individual stickleback and the planktonic diet contribution in this study (Table 5.5).

Table 5.5. Results of GLMs modelling of the relationships between dietary variation and functional properties of trophic traits of individual stickleback in 22 populations on North Uist. Population was also included as a factor. The models were fitted by using identity link function with normal errors. Probability values which are < 0.05 are in bold type.

Response	Fitted model	Wald	df	*p*	Estimate ±SE
Log (mean planktonic diet contribution)	Loch	7.06	20,119	**<0.001**	
	Displacement advantage of jaw closing	1.90	1,118	0.17	
	Gape	1.72	1,117	0.19	
	Jaw protrusion	2.17	1,116	0.14	
	Suction index	0.43	1,115	0.51	
	Kinematic transmission of opercular four-bar	0.16	1,114	0.69	
	Displacement advantage of jaw closing	0.00	1,113	0.99	
Log (mean benthic diet Contribution)	Loch	2.19	21,139	**0.004**	
	Suction Index	5.80	1,139	**0.02**	-28.1±11.7
	Mechanical advantage of jaw closing	4.96	1,139	**0.03**	-4.98±2.24
	Gape	1.48	1,138	0.23	
	Jaw protrusion	2.22	1,137	0.14	
	Mechanical advantage of jaw opening	1.27	1,136	0.26	
	Force transmission of opercular four-bar	1.26	1,135	0.26	
	Loch.(Mechanical advantage of jaw closing)	1.05	21,114	0.41	
	Loch. Suction index	0.86	21,93	0.65	

5.4.4 Differences in trophic functional variation between freshwater resident and anadromous fish

There were significant differences in functional properties between freshwater resident and anadromous fish except for suction index. Anadromous populations have relatively higher Jaw protrusion, gape, kinematic components (Disp. adv., jaw opening, KT. Opercular four-bar) than freshwater resident populations. On the other hand, force components (MA. adv., jaw opening FT. Opercular four-bar) were higher in freshwater resident populations than anadromous populations (Table 5.6). In addition, resident sticklebacks showed higher velocity in jaw closing than anadromous fish.

Table 5.6. t-test results comparing functional properties of trophic traits between freshwater resident and anadromous fish on North Uist. Probability values which are < 0.05 are in bold type.

Functional properties of trophic traits	Mean ±SE, residents	Mean ±SE, anadromous	t	d.f.	*p*
suction index	0.026 ± 0.0006	0.025 ± 0.002	0.47	218	0.64
Jaw protrusion	1.14 ± 0.01	1.22 ± 0.05	2.26	218	**<0.001**
Gape	3.36± 0.02	3.53 ± 0.1048	2.41	218	**0.017**
MA. adv., jaw opening	0.23± 0.001	0.22 ± 0.004	3.21	218	**0.002**
Disp. adv., jaw opening	4.32 ± 0.03	4.58 ± 0.09	3.53	218	**<0.001**
MA. adv., jaw closing	0.4291 ± 0.003	0.4595 ± 0.008	3.96	218	**<0.001**
Disp. adv., jaw closing	2.35 ± 0.02	2.195 ± 0.04	3.48	218	**<0.001**
FT. four-bar	0.14 ± 0.001	0.11 ± 0.003	8.79	218	**<0.001**
KT. four-bar	7.06 ± 0.07	8.94 ± 0.22	9.98	218	**<0.001**

5.4.5 Relationship between trophic morphology and environment

The mean mechanical properties of resident stickleback populations were associated with several aspects of the abiotic and biotic variation among the lochs in which they lived, especially the depth and area of lochs, benthic and planktonic diet contribution, and trout presence (Table 5.7).

In particular, there were significant associations between mechanical properties associated with feeding on plankton (positive relationship for suction index and the depth of lochs, positive relationship for kinematic transmission of opercular four-bar and planktonic diet contribution). Gape was negatively associated with trout presence and jaw protrusion was negatively associated with benthic diet contribution.

Significant association were also found between mechanical properties associated with feeding on benthos (negative relationship for mechanical advantage of jaw opening) and the depth of lochs and trout presence.

The mechanical properties did not tend to be associated with pH, fish density (CPUE), the relative density of competitors (Nine spined stickleback, *Pungitius pungitius*).

The results showed a possible effect of the evolution of measured trophic morphology of stickleback on density of Cladocera (chapter 2, 3). When loch depth and fish density were controlled, high density of cladocerans were found in lochs where stickleback had small gapes and high kinematic transmission in the jaw system (Table 5.8).

Table 5.7. Results of GLMs to evaluate the relationship between trophic functional properties and ecological factors in 19 freshwater (resident) populations of stickleback on North Uist. The models were fitted with normal errors and identity link functions. Probability values which are < 0.05 are in bold type.

Response	Fitted model	Wald F	df	*p*	Estimate
Suction index	Mean loch depth	8.16	1,15	**0.01**	0.003+0.001
	Loch area	5.66	1,15	**0.03**	-0.0003+0.0001
	Trout presence	1.46	1,14	0.25	
	Pungitius	0.63	1,13	0.44	
	pH	0.37	1,12	0.55	
	CPUE	0.16	1,11	0.69	
	Log (mean planktonic diet contribution)	0.01	1,10	0.91	
	Log (mean benthic diet contribution)	0.002	1,9	0.96	
Gape	Trout presence	9.73	1,16	**0.007**	-0.26±0.08
	Pungitius	4.53	1,15	0.05	
	Loch area	4.03	1,14	0.06	
	CPUE	1.25	1,13	0.28	
	pH	0.76	1,12	0.40	
	Log (mean planktonic diet contribution)	0.37	1,11	0.55	
	Log (mean benthic diet contribution)	0.19	1,10	0.67	
	Mean loch depth	0.01	1,9	0.92	
jaw protrusion	Log (mean benthic diet contribution)	8.55	1,16	**0.02**	-0.11±0.04
	pH	2.52	1,15	0.13	
	Trout presence	1.55	1,14	0.24	
	Log (mean planktonic diet contribution)	1.09	1,13	0.31	
	Mean loch depth	0.72	1,12	0.41	
	Loch area	0.16	1,11	0.69	

	Pungitius	0.17	1,10	0.69	
	CPUE	0.09	1,9	0.77	
Mechanical opening	Mean loch depth	7.31	1,15	**0.016**	-0.005±0.002
	Trout presence	5.29	1,15	**0.04**	-0.01±0.005
	pH	1.7	1,14	0.21	
	Loch area	0.21	1,13	0.66	
	CPUE	0.2	1,12	0.66	
	Log (mean benthic diet contribution)	0.15	1,11	0.70	
	Pungitius	0.03	1,10	0.87	
	Log (mean planktonic diet contribution)	0.0007	1,9	0.98	
Mechanical Closing	pH	4.86	1,16	**0.05**	-0.005±0.002
	Log (mean benthic diet contribution)	3.75	1,15	0.07	
	Log (mean planktonic diet contribution)	2.16	1,14	0.16	
	Pungitius	1.54	1,13	0.23	
	Loch area	0.63	1,12	0.44	
	Mean loch depth	0.96	1,11	0.35	
	Trout presence	0.19	1,10	0.67	
	CPUE	0.1	1,9	0.76	
kinematic opercular four-bar	Log (mean planktonic diet contribution)	3.72	1,16	**0.04**	0.26±0.14
	Log (mean benthic diet contribution)	1.83	1,15	0.195	
	Pungitius	1.09	1,14	0.313	
	pH	0.94	1,13	0.348	
	Mean loch depth	0.002	1,12	0.97	
	Trout presence	0.18	1,11	0.681	
	CPUE	0.11	1,10	0.745	
	Loch area	0.001	1,9	0.972	

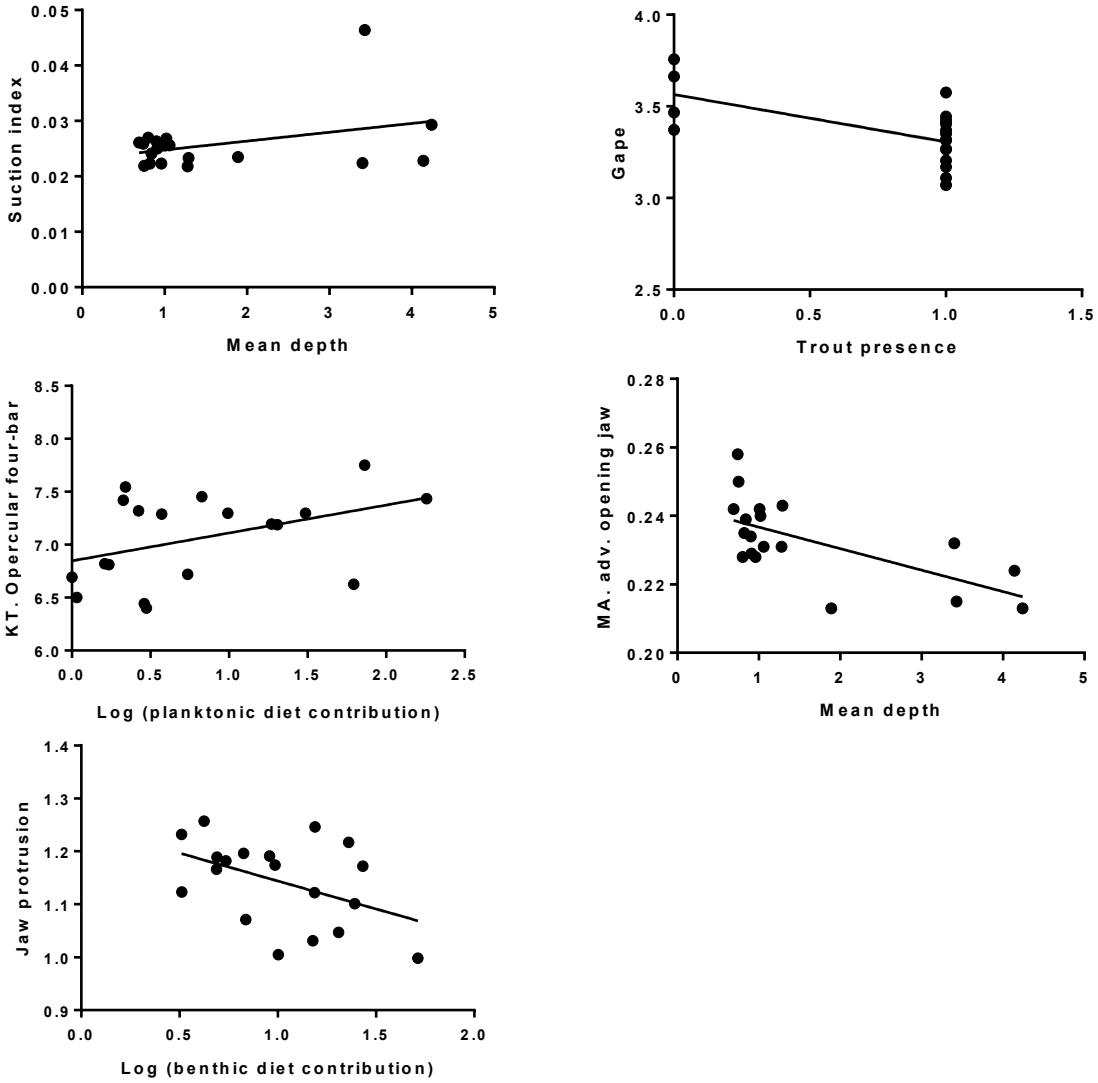


Figure 5.7. Relationships between properties of trophic traits and ecological factors for 19 freshwater stickleback populations on North Uist. The mean mechanical properties of resident stickleback populations were associated significantly with the depth and area of lochs, benthic and planktonic diet contribution, and trout presence. Solid lines are the fitted regression lines.

Table 5.8. Results of GLMs of the relationships between cladoceran density and two putative environmental explanatory variables (density of sticklebacks, loch depth) with properties of stickleback trophic traits. The models fitted with normal errors and identity link functions. Probability values which are < 0.05 are in bold type.

Response	Wald F	Wald F	df	*P*	
Log (Cladocera density)	CPUE	0.78	1,17	0.39	
	Suction index	0.38	1,16	0.55	
	Loch depth	0.00	1,15	0.97	
	Gape	9.33	1,17	**0.007**	-2.96±0.97
	Loch depth	0.05	1,16	0.83	
	CPUE	0.00	1,15	0.99	
	CPUE	0.78	1,17	0.39	
	Loch depth	0.20	1,16	0.84	
	Jaw protrusion	0.01	1,15	0.93	
	Displacement advantage of jaw opening	1.00	1,17	0.33	
	CPUE	0.47	1,16	0.5	
	Loch depth	0.16	1,15	0.69	
	CPUE	0.78	1,17	0.39	
	Displacement advantage of jaw closing	0.32	1,16	0.58	
	Loch depth	0.07	1,15	0.79	
	kinematic transmission of opercular four-bar	3.88	1,17	**0.04**	0.90±0.46
	CPUE	0.68	1,16	0.42	
	Loch depth	0.24	1,15	0.63	

5.5 Discussion

Substantial variation in trophic morphology traits and related mechanical properties in the adaptive radiation on North Uist were related to variation in the diet at the individual, and especially at the population level. Furthermore, variation in trophic morphology was predicted by environmental variation between lochs. Substantial phenotypic variation has previously been documented among stickleback populations in this adaptive radiation, but has not previously been directly related to functional consequences (MacColl *et al.*, 2013; Magalhaes *et al.*, 2016).

5.5.1 Within population variation

Our results show that the major axis of shape variation between fish is closely related to predicted variation in mechanical properties, especially the transmission of force. This makes good sense, given that in the evolutionary transition from marine to freshwater environments stickleback must also make the transition from diets dominated by zooplankton to diets dominated by benthic prey. Among freshwater populations on North Uist the extent of evolution towards stronger biting morphologies depends on the ratio of benthic-planktonic prey in the environment (Linde et al., 2004). This is further supported by the strong relationship between predicted functional properties of the trophic morphology of North Uist stickleback populations, and their actual diet as estimated from their stomach contents. Anadromous stickleback exhibited mechanical properties strongly associated with feeding on plankton.

Relationships between stomach contents and the trophic phenotypes of individual fish were generally weak, and were only significant with suction index and mechanical advantage of jaw closing. Because of this, I will not

dwell on their interpretation, except to say that there was some evidence of a tendency for a relationship between trophic phenotypes and the contribution of benthic items to the diet. This is consistent with shift to a benthic diet in freshwater compared to anadromous populations. If selection is efficient at shaping trait means to the available food in a loch, then we might well expect that there should be no strong relationship between individual trait variation and diet. This is because we would expect that heritable variation would be used up in the course of evolution, so that remaining intrapopulation variation is the result of plasticity or random developmental processes. In addition, while we might expect fish to be adapted to their overall diet, stomach contents provide only a snapshot of this, and what a fish eats on any one day might well be the result of a host of factors apart from its trophic apparatus, such as season, weather conditions, the habitat it is in, prey availability or presence of predators (Bellwood & Wainwright, 2002).

In this study, fish with a high proportion of benthic invertebrates in their diet had lower suction indices. Fish characteristically use suction feeding to capture rapidly moving and predator-sensitive prey such as the zooplankton *Cyclops* and *Diaptomous* (Kiørboe *et al.*, 1999; Holzman & Wainwright, 2009), and suction feeding is an important prey capture method in the ray-finned fishes (Oufiero *et al.*, 2012; McGee & Wainwright, 2013) including stickleback. However, suction index alone is often insufficient to predict diet, because it interacts with other morphological and behavioural adaptations, especially ram feeding. This is when fish increase their speed of approach to their prey either by moving themselves rapidly towards the prey, or rapidly protruding their jaws (Ferry *et al.*, 2015; Longo *et al.*, 2016). Variation in ram may overcome the significance of variation in

suction index in some species. This may explain why limnetic stickleback in Canadian lakes, which eat substantial amounts of plankton, actually have lower suction indices than benthic stickleback (McGee *et al.*, 2013). Furthermore, our results disagree with McGee *et al.* (2013) who showed that sticklebacks that feed mainly on benthic invertebrate have high suction indices.

Individual level feeding performance on benthos was related mainly to driven components of closing jaw. The results showed that fish have low mechanical advantage of closing jaw (high displacement advantage), showing that jaws able to close fast. This was associated with high proportion of benthic components in the diet. The feeding mechanism of the lower jaw in this study disagrees with studies that suggested a high velocity in the lower jaw of fish specified in consuming elusive prey not benthos (Westneat, 1994; Wainwright & Richard, 1995; Barnett *et al.*, 2006; De Schepper *et al.*, 2008; Cooper & Westneat, 2009). A possible explanation for these observations is that teleost mouths are highly kinetic over a prey capture effort (Westneat, 1994; Turingan *et al.*, 1995; Cooper & Westneat, 2009) and usually work as a friction device to prevent prey escaping (Bellwood & Wainwright, 2002).

A link between morphology and diet has been suggested for a wide range of trophic guilds. These discrepancies are important because they show that we cannot accurately determine fish guilds from a few ecomorphological traits. Another explanation for weakness in the association between morphology and distinctive use of resources lies in variation in foraging behaviour. Morphology might shape the manner in which fish feed, but not the taxa consumed, as has been proposed for both chaetodontid and cichlid

fish (Barel, 1982; Motta, 1988). The weak link is also illustrated clearly in *Diplodus* fish that have common morphological traits, but are extensively divergent in their respective diets (Sala & Ballesteros, 1997).

Liem (1980) suggested that several specialist fish feeders were also capable of feeding on a large range of prey items, and could be described as 'Jacks of all trades'. Wrasses provide a good example. Despite morphological differences among species, there exists only a weak relationship between morphology and diet (Bellwood *et al.*, 2006). Stickleback may present a further example of a "Jack of all trades". While selection in freshwater populations may favour trophic morphology that is adapted to a benthic diet, the fish are plainly still capable of often incorporating a large proportion of planktonic prey in their diet.

5.5.2 Among population variation

Anadromous stickleback on North Uist, which are likely good proxies of the ancestral morphology of freshwater fish, are mostly zooplanktivorous, in common with anadromous stickleback elsewhere in the world (Ostbye *et al.*, 2016). This clearly supports the notion that freshwater fish have adapted according to the habitats they have colonised (Motani *et al.*, 2013), in keeping with the ecological theory of adaptive radiation (Schluter, 2000).

The tradeoff between force and velocity of jaws was also clear between freshwater and anadromous populations. This is consistent with previous studies suggesting that anadromous stickleback spend most of their life in the ocean feeding on pelagic zooplankton (Williams & Delbeek, 1989). In contrast, freshwater fish feed on different prey sources in littoral and pelagic areas in an environment that is highly heterogeneous compared to the marine environment (Williams & Delbeek, 1989). Therefore, in these

contrasting environments, selection for different adaptive trophic morphologies may result from divergent niche use between marine and freshwater stickleback, and among freshwater stickleback populations. (Williams & Delbeek, 1989; Schluter, 2000; Berner *et al.*, 2010).

Morphological gape data indicate that size of the open mouth differs significantly between freshwater and anadromous populations. Divergence in gape size is strongly related to foraging efficiency in populations exploiting different diets (Schluter & McPhail, 1992). In the current study, large gape was found in fish that were expected to feed mostly on benthic invertebrates which agrees with other studies that revealed that large gape is typical of fish feeding on large food items (McGee *et al.*, 2013; Ferry *et al.*, 2015; Collins & Motta, 2017). In addition, larger gape may increase the hydrodynamic of opening the mouth, the area of the fish's buccal cavity and subsequently reduce suction pressure (Lauder, 1983; Osse, 1989; Van Wassenbergh *et al.*, 2009)

Closing jaw was the only component of the mechanical properties that showed lower velocity in anadromous populations compared with freshwater populations. This may be due to the feeding behaviour of marine fish that feed mainly using ram feeding, where the fish moves its jaws past the prey to capture it in its open mouth (Evans & Claiborne, 2005).

The analysis of the association between trophic morphology of fish populations and ecological factors revealed that both biotic and abiotic environment are associated with variation in feeding mechanisms. The analysis at population level showed clearly the relationship between diet and mechanical properties (suction index, jaw closing and opening, jaw

protrusion, gape) of stickleback beside trade-offs in feeding performance between force and kinematic transmission.

Area and depth of lochs may determine the predominant prey in stickleback diets. Stickleback populations that live in deep lochs have rapid strike ram-suction and small mouth gapes to enable them to feed mainly on zooplankton. On the other hand, low suction indices was associated with fish living in lochs with shallow-water benthic habitats, where bottom-dwelling crustaceans and other invertebrates are common prey items.

Jaw protrusion was reduced in populations with a greater benthic contribution to their diet. This is consistent with studies which have indicated that it is greater in fish that feed mainly on plankton (McGee *et al.*, 2013; Higham *et al.*, 2017). Adaptive divergence in stickleback may result in greater projection of the oral jaw in pelagic feeding (limnetic) than benthic populations (Hulsey & García de León, 2005; Bellwood *et al.*, 2015), which may increase the velocity of fish attacking prey (Gibb, 1997). However, I found no relationship between jaw protrusion and planktonic contribution to diets. It is possible that estimates of planktonic contributions to diet based on stomach contents at a single time point are unreliable, especially given the seasonal, cyclical nature of plankton abundance.

A wide range of mechanical advantage and displacement advantage of the lower jaw was found in this study, although there was a bias towards fish being able to move the lower jaw forcefully rather than rapidly during jaw opening (Westneat, 1994; Wainwright *et al.*, 2004). The results revealed that jaw force was associated with fish feeding on benthic invertebrate and littoral macroinvertebrates which may need to be extracted from the substrate where they might burrow or be attached (Westneat, 2004).

The results in this study agree with other examples of tradeoffs between force and velocity of fish jaws. In labrid fish species evasive prey feeders have high velocity transmission in their lever systems. Others are able to apply force strongly to crush hard bivalve prey such as snails and other benthic invertebrates (Wainwright, 1987; Wainwright, 1988).

My results suggest that the presence of predators (brown trout, Salmo trutta) may make it challenging to secure food under the risk of predation, and cause a reduction in feeding efficiency (Lehtiniemi, 2005; Siepielski *et al.*, 2016). This, in turn, may cause a tendency of individuals to improve their ways of searching for different and high-value food sources by improving their feeding performance to reducing cost and time spent in their foraging. This may be achieved by targeting swimming prey rather than extracting benthic prey.

On the other hand, kinematic transmission of Opercular four-bar system in planktonic feeders suggests more output rotation is given by more input rotation which enables fish to open their mouth more rapidly compared with benthic feeders (McGee *et al.*, 2013).

The coefficient of force and velocity in four-bar biomechanical model permit to additional outputs through the feeding mechanism of force and velocity. It has also been noticed that the divergence has relatively the same trade in power of simple lever system. This similarity between the two systems may come from that both of them are sharing jaw opening inlever in simple lever system which equals to output link in Opercular four-bar system. This combination between the two systems in this component may also reveal the low force and velocity transmission used in closing jaw inlever as this component is not connected directly to transfer dynamic motion.

Differences in opercular four bar system may be due to expansion of dorsoventral of oral jaw.

Finally, it seems that fish-zooplankton interactions may show evidence of eco-evolutionary feedbacks. Results suggested an association between the extent of evolution of measured trophic morphology (gape and velocity of jaw opening) of and increased cladoceran density. Cladocerans produce more offspring and have higher reproductive investment (Stibor, 1992) under the intensive predation of fish evolved to feed mostly on plankton. Any resulting increase in the productivity of cladoceran populations may further increase natural selection on fish trophic traits used to capture such prey (Kahilainen *et al.*, 2011).

In conclusion, this chapter has shown that besides the wide phenotypic variation in body shape and armour already reported in stickleback populations on North Uist (Magalhaes *et al.*, 2016), there has been substantial trophic diversification. The transition of stickleback from marine to freshwater environments highlights how stickleback trophic traits vary among populations in ways linked to resource use. In general, individual fish only weakly matched their trophic traits to their diet. However, evolutionary adaptation was more obvious at the population level from the relationships between traits and environments. These results support the role of trophic utility in divergent selection of feeding performance during adaptive radiation of fish in post-glacial lakes.

Chapter 6. General discussion

Eco-evolutionary dynamics describes the interaction between ecology and evolution on contemporary timescales (Hendry, 2016). The idea has been widely applied to the study of contemporary evolution in predator-prey communities. It has been generally understood that both the structure and the dynamics of prey communities can be affected and thus changed by their predators to bring about predator evolution (Palkovacs & Post, 2008). These interactions in predator-prey systems may result in patterns of ecological and evolutionary diversity, such as adaptive divergence, divergence in predator foraging traits and variation in ecological dynamics of prey communities (Palkovacs & Post, 2008).

In this study, I investigated interactions that lead to ecological changes and drive either contemporary evolution (eco-to-evo) or vice versa (evo-to-eco). These interactions can feedback to influence one another: ecological change can cause evolutionary change, which in turn drives ecological change (Schielke *et al.*, 2011). The two main components of the interactions I investigated that determine eco-evolutionary dynamics in North Uist loch ecosystems were the three-spined stickleback (*Gasterosteus aculeatus*) (as the most ubiquitous predator in aquatic ecosystems) and zooplankton communities (as the main representative of prey communities).

Many studies have explored eco-evolutionary interaction in small experimental mesocosms (Hairston *et al.*, 2005; Palkovacs & Post, 2009; Bassar *et al.*, 2010; Palkovacs *et al.*, 2011; Schoener, 2011; Matthews *et al.*, 2016). In this study, the small water bodies on North Uist allowed us to study eco-evolutionary dynamics in small ecosystems in which lochs

represent small natural mesocosms that vary in both their biotic and abiotic factors.

Extreme variation of ecological factors between lochs in the east and west of the island has likely driven rapid selection of most phenotypic traits of North Uist sticklebacks (MacColl *et al.*, 2013; MacColl & Aucott, 2014; Magalhaes *et al.*, 2016) and leads to an expectation of changes in to other functional traits. I sought to cover the essential components that regulate the eco-evolutionary dynamics of fish and zooplankton by investigating the following aspects:

1- I evaluated taxonomic assemblages and species composition of zooplankton both within and between populations in response to ecological factors. To examinee eco-evolutionary feedback in zooplankton communities, we first need to know what those communities include to appropriately identify the most important species of zooplankton and determine the differences in diversity and distribution.

2- What is the relative importance of top-down and bottom-up control in driving demographic and population dynamic variation among prey communities?

3- Is there a genetic component to interpopulation variation in life history responses to predation?

4- Various changes in the evolution of prey may further influence ecological factors, including dietary preferences. This change may have an unexpected impact on ecological outcomes at various levels of the organisation, which will shape the evolution of predator foraging traits and functional feeding systems.

These aspects of the project are addressed below, with a particular focus on the results found in this work.

6.1 Composition and distribution of zooplankton on North Uist

The lochs of North Uist support diverse and abundant zooplankton communities, which are associated with a wide variation in biotic as well as abiotic factors among lochs. Both abundance and diversity varied greatly between locations.

In this study 39 zooplankton species were identified. Zooplankton species richness was higher compared with other studies on large lakes in Scotland, including 15 species in Loch Rusky (Romo, 1990), and 23 species in Loch Leven (Gunn & May, 1999). Rotifera species richness and abundance are higher in freshwater bodies than other groups of zooplankton (Florescu & Moldoveanu, 2017). Likewise, the Northern Hemisphere often exhibits high species richness and abundance of Rotifera, which may be due to their short life span, high parthenogenetic reproductive rate and their generalised feeding (Wallace, 2002; Moreira *et al.*, 2016). The results of this study agree with other studies that found Rotifera often exhibit high densities in the Northern Hemisphere (Segers, 2008). Large lochs in Scotland have also reported dominance of Rotifera (Romo, 1990; May & O'Hare, 2005).

Small-bodied cladocerans such *as Bosmina coregoni*, have exhibited remarkably high densities in some lochs, which is likely because predation is highly selective on large-bodied Cladocera (Brooks & Dodson, 1965; Skoglund *et al.*, 2013). *Bosmina coregoni* was recorded as the dominant crustacean zooplankton in most of Scotland's large lochs (Gunn & May, 1999). *Bosmina* density, besides *Daphnia*, also showed a high consistency between years (2014-2016). In addition, most of the dominant crustacean

zooplankton in my study were recorded as the most frequent species in other large lochs in Scotland, especially *Bosmina coregoni* and *Daphnia hyalina* (Gunn & May, 1999).

Notably, large Cladoceran species, which normally prefer either littoral or deep habitats, were scarce in North Uist's lochs. Indeed, large-bodied species of Cladocera are usually found only sporadically in their habitats (Gillooly & Dodson, 2000; Meerhoff *et al.*, 2007; Dejenie *et al.*, 2012).
In keeping with previous studies, I found a greater abundance of cyclopoid compared to calanoid copepods. Similar to cladocerans, this is probably because the small size of cyclopoids enables them to avoid planktivorous fish (Brooks & Dodson, 1965; Skoglund *et al.*, 2013).
Interestingly, fish presence seemed to influence the composition of zooplankton groups in only one subset of lochs containing fish. A significant relationship was recoded only between Cladocera density and fish density when lochs without fish were excluded from the analysis. There was no clear association between fish density and Rotifera and Copepoda groups in this study. A weak correlation between zooplankton density and fish occurrence may be due to our pattern of sampling in the short term, which is concentrated on spatial replication. This may well have failed to catch all lochs at the same points in their annual cycles. This happens when they are known for having large quantities of zooplankton and could either overestimate the importance of exceptional events or miss them altogether (d'Alcalà *et al.*, 2004).
In addition, different diversity profile curves of zooplankton assemblages were observed in this study. Uneven or patchy distribution was observed in small lochs, both with and without fish, compared with a relatively high evenness in large lochs with fish. The role of predation was not significantly

related to the diversity of species, but it might have an indirect impact on controlling the distribution of zooplankton across populations by reducing the competition and increasing evenness (Thorp & Cothran, 1984; Xie & Yang, 2000; Donald *et al.*, 2001)

Zooplankton community structure was relatively associated with the measured environmental factors. Species richness was higher, and copepods abundance was lower in acidic lochs. This agrees with Hessen & Nilssen (1983), who found that copepods prefer to live in the peak of the eutrophic system in concurrence with a high pH. The results of species richness in my study contrasts with the findings of Wetzel (2001), who found lower species richness in dystrophic and acid ecosystems

6.2 Cladoceran life history is driven mainly by top-down more than bottom-up control on North Uist

There has been a long debate on how bottom-up (resources) and top-down (predation) factors structure ecological communities (Hunter & Price, 1992). Predation by fish largely affects zooplankton survival, which is likely to favour feedback that will subsequently increase the rate of zooplankton reproduction (Ślusarczyk, 1997; Riessen, 1999), but abundant resources should favour the same pattern. This study suggests that top-down effects have a major impact on cladoceran life histories, with higher predation favouring fast life histories and higher reproductive investment. This pattern is not significantly affected by any variation in resource conditions. My results are compatible with the predictions of life history theory—that low predation populations increase egg production, and females mature early and display short lifecycles that might increase the rate of survival to maturity (Stearns, 1976). The increase in mean clutch size in lochs with fish

shows additional effort being invested in producing small eggs, which might allow small juveniles to evade predators. In contrast to previous studies (Brooks & Dodson, 1965; Galbraith Jr, 1967), there was no connection on North Uist between the occurrence of fish and the average size of cladocerans. Our findings agree with Gliwicz & Boavida (1996) who found that size at maturation of *Daphnia* within lakes that are stocked with fish was half that in fishless lakes. Moreover, predation was linked with the egg size of planktonic cladocerans which is considered an ordinary phenomenon when fish predation increases (Lampert, 1993).

In the presence of fish *Daphnia* and *Bosmina* are also more likely to produce ephippia (resting eggs) than populations in fishless lochs, which continue to produce offspring. This again suggests a longer lifecycle. In this study, most ephippia were found during late spring. It is likely that ephippia production is an evolved response to the low survival of parthenogenetic cladoceran females at this time of the year, which may be a reaction to chemical cues discharged by fish, higher temperatures and/or long-day photoperiods (Pijanowska & Stolpe, 1996). A plausible alternative explanation is that high phytoplankton growth during late spring and summer may increase zooplankton density when plenty food is available. By contrast, predation pressure increases considerably on abundant planktonic cladocerans during this period of the year (Townsend *et al.*, 1986; Velthuis *et al.*, 2017) This increases mortality (Lampert *et al.*, 2014), which might initiate the diapause phase (Lees, 2016).

Finally, it is known that reproductive patterns in Cladocerans are strongly linked with indices of predation threat and are not entirely independent of other environmental factors (Maciej Gliwicz, 2002). Although the association

between reproductive parameters and resource availability is unclear, when severely limited, they constrain life history responses to fish (Hülsmann *et al.*, 2004; Gliwicz & Maszczyk, 2007).

6.3 Transgenerational life history variations in cladocerans on North Uist

Differences in life history parameters between cladoceran populations remained consistent after three generations in common garden conditions, which strongly suggests that they have an evolved, heritable basis. In line with previous findings, cladocerans from populations experiencing fish predation were observed to have faster growth, maturity and a large clutch size at a young age. This may be a strategy for successful establishment and rapid increases in populations that are at risk of predation (Ślusarczyk, 1997; Svensson & Gorokhova, 2007). Third generation offspring size and embryonic development were similar to that of their parents while young. However, the correlation in growth rate became weaker with maturity.

A trade-off in offspring size has been predicted as a response by prey to predator selectivity. Decreasing size at birth is assumed for offspring that are highly vulnerable to fish (Dodson, 1974; Lynch, 1980). Conversely in fishless lochs larger offspring size may be favoured to avoid predation by invertebrates (Lüning, 1992). Under these circumstances, mortality is expected to increase the production of more offspring, a faster growth rate and earlier reproduction (Walsh & Post, 2012; Packer & Walsh, 2017). However, different findings regarding the size of neonates were observed. While Stibor (1992) found that small adults give birth to small neonates, our study found that the size of neonates depended mainly on fish presence not size of mothers. Finally, the results showed enough evidence that

zooplankton on North Uist may have an evolved, heritable basis since last glaciation approximately 10,000-15,000 years ago when species invaded new freshwater environments (Dynesius & Jansson, 2000; Jordan *et al.*, 2010). This establishes the view for co-evolutionary or eco-evolutionary feedback between Cladocera and their stickleback predators.

6.4 Stickleback feeding performance: Linking morphology to feeding specialisation

The previous two sections described the effects of fish on both the abundance and life history strategies of zooplankton, especially cladocerans. Here, I examine the other side of the eco-evolutionary dynamics equation: How is variation in prey associated with the predator's evolution?

In the adaptive radiation on North Uist, substantial variation in trophic morphology traits and derived mechanical properties are associated with diet variation at the individual level and more strongly at the population level. In general, in the evolutionary transition from marine to freshwater habitats, fish have experienced a transition in diet from zooplankton to benthic prey, and this has driven the evolution of trophic morphology. A comparison between anadromous and freshwater resident populations revealed that anadromous populations, which feed mainly on plankton, have high jaw velocity, while freshwater populations, which feed mainly on benthic organisms, have high jaw force. This is likely a result of adaptation to the contrasting environments of their diets (Williams & Delbeek, 1989; Schluter, 2000; Berner *et al.*, 2010; Ostbye *et al.*, 2016).

Within freshwater, populations with a high proportion of benthic invertebrates in their diet had reduced mechanical jaw closing, which

contrasted other studies that suggested a high velocity in the lower jaw of fish that are specified to consume elusive prey but not benthos (Westneat, 1994; Wainwright & Richard, 1995; Barnett *et al.*, 2006; De Schepper *et al.*, 2008; Cooper & Westneat, 2009). This may be related to a high jaw kinetic that is used for prey capture with teleost mouths, which prevent prey from escaping (Turingan *et al.*, 1995; Bellwood & Wainwright, 2002; Westneat, 2004).

I also found that benthic-feeding populations had lower suction indices. This contrasts with McGee *et al.* (2013), who found that sticklebacks that feed on benthic invertebrates had high suction indices. Studies also revealed that suction feeding could be accounted for by other feeding mechanisms and may be associated with other behavioural and morphological adaptations especially ram feeding (Ferry *et al.*, 2015; Longo *et al.*, 2016).

Despite the strong patterns of association between diet and trophic mechanical properties across fish populations, at the individual level relationships were weak. While a wide range of studies show relationships between morphology of fish and diet, there are certain discrepancies as well (Longo *et al*., 2016). A possible explanation may be the stickleback's 'Jack of all trades' foraging behaviour, which may allow individuals a generalist diet consisting of certain benthic and planktonic crustaceans (Liem, 1980). Furthermore, factors other than trophic morphology such as prey selectivity and prey availability are likely to affect the diet of individuals (Bellwood & Wainwright, 2002).

The mechanical properties of resident stickleback populations were associated with both the abiotic and biotic attributes of the lochs. Depth of the lochs, plankton contribution and trout presence in the diet were the

main factors determining a trade-off between the force and the velocity of the simple lever system of the lower jaw and suction index, while the opercular four-bar jaw system was associated with the benthic's diet contribution.

The results of this study agreed with other examples of the trade-off between force and velocity in fish jaw systems. Benthic feeders actively apply force through their jaw system, while evasive prey feeders have high velocity transmission in their lever systems (Wainwright, 1987; Wainwright, 1988). Finally, investigating additional effects of fish trait evolution on Cladocerans revealed possible eco-evolutionary feedback between fish and zooplankton: fish in lochs with a high density of zooplankton have a small gape and a highly kinematic transmission in their jaw system.

6.5 Possible evidence of eco-evolutionary feedbacks in fish-zooplankton communities on North Uist

Although there are many examples of eco-evolutionary dynamics, it might be difficult to predict eco-evolutionary consequences and obtain clear eco-evolutionary feedback evidence (Fussmann *et al.*, 2007; Kinnison *et al.*, 2008; Ferriere & Legendre, 2013).

The proposed key players in eco-evolutionary feedback in this study are the stickleback (predator) and their primary prey (zooplankton). The evidence showed that some aspects that might lead to that set of fish and zooplankton responses may have come as a result of their involvement in eco-evolutionary feedback processes. Here, I review some of the results from my study that could highlight the eco-evolutionary feedback on North Uist.

Many lochs on the island were colonised by sticklebacks after the last glaciation, which was 10,000-15,000 years ago. Therefore, throughout their evolutionary history, some populations may have begun to exploit and affect their new environment inside these natural barriers (Jones *et al.*, 2013). The first effect that might be expected is consuming zooplankton, the main food for fish in aquatic ecosystems.

Under such circumstances, a reduction in prey survival may occur, and selection may promote genotypes that are capable of reproducing at a smaller size and at a younger age as well as at a higher reproductive rate (Gadgil & Bossert, 1970; Law, 1979; Stibor, 1992; Charlesworth, 1994; Gårdmark & Dieckmann, 2006; Walsh & Post, 2011).

Cladoceran species in lochs with fish went through similar pronounced life history changes and had a higher tendency to have a short lifecycle, which was primarily related to fish predation (chapter 3).

Thus, it seems that constant exposure of fish to zooplankton may allow the zooplankton to influence the evolution of subsequent generations (chapter 4) and sustain eco-evolutionary feedback. This is considered a potential contributor to co-evolutionary or eco-evolutionary feedback, whose existence is predicated on such genetic responses in populations (Becks *et al.*, 2012).

As a consequence, there is a possibility that the life maintenance of fish required afferent feedback (Burleson *et al.*, 1992). Sticklebacks may have enhanced their feeding performance to adapt to the most frequent variation in the trophic level.

Evidence from chapter 5 suggest that various aspects may have occurred that may lead to a strong selection of traits related to foraging on different prey items, including plankton. This variation in trophic traits represents the second part of the loop, when changes in prey feedback can shape the evolution of predator foraging traits and the functional components of the main foraging apparatus (Palkovacs & Post, 2008; Duffy & Forde, 2009; Palkovacs & Post, 2009; Kahilainen *et al.*, 2011; Palkovacs *et al.*, 2011; Walsh & Post, 2011; Hendry, 2016).

Although, sticklebacks on North Uist develop their foraging trait to feed on different prey items, their traits divergence related to feeding on plankton is significantly differentiated (chapter 5). However, collecting evidence from natural systems to connect eco-evolutionary feedback elements is a big challenge when it is related to the uncontrolled wide variation in environmental factors.

Conclusion

This study has investigated the mechanisms of eco-evolutionary feedback in fish-zooplankton communities. Eco-evolutionary feedback requires that populations change their environment, which may then influence the subsequent evolution of the population (Post & Palkovacs, 2009). This study can be considered as the first intensive study investigating eco-evolutionary dynamics in fish-zooplankton community, including all essential components that regulate this model in small natural systems, with a wide range of environmental factors.

Initially, exploring the structure and the composition of zooplankton on North Uist represented an essential contribution to knowledge; zooplankton communities in this area have not been investigated for nearly a century. Zooplankton communities of small lakes in the UK (and indeed Europe) are poorly known, despite being a major component of freshwater biodiversity, which makes it valuable as an indicator species and provides examples of important consequences of the functions of aquatic ecosystems.

It is known that predators can influence their environment through their consumption of other organisms (Post & Palkovacs, 2009). My results showed that zooplankton alter their life history parameters mainly in habitats containing a higher density of fish. Cladocerans, an important zooplankton group, mature early, release their offspring faster and at a smaller size as well as have a large clutch size, small eggs and a short lifecycle compared with places with either lower fish density or no fish. This variation in life history parameters was experimentally revealed as an example of transgenerational epigenetic inheritance. This also agrees with Yoshida *et al.* (2003) who showed that organisms' effects on the ecosystem

could evolve and persist across generations. By contrast, the results showed differences in feeding mechanisms that may strongly influence the zooplankton community. This includes modification in trophic traits and their derived components, which enable fish to enhance feeding mechanisms.

In fish, the composition of the diet in this study was often correlated with the pattern of trophic traits and their derived components used for feeding. It was also found that the evolution of some fish traits may affect the prey community's structure as a sign of eco-evolutionary feedback between the predator and the prey.

The results agreed with the eco-morphological hypothesis that suggests that variation in foraging traits of fish strongly affects diet and differences in both feeding ability and prey selection (Wainwright & Richard, 1995).

Finally, this study highlighted the relationship between environmental variables and the population dynamics of zooplankton and divergence in fish trophic traits. The results showed a strong association between loch depth and the life history of Cladocera and the mechanical properties of the stickleback's jaw.

This may be because depth determines the intensity of trophic structures and fauna in water ecosystems (Narumalani *et al.*, 1997; Covich *et al.*, 1999; Jeppesen *et al.*, 2000) which can potentially alter species in associated food webs. This finding also supported the main role of the geographic standpoint in shaping outcomes of continuous ecological interactions as an example of mosaic coevolution theory (Thompson, 2005).

Future work

The current study investigated the potential for eco-evolutionary interaction between zooplankton and fish on North Uist. I focused mainly on variation in trophic morphology of stickleback and life history parameters in Cladocera as well as investigating the overall patterns in community composition of Copepod and Rotifera.

Today's theoretical ecologists are extending eco-evolutionary studies to explore the functioning and eco-evolutionary dynamics of multispecies communities (Fussmann *et al.*, 2007). In future work it will be interesting to explore the population dynamic variation of zooplankton groups (Rotifera and Copepoda) to better understand eco-evolutionary dynamics on North Uist. In addition, I strongly recommend to do a common garden experiment in aquatic mesocosms on North Uist which may reveal more on this aquatic systems.

I investigated the morphology of the jaws of stickleback, which form only part of the trophic apparatus. Another essential component are the gill-rakers. Variation in these, and their functional consequences, are well known in stickleback, but it would be interesting to understand the extent to which jaws and gill-rakers are integrated across individuals and populations. Furthermore, the way in which tools are used is almost as important as the tools themselves. An important missing part of our understanding of fish trophic morphology is how it is used behaviourally, and to what extent, morphological and behavioural variation are related. This would require detailed observations of fish feeding, including the use of high-speed video to quantify biomechanical function during strikes on prey, and how the

transfer force and velocity in fish jaw system is used in the capture of different kinds of prey.

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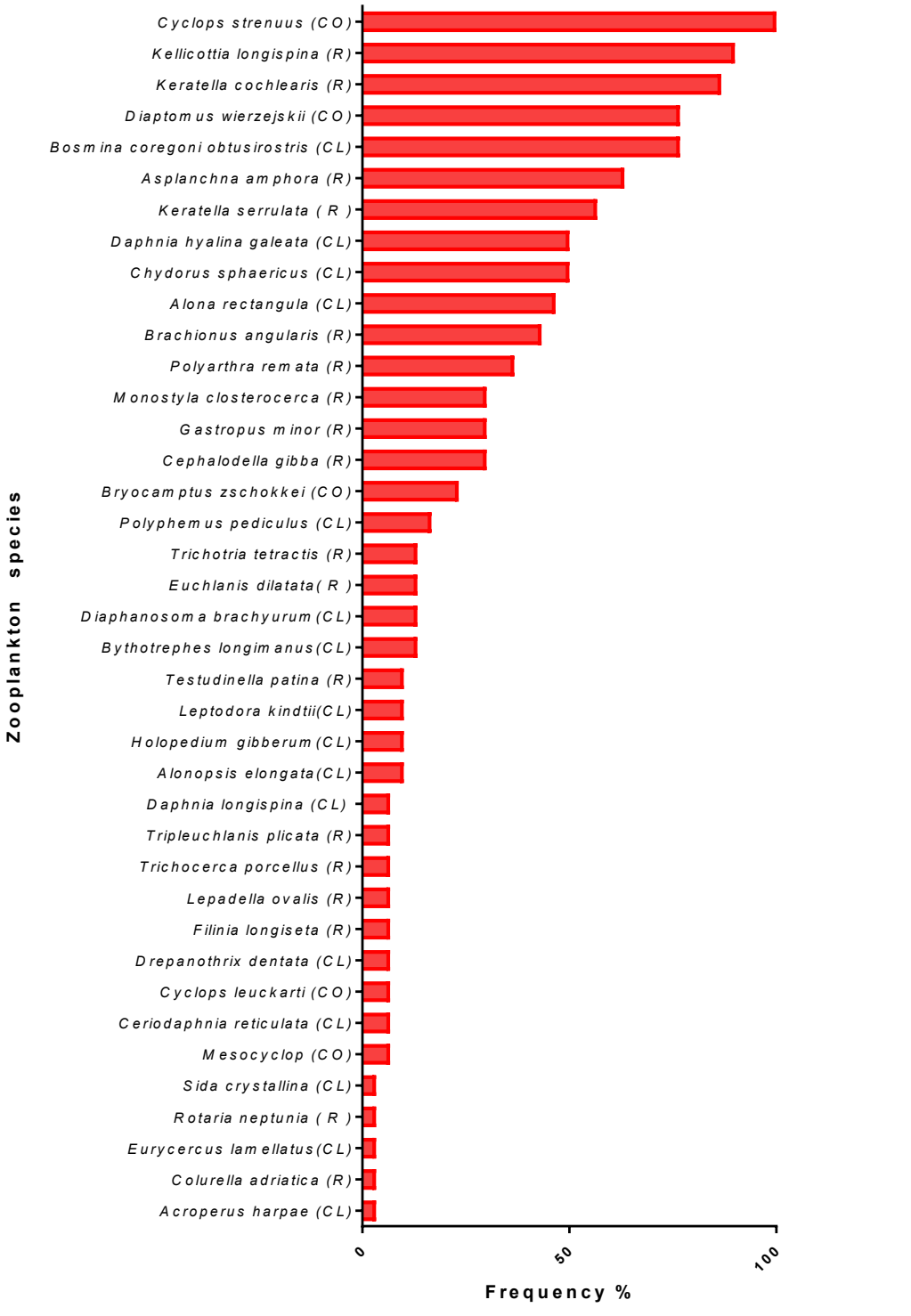
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Appendices



Appendix 2.2. Frequency of zooplankton species in the 30 lochs on North Uist explaining how many times in percentage each species appeared. Species coded with (R, CL, CO) representing Rotifera, Cladocera, Copepod respectively.

Appendix 3.1. Life history parameters mean clutch size and egg size (±SE), minimum size gravid female, the proportion of gravid females as 95% confidence interval for 23 *Bosmina* populations on North Uist. CI=confidence interval.

Loch	Clutch size	egg size (mm)	Minmum size of gravid female(mm)	Percentage of gravid females (%)
Aong	3.1±0.43	0.0139±0.0007	0.421	20.8 (10.1, 32.4)
Aroi	2.7±0.37	0.0127±0.0007	0.464	16.0 (7.1, 27.8)
Bdrm	3.9±0.69	0.0148±0.0006	0.368	50.0 (35.9, 64.1)
Bhar	1.7±0.30	0.0155±0.0006	0.409	25.0 (13.0, 36.9)
Cdrm	5.5±0.22	0.0151±0.0006	0.400	60.0 (45.0, 72.2)
Chru	5.5±0.54	0.0080±0.0002	0.340	90.0 (78.2, 96.0)
Fada	4.5±0.53	0.0129±0.0007	0.316	80.0 (65.1, 88.5)
Fhai	3.3±0.45	0.0129±0.0005	0.320	90.0 (78.2, 96.0)
Gill	2.2±0.36	0.0137±0.0007	0.231	50.0 (35.9, 64.1)
Grog	5.8±0.68	0.0137±0.0007	0.280	61.0 (45.0, 72.2)
Host	4.3±0.65	0.0157±0.0009	0.452	40.0 (26.1, 53.0)
Maig	1.3±0.15	0.0164±0.0003	0.380	49.0 (33.3, 61.1)
Shos	2.4±0.31	0.0156±0.0006	0.520	33.0 (19.5, 45.0)
Stru	8±0.97	0.0104±0.0003	0.280	80.0 (65.1, 88.5)
Torm	2.5±0.50	0.0150±0.0013	0.389	33.0 (19.5, 45.0)
FL01	2.7±0.50	0.0190±0.0007	0.442	66.7 (52.0, 78.2)
FL03	2.3±0.21	0.0203±0.001	0.652	16.7 (7.1, 27.8)
FL05	3.1±0.18	0.0107±0.0006	0.450	50.0 (35.9, 64.1)
FL06	1.9±0.23	0.0168±0.0005	0.515	10.7 (4.0, 21.8)
FL09	2.3±0.30	0.0122±0.0004	0.452	50.0 (35.9, 64.1)
FL10	1.7±0.15	0.0145±0.0007	0.505	25.0 (13.0, 36.9)
FL11	3±0.33	0.0121±0.001	0.353	40.0 (26.1, 53.0)
FL12	2.8±0.33	0.0142±0.001	0.429	12.0 (4.7, 22.8)

Appendix 3.2. Life history parameters mean clutch size and egg size (±SE), minimum size gravid female, the proportion of gravid females as 95% confidence interval for 17 Daphnia populations on North Uist.

Loch	Clutch size	*Daphnia* egg size(mm)	Minimum size gravid female(mm)	Percentage of gravid females (%)
Aong	4±0.577	0.0249±0.0008	1.25	86.0 (73.2, 93.3)
Aroi	3±0.683	0.0304±0.0009	1.29	45.0 (29.9, 57.0)
Bdrm	3.5±0.619	0.0313±0.0007	1.16	50.0 (35.9, 64.1)
Bhar	3.5±0.269	0.0284±0.0005	1.45	66.0 (52.0, 78.2)
Fada	5.8±0.757	0.0217±0.0003	1.08	16.0 (7.1, 27.8)
Fhai	3±0.258	0.0288±0.0018	1.31	50.0 (35.9, 64.1)
Gill	6.2±1.073	0.0215±0.0010	1.39	20.0 (10.1, 32.4)
Grog	8.6±0.499	0.0159±0.0014	1.25	33.0 (19.5, 45.0)
Host	3.5±0.477	0.0336±0.0010	1.45	90.0 (78.2, 96.0)
Maga	12±0.775	0.0191±0.0012	1.12	70.5 (56.0, 81.2)
Maig	2.3±0.260	0.0328±0.0003	1.18	43.0 (29.9, 57.0)
Shos	4.5±0.307	0.0403±0.0018	1.47	85.0 (70.2, 91.5)
Stru	12±1.348	0.0188±0.0006	1.12	80.0 (65.1, 88.5)
Torm	5.5±0.898	0.0220±0.0009	1.31	80.0 (65.1, 88.5)
FL06	4±0.258	0.0360±0.0013	1.92	35.0 (22.8, 49.0)
FL10	1.1±0.100	0.0428±0.0006	1.49	30.0 (18.8, 44.0)
FL12	1.8±0.249	0.0390±0.0014	1.65	20.0 (10.1, 32.4)

Appendix 5.1. Average density of food categories of stickleback diet of 22 fish populations on North Uist.

Loch	Aong	Aroi	Bdrm	Bhar	Buai	Cdrm	Chru	Fada	Fhai	Gill	Grog	Host	Iala	Maga	Maig	Reiv	Shos	Stru	Torm	Faik	Obse	Tros
Bosmima coregoni	0	40.6	355.6	7.3	0	118.6	0	0.7	0	3.6	0	0.2	0	0	5.5	0	0	7.5	7.3	0	0	155.3
Alona sp.	1.2	0	0	0.8	0.1	0.4	0	1.5	1.2	0	0	0.6	4.1	0	0.2	0	1.9	1.7	0.1	0	0	0
Drepanothrix denata	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daphnia sp.	0	0	1	1.5	0	0	0	0.1	0	0	0	10.9	0	0.1	1.1	0	0.6	0	0	0	0	0
Chydorus sphaericus	0.9	0.5	0	0.1	0.5	0	0	0.2	0.3	0	0	0	2	0	0.5	0	0.9	1	0.1	0	0	0
Polyphemus pediculus	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0
Bythotrephes longimanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.4	0	0	0	0	0
Simocephalus sp.	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Evadne nordmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.6	0
Diaptomus sp.	0.2	0	0	0.1	0.7	0	0	0	0	0	0	0.7	0	0	5.4	0	0.4	0	0.1	0	0	0
Cyclops sp.	0.4	0	0.1	1	0.8	0.2	0.1	0.12	0.1	0	0.6	0.5	0.1	0.6	6.5	0	51.1	0	0.1	1.2	27.7	13.6
Harpaticoid copepod	1.5	0	0	0	0	0	0	0.4	0.3	0.3	0	0	1	0.1	0.1	0	0	0.8	0.1	0	0	0
Stickleback eggs	1.8	0.1	0	0	0.2	0	0.1	0	0.9	0	1.2	1.3	0.6	2.1	0	0	0	0	0.5	0	0.3	0
Pond Snail (Lymnaeidae)	0	0	0	0	0	0	0.1	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0
Pea mussel (Sphaeriidae)	0	0	0	0	0.1	0	0.3	0	0	0	0.1	0	0	0	0	0	0	0.1	0	0	0	0
Ostracoda	0.2	0	0.1	0.1	0	0	0.5	0	0.4	0	0	0	0.4	0	0	0.3	0.4	0	0.1	0	1.7	0
Mysis relicta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0.1	0.1	0
Gammarus duebeni	0	0	0.2	0	0	0.2	0.6	0	0.6	0.4	0.6	0.5	0	0.7	0	1.9	0	0	0.5	0.3	0.1	0

Diptera larvae	0	0	0	0	0	0	0	0.2	0.1	0.2	0	0	0	0	0	0	0.1	0	0.1	0	0	0
Non-biting midge (adult)	0	0	0.1	0	0.7	0.1	0	0	0.2	0	0	0.1	0	0	0	0.1	0	0	0	0	0	0
Midge larvae	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	2.6
Chirionomid (Tanypodinae)	1.1	2.8	2.7	1.5	0.5	0.7	4.8	0.7	0.9	0.4	1.6	2	0.2	1.7	9.4	4.4	13.8	0.8	0.1	0.1	0	0
Chironomid (Orthocladiinae)	0	0	0	0.1	0	0	0	0	0	0	0.4	0	0	0	0	0	0	0	0	22	0	0
midge pupae	0.1	0	0	0	0	0	0	0.1	0.2	0	0	0.5	0	0	0	0	0	0	0	25	0	0
Caddis fly larvae: *Triaenodes bicolor* : Leptoceridae	0	0.3	1.1	0	1.5	0.5	0.2	0.2	0.1	0	0.6	0.2	0	0.2	0	0	0.2	0	0	0.1	0	0
Caddis fly larvae: *Tinodes waeneri*	0	0	0.3	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayfly nymph: Caenidae	0	0.1	0.4	0	0.1	0	0.2	0.1	0.1	0	0	0	0	0	0	0.2	0.8	0	0.1	0	0	0
Worm	0	0	0	0	0.1	0	0	0.3	0.2	0	0.1	0.3	0	0.1	0.2	0	0	0	0	0	0	0
Leech	0	0	0	0	0	0	0.3	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0.07	0
Mite	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crab zoea larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
crustacean alrvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	0

Appendix 5.2. Frequency of food categories of stickleback diet of 22 fish populations on North Uist.

Loch	Aong	Aroi	Bdrm	Bhar	Bual	Cdrm	Chru	Fada	Fhai	Gill	Grog	Host	Iala	Maga	Maig	Reiv	Shos	Stru	Torm	Faik	Obse	Tros
Bosmima coregoni	0	5	10	7	0	9	0	2	0	2	0	2	0	0	7	0	0	1	4	0	0	10
Alona sp.	5	0		4	1	1	0	4	4	0	0	4	8		1		5	2	1	0	0	0
Drepanothrix denata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daphnia sp.	0	0	1	3	0	0	0	1	0	0	0	9		1	3	0	1	0	0	0	0	0
Chydorus sphaericus	6	1	0	1	3	0	0	1	2	0	0	0	5	0	2	0	2	1	1	0	0	0
polyphemus pedicalis	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Diaptomus sp.	1	0	0	1	2	0	0	0	0	0	0	4	0		4	0	1	0	1	0	0	0
Cyclops sp.	1	0	1	4	5	2	1	1	1		5	3	1	3	7		8	0	1	3	10	9
Harpaticoid copepod	4	0	0	0	0	0	0	1	1	1	0	0	3	1	1	0	0	2	1	0	0	0
Bythotrephes longimanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Simocephalus sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Evadne nordmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Stickleback eggs	3	1	0	0	2	0	1	0	2	0	1	3	2	3	0	0	0	0	1	0	3	0
Pond Snail (Lymnaeidae)	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pea mussel (Sphaeriidae)	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
Ostracod	2	0	1	1	0	0	2	0	2	0	0	0	1	0	0	1	3	0	1	0	3	0
Mysis relicta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	0
Gammarus duebeni	0	0	1	0	0	2	4	0	4	4	5	2	0	5	0	5	0	0	5	1	1	0
Diptera larvae	0	0	0	0	0	0	0	2	1	2	0	0	0	0	0	0	1	0	1	0	0	0
Non-biting midge (adult)	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
Midge larvae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	8
Midge larvae: chirionomid (Tanypodinae)	5	7	6	6	2	3	8	3	4	3	4	6	2	5	3	5	7	3	1	1	0	0
Midge larvae: chironomid (Orthocladiinae)	0	0	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	5	0	0
midge pupae	1	0	0	0	0	0	0	1	2	0	0	4	0	0	0	0	0	0	0	4	0	0

Caddis fly larvae: *Triaenodes bicolor:* Leptoceridae	0	1	3	0	8	2	2	2	1	0	5	2	0	1	0	0	2	0	0	1	0	0
Caddis fly larvae:*Tinodes waeneri*: Limnephilidae	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayfly nymph: Caenidae	0	1	2	0	1	0	2	1	1	0	0	0	0	0	0	2	3	0	1	0	0	0
Worm	0	0	0	0	1	0	0	2	1	0	1	2	0	1	1	0	0	0	0	0	0	0
Leech	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Mite	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crab zoea larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
crustacean larvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0

Appendix 5.3. Mean plankton and benthic prey contribution in fish diet according to size-numeric score (±SE) of 22 fish populations on North Uist.

Fish population	Planktonic diet contribution	Benthic diet contribution
Aong	2.73±1.40	5.87±2.04
Aroi	17.61±10.35	14.48±4.12
Bdrm	179.70±148.87	23.67±8.39
Bhar	8.82±3.79	3.88±1.53
Buai	1.88±1.09	26.10±8.95
Cdrm	61.28±26.53	8.09±2.93
Chru	0.08±0.08	21.91±6.81
Fada	1.66±0.69	4.45±1.16
Faik	1.53±0.81	9.60±3.65
Fhai	1.12±0.36	14.08±3.98
Gill	1.98±1.67	5.70±2.08
Grog	0.72±0.27	14.40±2.81
Host	19.31±6.78	9.06±3.27
Iala	1.20±0.53	8.69±1.34
Maga	0.62±0.33	19.39±4.32
Maig	29.72±9.27	3.90±1.85
Obse	37.99±16.04	3.76±1.90
Reiv	0.00±0.000	50.66±23.37
Shos	71.98±29.80	3.22±1.79
Stru	5.71±4.47	2.24±1.13
Torm	4.46±3.25	2.25±1.13
Tros	95.79±28.66	7.20±1.67

Appendix 5.4. Data of suction index component traits, jaw protrusion and mouth gape of 22 fish populations on North Uist.

Population code	Buccal length (mm)	Neuro cranium outlever (mm)	Epaxial hight (mm)	Epaxial width (mm)	Jaw protrusion (mm)	Mouth gape (mm)
Aong	11.202±0.108	12.239±0.116	2.119±0.081	4.184±0.058	1.071± 0.042	3.204±0.067
Aroi	11.359±0.169	12.484±0.153	2.005±0.052	4.432±0.072	1.246± 0.067	3.110±0.093
Bdrm	11.386±0.173	12.878±0.169	2.208±0.127	4.125±0.082	1.101± 0.049	3.265±0.060
Bhar	11.521±0.094	12.588±0.109	2.137±0.068	4.212±0.041	1.166± 0.039	3.576±0.088
Buai	11.917±0.091	13.080±0.067	2.187±0.089	4.621±0.050	1.172± 0.037	3.663±0.058
Cdrm	11.964±0.175	13.136±0.176	2.160±0.094	4.255±0.082	1.191± 0.042	3.366±0.073
Chru	12.312±0.191	13.264±0.191	2.230±0.061	4.339±0.062	1.217± 0.041	3.757±0.096
Fada	11.517±0.097	12.550±0.083	2.230±0.056	4.237±0.048	1.182± 0.023	3.444±0.065
Fhai	11.394±0.113	12.438±0.134	1.993±0.052	4.036±0.115	1.031± 0.043	3.428±0.078
Gill	11.056±0.218	11.985±0.241	1.994±0.051	3.955±0.100	1.196± 0.027	3.318±0.089
Grog	11.757±0.128	12.822±0.105	2.213±0.064	4.263±0.104	1.122± 0.034	3.350±0.076
Host	11.681±0.095	12.765±0.092	2.211±0.062	4.225±0.089	1.005± 0.044	3.314±0.069
Iala	11.083±0.142	12.226±0.147	2.183±0.054	4.138±0.041	1.174± 0.039	3.071±0.110
Maga	11.722±0.154	12.913±0.182	2.044±0.063	4.296±0.082	1.047± 0.036	3.372±0.082
Maig	11.533±0.207	12.620±0.236	2.170±0.051	4.246±0.082	1.189± 0.041	3.169±0.095
Riev	11.095±0.146	12.042±0.159	2.007±0.139	4.011±0.098	0.998± 0.048	3.371±0.102
Shos	11.446±0.180	12.479±0.166	2.063±0.073	4.015±0.135	1.257± 0.033	3.382±0.152
Stru	11.938±0.115	13.014±0.126	2.135±0.030	4.793±0.109	1.232± 0.032	3.467±0.058
Torm	11.548±0.069	12.688±0.089	2.840±0.038	4.405±0.126	1.123± 0.043	3.267±0.085
Faik	11.530±0.221	12.531±0.250	2.024±0.163	4.203±0.057	1.175± 0.069	3.404±0.119
Obsm	11.373±0.306	12.412±0.313	2.100±0.187	4.145±0.031	1.163± 0.096	3.424±0.214
Tros	11.900±0.367	12.904±0.362	2.490±0.130	4.383±0.140	1.319± 0.100	3.789±0.188

Appendix 5.5. Trophic traits components of mechanical and displacement advantage of opening and closing jaw and opercular four-bar system of 22 fish populations on North Uist.

Population code	Opening inlever (mm)	Closing inlever (mm)	Opening-closing outlever (mm)	Input link (mm)	Output link (mm)	Coupler link (mm)	Fixed link (mm)
Aong	0.680±0.009	1.246±0.034	2.848±0.051	12.239±0.116	3.204±0.067	2.119±0.081	4.184±0.058
Aroi	0.680±0.012	1.271±0.049	2.977±0.069	12.484±0.153	3.110±0.093	2.005±0.052	4.432±0.072
Bdrm	0.662±0.015	1.086±0.041	2.873±0.067	12.878±0.169	3.265±0.060	2.208±0.127	4.125±0.082
Bhar	0.675±0.007	1.306±0.034	3.022±0.074	12.588±0.109	3.576±0.088	2.137±0.068	4.212±0.041
Buai	0.747±0.020	1.325±0.023	3.136±0.096	13.080±0.067	3.663±0.058	2.187±0.089	4.621±0.050
Cdrm	0.727±0.008	1.267±0.032	3.010±0.090	13.136±0.176	3.366±0.073	2.160±0.094	4.255±0.082
Chru	0.768±0.016	1.419±0.035	3.285±0.091	13.264±0.191	3.757±0.096	2.230±0.061	4.339±0.062
Fada	0.672±0.009	1.307±0.018	2.875±0.044	12.550±0.083	3.444±0.065	2.230±0.056	4.237±0.048
Fhai	0.645±0.009	1.242±0.028	2.830±0.025	12.438±0.134	3.428±0.078	1.993±0.052	4.036±0.115
Gill	0.735±0.020	1.246±0.044	3.044±0.082	11.985±0.241	3.318±0.089	1.994±0.051	3.955±0.100
Grog	0.695±0.014	1.279±0.041	2.877±0.048	12.822±0.105	3.350±0.076	2.213±0.064	4.263±0.104
Host	0.631±0.017	1.231±0.031	2.970±0.093	12.765±0.092	3.314±0.069	2.211±0.062	4.225±0.089
Iala	0.714±0.015	1.283±0.034	2.857±0.070	12.226±0.147	3.071±0.110	2.183±0.054	4.138±0.041
Maga	0.659±0.012	1.258±0.036	2.902±0.096	12.913±0.182	3.372±0.082	2.044±0.063	4.296±0.082
Maig	0.682±0.011	1.240±0.036	2.944±0.074	12.620±0.236	3.169±0.095	2.170±0.051	4.246±0.082
Riev	0.797±0.013	1.255±0.036	3.099±0.049	12.042±0.159	3.371±0.102	2.007±0.139	4.011±0.098
Shos	0.644±0.013	1.444±0.051	2.996±0.051	12.479±0.166	3.382±0.152	2.063±0.073	4.015±0.135
Stru	0.676±0.007	1.274±0.026	2.930±0.052	13.014±0.126	3.467±0.058	2.135±0.030	4.793±0.109
Torm	0.631±0.008	1.193±0.019	2.965±0.054	12.688±0.089	3.267±0.085	2.840±0.038	4.405±0.126
Faik	0.624±0.021	1.196±0.056	2.765±0.112	12.531±0.250	3.404±0.119	2.024±0.163	4.203±0.057
Obsm	0.658±0.016	1.377±0.057	2.930±0.116	12.412±0.313	3.424±0.214	2.100±0.187	4.145±0.031
Tros	0.622±0.016	1.427±0.061	3.005±0.073	12.904±0.362	3.789±0.188	2.490±0.130	4.383±0.140