

LIFE HISTORY EVOLUTION IN THREE-SPINED STICKLEBACK

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Abstract

A main challenge in evolutionary ecology is to elaborate the main ecological factors that vary in a study system and analyse how those factors shape the phenotype of organisms in their natural environment. These ecological variables can have complex effects since most life history traits are correlated. Here I examined the effects of ecological factors on life history traits, and the relationship among life history traits of North Uist three-spined stickleback, *Gasterosteus aculeatus* (hereafter stickleback). Both abiotic and biotic factors that are commonly measured in aquatic systems, and are known to vary greatly between North Uist lochs, were examined. The traits analysed were body size, age and size at maturation, proportion of fish over one year old, and the fecundity rate. My results showed that there was strong variation in water chemistry and inter-specific competition among the lochs on North Uist. Lochs on the west side of the island were characterised by higher sodium and calcium ion concentrations, higher pH and conductivity, and increased inter-specific competition. As a result, western populations had a faster absolute growth rate, larger body size, and a higher proportion of older fish, indicative of a longer life span. Although western populations had a larger body size, they tended to mature later with a smaller gonadosomatic index. They also favoured a larger number of eggs, but with a smaller egg size. Variations within population showed that larger females had greater fecundity but small egg size. Both plasticity and evolution appear to have contributed to these life history variations. Overall, variation in life history of North Uist stickleback was largely related to measures of resources availability and interspecific competition, except for egg size which was mostly associated with variation in predator density.

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Contributions

This thesis is substantially my own work with some contributions from other people as below:

1. Andrew MacColl gave advice on strengthening the ideas for most of the chapters, helped with choosing the populations for sampling, afforded advices for statistical analysis, provided feedback for discussion, and conducted an English proofread for the whole of the thesis.
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Chapter 1. General Introduction

In this thesis, I conducted a detailed study on the life history evolution of three-spined stickleback fish (*Gasterosteus aculeatus* Linnaeus 1758) (hereafter stickleback). This study involved 28 wild freshwater populations of stickleback that are mainly from the Scottish island of North Uist. The main aim of this research was to unravel the impact of complex ecological factors, selection and trade-offs on the life history evolution of stickleback. In addition to the main aim, this study has also examined the effect of maternal quality on the reproductive strategies of this fish and provided a refinement of ageing methods based on the seasonal growth of otoliths.

1.1 The evolutionary ecology and life history of organisms

The relationship between ecological constraints and the evolution of life history has been researched in many organisms (e.g. Stearns, 1992; Charnov 1997; Roff, 2001; Jorgensen et al., 2011; Rollinson & Hutchings 2013). It is widely recognised that the life history of organisms is not a fixed condition, but varies depending on the ecological constraints experienced by the organism (Partridge & Harvey, 1988; Baker et al., 1998; Taylor et al., 2011; Morrongiello et al., 2012; Rollinson & Hutchings, 2013). Some of these ecological effects may only persist for short periods as a part of phenotypic plasticity (e.g. Day *et al.*, 1994; Plaistow *et al.*, 2004; Bårdsen *et al.*, 2011) while others can lead to a permanent effect due to selection and evolution (e.g. Heino & Godo 2002; Wund et al. 2012; Hunter et al. 2015).

Many traits can be described as life history characters. Stearns (1992) states that life history traits include body size, number and size of offspring, growth rate, age and size at maturation, age and size at senescence, and life span. Body size, age at first breeding, size of offspring and reproductive effort are perhaps the most important life history attributes (Arendt 1997; Charnov 1997). Determination of age and size at maturation has the most crucial impact on fitness for both adults and offspring (Stearns & Koella, 1986; Roff, 2001; Rollinson & Hutchings, 2013b). However, maturation time in most of organisms is highly dependent on another trait: body size (Stearns & Koella 1986; Roff 1992; Heino et al. 2002). Some organisms have specific traits that have consequences for life history traits such as nest location and construction in bees (Williams *et al.*, 2010), voltinism (Altermatt, 2010) and movement behaviour in butterflies (Stevens *et al.*, 2012), and hibernation activity in some mammals (Turbill *et al.*, 2011). In fishes (especially stickleback), trophic morphology

(Schluter, 1995), size and body depth (Schluter, 1995; Berner et al., 2011), armour (Leinonen et al., 2011), and pelvic girdle structure (Baker et al., 1998) can be related to fitness and life history.

Among ecological factors, resources and predation have been suggested as the main factors affecting the life history of organisms (Fig.1.1). Resources have a direct positive correlation with growth rate (Arendt & Reznick, 2005) and thus body size, earlier and larger size of maturation (Stearns & Koella, 1986; Reznick *et al.*, 1990; Heino *et al.*, 2002), higher fecundity (Stearns 1992; Roff 1992), and longer life span (Howells *et al.*, 1983). Non-selective predation will depress growth rate (Arendt, 1997), force earlier and smaller size of maturation (Heino & Godo, 2002; Beckerman *et al.*, 2010), and reduce life span (Stearns 1992). In contrast with non-selective predation, juvenile stage predation triggers organisms to increase growth rate, especially during vulnerable stages (Arendt 1997), and increases age and size at maturation (Stearns & Koella 1986). In addition to these main factors, competition (Mittelbach, 1988; Arendt, 1997; MacColl *et al.*, 2013), temperature (Zera & Harshman, 2001; Kuparinen *et al.*, 2011; Green *et al.*, 2014), pollution, and chemicals (Kraak 2007) have also been widely recognised to influence the life history of organisms.

While the role of environmental factors may seem clear when they are considered individually, in the real environment, factors are correlated with each other (MacColl, 2011). This can have consequences for the evolution of life histories and surprisingly the understanding of these complex relationships is still limited. A study by Arendt & Reznick (2005) of Trinidadian guppies (*Poecilia reticulata*), for example, showed that resources are more important than predation in shaping the growth rate. When resources are abundant, the effect of predation can be neglected. However, a contrasting pattern was found by Martin et al. (2011) for the growth rate of broods of Passerine birds. They found that high predation risk can force individuals to grow faster even when they have lower resources. A recent study by MacColl et al (2013) on stickleback proposed a model that body size is mostly shaped by the density of competitors, with pH of their aquatic environment (perhaps indicating resource availability) as a secondary factor, while another proxy of resource availability (alkaline metal concentrations) did not have a direct effect. They found that resources only had a direct effect on the abundance of competitors and the size of predators. A better understanding of the relationship between life history traits and the complex of ecological conditions and constraints, is required to predict populations' responses to environmental changes (Kuparinen, 2011).

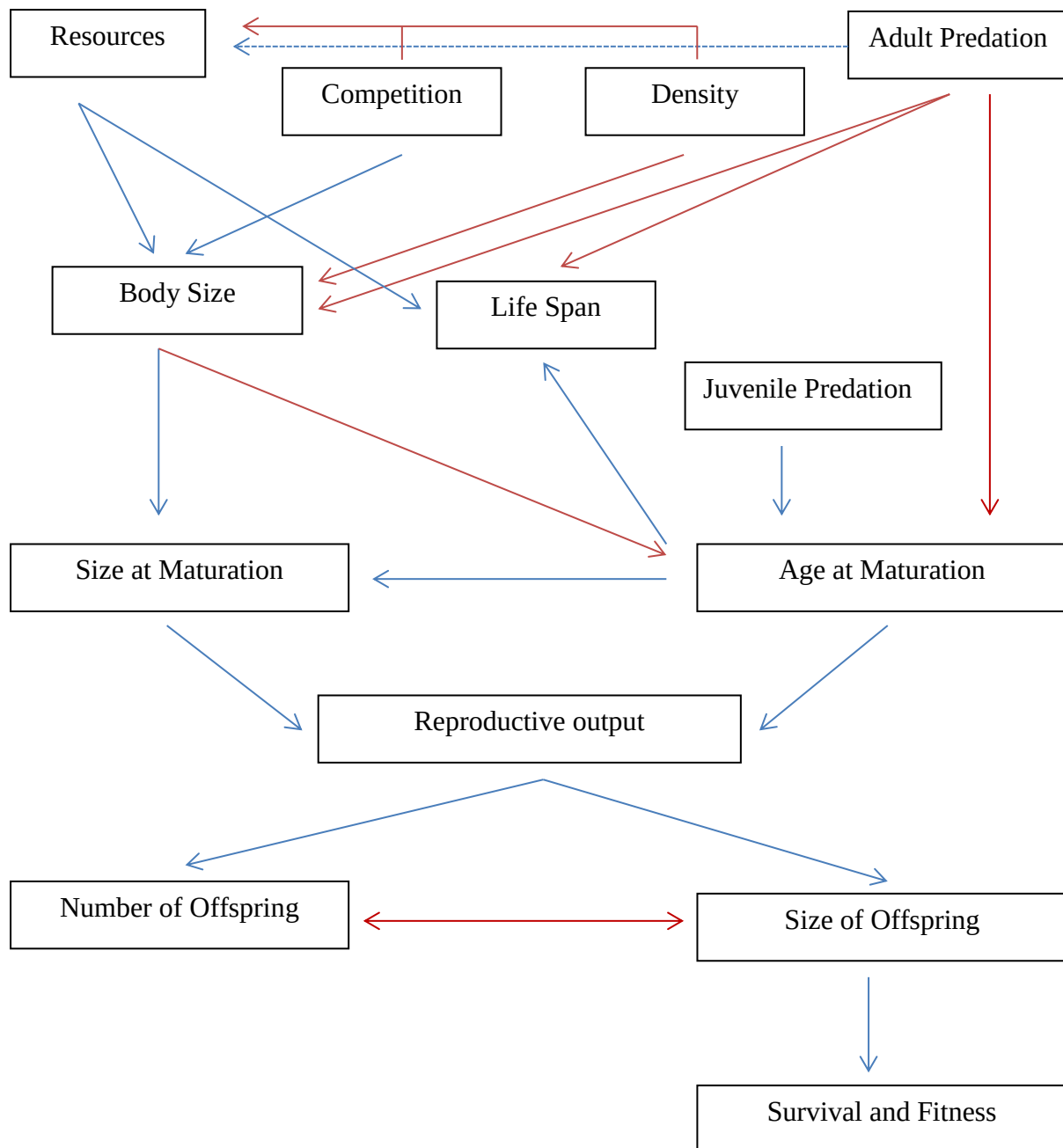


Figure 1.1. Diagram showing the theoretical relationship of ecological factors and life history traits, and between the life history traits themselves. Blue line= positive effect, red line=negative effect, dashed blue line=indirect positive effect, red line double arrow=trade off. The diagram was re-drawn from Smith & Fretwell (1974; Stearns & Koella, 1986; Roff, 1992; Stearns, 1992; Arendt, 1997; Einum & Fleming, 1999).

Apart from the effect of ecological factors on life history traits, examining the degree of evolution of these traits is also crucial and remains a main challenge in life history research

(Kraak, 2007; Merilä & Hendry, 2014). The most important reason for the continuing work in disentangling plasticity and evolution is to determine whether current phenotypes are evolutionary (genetically based) or simply immediate, plastic responses to environmental variation. Estimating the extent of genetic contributions will be useful in mapping the pattern of population divergence through time, while the presence of phenotypic plasticity, including life history traits, could be an indication of recent instability of the environment (Heino & Dieckmann, 2008). Moreover, disentangling these two factors is also required to predict the fitness of organisms in facing environmental fluctuation (Oomen & Hutchings, 2016). As heritable, genetic change requires long time periods, fitness of organisms in fluctuating habitats mostly depends on the degree of their plasticity (Via *et al.*, 1995; Dieckmann & Heino, 2007). Indeed, this kind of study has been widespread among many taxa of organisms (Mousseau & Roff, 1987; Crozier & Hutchings, 2014), but the evolutionary aspects can be very specific depending on the species and the ecological factors that drove the selection.

1.2 Life history traits and trade-offs

The study of life history is made more complex by the possibility of trade-offs among life history traits. Simply put, evolutionary change in one trait can influence, or be influenced by, another trait or fitness (see Charnov & Berrigan, 1990; Charnov & Berrigan, 1991; Charnov, 1997; Moser, 2012) (Fig. 1.1). It is likely that such trade-offs will result in trait optimization that depends on fitness costs and benefits (Charnov, 1997; Roff, 2001, Stearns 1992). The principal life history trade-off among organisms is that between growth, reproduction and maintenance (e.g. of the immune system) (Reznick, 1983; Biro *et al.*, 2006; Berner & Blanckenhorn, 2007). Increasing investment in any of these traits may reduce investment in the others since there is a partition of the available resources to all of the existing traits.

A popular example of a trade-off in life history traits is that between the age and size at maturation of an organism. It is generally better to mature sooner, because (a) individuals are then more likely to reach maturation and (b) they will have grand-offspring sooner. However, many organisms increase in size as they age and size at maturation is likely to have fitness consequences in many organisms (Roff, 1992; Stearns, 1992), especially those, like fish, whose growth is not deterministic. There are two gains for an organism in delaying maturation: increasing initial fecundity and reducing juvenile mortality rate (Stearns, 1992). Fecundity will increase because older organisms are often bigger and bigger size will lead to greater number or larger size of propagules. Larger propagules will lead to bigger offspring

and may reduce the juvenile mortality rate. Besides delaying age at maturation, fecundity also can be enhanced by increasing growth rate. Increasing growth rate will produce bigger size at maturation even at the same age (Roff, 1992).

In the real environment, the pattern of life history trade-offs is quite often determined by the ecological factors surrounding the organisms. An established model by Smith & Fretwell (1974) pointed out that in low-quality environments, females tend to prioritise the investment per offspring even when reducing the number of offspring as a consequence. Increasing investment per offspring in fluctuating environments is thought to achieve higher fitness (Smith & Fretwell, 1974) due to larger initial size (Stearns, 1992), faster growth (Einum & Fleming, 1999; Murphy *et al.*, 2014) and/or a lower risk of predation (Biro *et al.*, 2006; Bell *et al.*, 2011) in comparison to smaller siblings. However, the trade-off in fecundity-offspring size probably applies less in aquatic environments, especially in oxygen-poor conditions. As larger offspring are more vulnerable to hypoxia during incubation, females may tend to optimise (not maximise) their offspring size in aquatic conditions (Hendry *et al.*, 2001).

1.3 Three-spined stickleback as a model organism in evolutionary ecology

The three-spined stickleback has been used widely as a model organism in evolutionary ecology studies (e.g. Bell, 1976; Schluter, 1995; Nosil & Reimchen, 2005; Berner *et al.*, 2008; MacColl *et al.*, 2013). It makes a good model because of its rapid divergence between populations in short periods. Extensive variation among freshwater populations in genetics and phenotypes has been reported for this species (Peichel *et al.*, 2001), resulting from only ~15,000 years of evolution (Bell & Foster 1994; but see Baker *et al.* 1998 i.e. 20,000 years), since the species invaded freshwater following deglaciation. Stickleback can be easily found in large numbers in almost all aquatic environments across the northern hemisphere. In addition, the species is easily raised in natural and laboratory conditions. These factors have led to them being favoured as an organism for comparative studies in morphology and genetics (Bell & Foster, 1994).

As in other phenotypic traits, stickleback also exhibit great variation in their life history traits. Adult body size, which usually represents size at maturation, varies greatly from 23-100mm (Reimchen, 1992; Moser *et al.*, 2012; MacColl *et al.*, 2013). MacColl *et al.* (2013) found that some freshwater stickleback mature at only 7% of the body weight of their ancestors, anadromous stickleback. Age at maturation of this fish ranges from one to several years (Jones & Hynes, 1950; Snyder & Dingle, 1989; Leaver & Reimchen, 2012; Defaveri &

Merila, 2013). A comparison of published data on the reproductive aspects of stickleback by Oravec & Reimchen (2013) found differentiation in egg size up to 86% of largest egg diameter or 546% of egg volume. Fecundity ranges from 23 to 506 eggs (Oravec & Reimchen, 2013). The longest life span ever reported for a stickleback is 8 years (Reimchen, 1992), but the majority of studies have found life spans no longer than 6 years (Gambling & Reimchen, 2012; Defaveri & Merila, 2013), and many stickleback populations are essentially annual (Baker, 1994; Katsiadaki *et al.*, 2007; Gambling & Reimchen, 2012). In addition to life history traits, there is great variation in body armour (spines and ectodermal plates) (Bell, 1976), length and number of pectoral and dorsal spines, the trophic morphology, including gill rakers (Schluter, 1995, Nosil & Reimchen, 2005) and body shape. The variation in other phenotypic traits can influence the life history since there is a trade-off in allocation of resources in each type of investment (Berner & Blanckenhorn, 2007), including phenotypic investment.

Life history variation of stickleback is linked to multiple factors, ranging from the proximate genetic causes to the ultimate ecological constraints. The genetic basis of the differentiation of body size, for example, was reported by Leinonen *et al.* (2011; Mccairns & Bernatchez 2012; Magalhaes *et al.* 2016). By doing a common garden experiment, Snyder & Dingle (1989; Snyder, 1991) found that genetic factors significantly contributed to the differentiation of maturation time and the presence of the egg size-fecundity trade-off in Californian stickleback. A study by Kalbe *et al.*, (2009) on German stickleback also found that the reproductive success and the survival of this fish was related to major histocompatibility complex (MHC) genes.

From an ecological perspective, body size variation in the stickleback has been argued to be caused by competition (MacColl *et al.* 2013), habitat type (lake, stream or marine) (Moser *et al.* 2012), and parasitism (Giles 1981). Baker's *et al.* (2011) study found that increasing lake productivity shifted age and size at maturation of Alaskan stickleback to mature younger and at a smaller size. However, although selecting for earlier maturation, productivity was positively associated with larger reproductive investment of the Alaskan populations (Baker *et al.*, 2011). A lab based study by Ali & Wootton, (1999) disclosed that a longer spawning interval in this fish was significantly associated with lower resource availability.

Although life history of stickleback is well studied as explained above, a comprehensive study linking the effects of complex ecological factors in a big data set of wild stickleback is still lacking. The difficulty with this kind of study is getting enough data from wild

populations with sufficient variation in the prominent ecological factors i.e. resources and predation. In this study, I unravelled the confounding effects of resources, predation and intra- and inter-specific competition in the life history variation of 27 populations of freshwater stickleback from North Uist, Scotland.

1.4 The study area and the populations involved

This study involved populations of stickleback from the island of North Uist, Outer Hebrides, Scotland. North Uist is a small island of only 303 km² in the Western Isles of Scotland. There are more than 180 lochs (lakes), and many smaller lochans (ponds), on the island, ranging from alkaline in the west to acidic in the central and eastern parts of the island (Giles, 1981). These lochs vary greatly in size but are generally small and shallow (MacColl *et al.*, 2013). Many of the lochs are completely isolated from the marine environment, while others are tidal or have streams running to the sea. The shell-rich sand in the west of this island has a high level (more than 80% in some places) of CaCO₃ (Kneale & Viles, 2000), giving rise to ‘machair’ habitat (short grassland rich in plant species). Ballantyne (2010) stated that the northwest of Scotland, including North Uist was probably completely deglaciated by 15,000 years ago. Therefore, the evolution of freshwater stickleback on this island is assumed to have begun by that time as a result of the invasion of fresh-water bodies by marine populations shortly after this island was deglaciated (MacColl *et al.*, 2013).

The study of life history evolution, especially with stickleback as a model species, becomes valuable in North Uist because this fish is found in most waterbodies across the island, and both aquatic environments and the stickleback that inhabit them have a big range of variety. Lochs in North Uist have a big range of calcium availability and pH (Giles, 1981; MacColl *et al.*, 2013; Spence *et al.*, 2013; MacColl & Aucott, 2014) which correlates with resource availability (Waterston *et al.*, 1979), while the stickleback vary in their type (marine, resident and freshwater) and body size (MacColl *et al.* 2013), body shape, plate and spine traits, (Magalhaes *et al.*, 2016), immune system profile (Robertson *et al.*, 2016) and genetic structure (Magalhaes *et al.*, 2016). Populations of stickleback in some lochs also differ in the number of their main competitor, nine-spined stickleback (*Pungitius pungitius*) (MacColl *et al.* 2013), their parasite prevalence (De Roij *et al.*, 2011; El Nagar & MacColl, 2016), and size and density of their main predator, the brown trout (*Salmo trutta*) (Spence *et al.*, 2013; MacColl & Aucott, 2014).

Twenty seven freshwater populations (Fig. 1.2) of North Uist stickleback were involved in this study. These populations were chosen across a range of ecological factors namely: resources, competition and predation, and their body size (MacColl *et al.*, 2013). Two of these populations, Reiv and Bhar, were involved in almost all of the chapters (Chapter 6 without Bhar) since they have a very great contrast in environmental factors e.g. resources, predation and competition, and body size (Fig. 1.3). Reiv is located on the west side of the island with high resources, high density and a larger body size of stickleback, and high competition but without predation. On the other hand, Bhar is located more centrally and characterised by poor resources, low density and a smaller body size of stickleback, no *P. pungitius* but high trout density.

In addition to the Uist populations, Tottle Brook, Nottingham (52°93'N; 1°20'W) stickleback were sampled only for comparison of otolith growth over the contrast in latitude. There is no information about the ecological factors and stickleback phenotypes on this small stream. However, due to the its running water habitat and longer daylight comparing to Uist, this population is believed to have good resources, and the stickleback are large and long-lived.



Figure 1.2. Map of North Uist showing the 27 lochs/populations of freshwater stickleback that were involved in this study. The map was adapted from Google, access date 10th Sept 2016. White line shows the main road of North Uist.

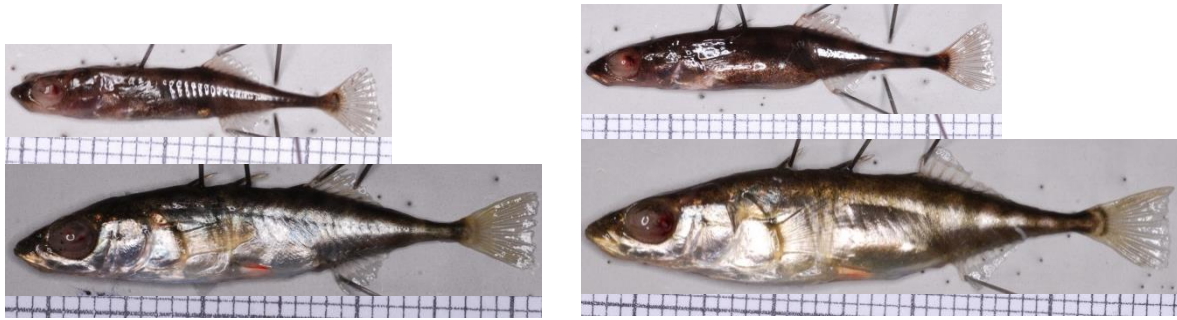


Figure 1.3. Comparison of body size and shape of three-spined stickleback from two contrasting populations on North Uist. Top=Bhar, Bottom=Reiv. Left=the smallest male caught within each population, right=the smallest matured female. Fish have had their eyes removed for parasite assays. Photo credit: Daniel D'Agostino/MacColl lab.

1.5 Thesis outline and brief description of the following chapters:

Chapter 2: Otolith development in wild populations of stickleback: Jones & Hynes method does not apply to most populations

Otolith reading is the main methodology for determining the age of most fishes. The main paper for this method in stickleback is Jones & Hynes (1950). Since I found that the Jones & Hynes (1950) method is contradicted by Borland (1986) and general otolith patterns in other fishes (Williams & Bedford, 1974; Jones, 1992; Uriarte *et al.*, 2016), and because a good knowledge of age is central to understanding life history, I decided to make a comprehensive study of this method. This chapter involved consecutive sampling of stickleback through the year in two different locations: Nottingham and North Uist.

Chapter 3: Role of body size and ecological factors in maturation of freshwater stickleback

Body size, and age and size at maturation are prominent life history traits. This is because they are clearly influenced by ecological factors and they play a key role in life history trade-offs. Larger body size is believed to correlate with earlier maturation and larger reproductive effort, but shorter life span (Reznick, 1983; Stearns & Koella, 1986; Roff, 1992; Biro *et al.*, 2006). In this chapter, I analysed how complex ecological factors drove the evolution of body size, maturation time and life expectancy in 24 freshwater populations of stickleback from

North Uist. Of course, I also incorporated the effect of life history trade-off in the pattern of this evolution.

Chapter 4: Ecological correlates of reproductive strategies of freshwater stickleback

In contrast to body size, age and size at maturation and life span that are highly influenced by ecological factors, reproductive investment is mostly shaped by the other life history traits i.e. body size and maturation time. Since this trait has a direct positive correlation with body size (Stearns 1992; Roff 1992), higher growth rate and late maturation will have similar consequences. From an ecological perspective, populations which are living in adverse environments will increase investment per offspring, and quite often reduce fecundity as a consequence (Smith & Fretwell, 1974; Merilä & Hendry, 2014). In this chapter, I analysed the effect of ecological factors, aspects of maternal phenotype, and life history trade-offs on the reproductive strategies of 18 populations of North Uist stickleback. There are four aspects of fecundity that are involved in this study: total reproductive output, gonadosomatic index, egg number and egg size.

Chapter 5: Heritability of growth in North Uist freshwater stickleback

As stated in the first section of this chapter, disentangling the effects of evolution and plasticity remains an important question for evolutionary biologists. Here, I calculated the degree of heritability of growth rate among six population of North Uist stickleback by conducting a full sib-half sib common garden experiment. I also observed the juvenile mortality rate of these populations in the laboratory during 270 days of the experiment.

Chapter 6: Resources are more important than predation in driving the size of maturation on freshwater three-spined stickleback

Resources and predation have a similar effect in reducing the age of maturation of organisms, but have a contrasting impact on size of maturation. Higher resources select for a larger size of maturation, while predation has the opposite effect. An interesting question is, if these two factors covary, which is more important in shaping the size of maturation. I answered this question by analysing the maturation event of six populations of North Uist stickleback. This maturation event has been analysed using two different methods: probabilistic maturation reaction norms (PMRN) that are mostly shaped by evolution (Heino *et al.*, 2002) and traditional reaction norms that are shaped mostly by plasticity (Stearns & Koella, 1986).

Chapter 7: General discussion

Here I linked the findings from each chapter to describe a general pattern of evolution of life history in stickleback, especially those from North Uist. Most of this discussion focuses on the main finding of new ageing method in the stickleback, the impact of complex ecological factors on the prominent life history traits i.e. body size, maturation time, reproductive effort and life expectancy, trade off among life history traits themselves and the degree of selection on growth rate and maturation time.

To summarise this general introduction I want to highlight that even though the impacts of ecological factors are fairly clear when they are independent, when they covary there may be complex effects that still need unravelling. These complex effects becomes more complicated by life history trade-offs. Briefly, in some cases, the effect of ecological factors can be negated by the stronger impact of life history trade-off or vice versa. In this thesis I analysed the complex effect of resources, predation and competition on prominent life history traits namely: body size, age and size at maturation, life span (Chapter 3), and reproductive investment (Chapter 4). I also examined the presence of trade-offs (Chapter 3, 4 and 6), and disentangled the role of phenotypic plasticity and genetic aspects among those traits (Chapter 5 and 6). In addition, I also re-observed the ageing method in this fish by comparing the established models of otolith growth in stickleback with other fishes (Chapter 2). The main questions/hypotheses of this study are summarised in Table 1.1.

Table 1.1. Summary of hypotheses/questions in this thesis

Chapter	Main question/hypotheses
II	a. Does otolith growth in stickleback follow Jones & Hynes (1950) or the general models from other fishes (Williams & Bedford, 1974; Borland, 1986; Jones, 1992)?
III	a. How do ecological factors i.e. resources, predation and competition interact in shaping the body size, maturation time and life expectancy of stickleback? b. Stickleback with larger body size will mature earlier and at larger size (Stearns & Koella, 1986; Roff, 1992; Heino <i>et al.</i> , 2002).
IV	a. Larger mothers have larger ovaries and fecundity, but smaller egg size (Hendry <i>et al.</i> , 2001). b. How do the confounding effect of ecological factors act upon the reproductive traits of stickleback? c. There is a trade-off between fecundity and egg size (Smith & Fretwell, 1974).
V	a. Variation growth rate has a heritable basis (Mousseau & Roff, 1987; Merilä & Hendry, 2014).
VI	a. Which factor is more important in shaping size at maturation: resources or predation?

Chapter 2. Otolith development in wild populations of stickleback: Jones & Hynes method does not apply to most populations.

2.1 Abstract

Accurate ageing of fish by reading otoliths has been central to our understanding of fish growth, life-history, demographics and ecology for at least a century. Three-spined stickleback (*Gasterosteus aculeatus*) (hereafter ‘stickleback’) have increasingly become a model organism in many areas of biology, but reading of their otoliths has not been widely used until recently. Although a method proposed by Jones & Hynes’s (1950) has been established and cited as the main reference for otolith reading in stickleback for more than a half of century, their method is contradicted by the findings of later researchers. These contradictions can be characterised by three questions: i) Does a transparent ring form in the otolith in the first summer of juvenile stickleback?; ii) Does a second opaque ring develop before their first winter?; and iii) When do transparent and opaque rings generally form? Here, I critique the Jones & Hynes (1950) method by analysing sequential samples of otoliths from three wild populations of stickleback from North Uist, Scotland and Nottingham, England. Contrary to Jones & Hynes (1950), but coincident with the finding of later researchers, our results showed that: i) no true transparent ring formed in the otolith of the stickleback during their first summer. The first transparent ring was likely starting to be formed by the end of summer and was completed by the end of their first winter; ii) There was no second opaque growth in the otoliths of the stickleback before they passed their first winter. The second opaque growth was just starting to appear by early April of the second year in the southern population i.e. Nottingham, but later, by May, in the northern populations i.e. North Uist; iii) Formation of the opaque ring in stickleback mostly occurs in spring and summer, with younger fish starting earlier. In contrast, the formation of transparent rings is mostly during autumn and winter, but in some individuals can be more widespread through the year, possibly as a result of reproductive investment.

Keywords: otolith, seasonal ring, ageing, three-spined stickleback

2.2 Introduction

The determination of age in fishes by counting of annual rings on otoliths has been well established among researchers and fisheries managers for decades (e.g. Reibisch, 1899 as cited in Jackson, 2007; Jones & Hynes, 1950; Panella *et al.*, 1971; Williamns & Bedford, 1974; Swan & Gordon, 2001; Uriarte *et al.*, 2016; review in Campana, 2005). The method is still widely used (e.g. Koeda *et al.*, 2016; Matta *et al.*, 2016; Uriarte *et al.*, 2016), even though more recent methods for otolith analysis, such as daily increment quantification, microchemistry, carbon dating, plasma mass spectrometry and three dimensional x-ray scanning are more powerful for reliable age estimation (Thorrold & Shuttleworth, 2000; Campana & Thorrold, 2001; Jones & Chen, 2003; Hippel *et al.*, 2013; Mapp *et al.*, 2016). In comparison to these more recent methods, reading of annual rings is still useful in ecological studies because of the need for large and low-cost data sets. Reading of annual rings in otoliths is also easier to apply and provides better age estimation than comparable methods such as the reading of rings on spines or scales (Erickson, 1983; Buckmeier *et al.*, 2002). Significant problems with all these methods, that can lead to inaccurate age determination, are the presence of smear or fake ring “checks”, and inconsistent patterns from year to year, place to place and/or between fish species (Williams & Bedford, 1974). Checks are likely to be the result of short-term alterations in growth as a result of brief decreases (or increases) in otherwise good (or poor) resource conditions (Panella, 1971; Campana & Neilson, 1985; Uriarte *et al.*, 2016).

The method of reading annual rings of otoliths to obtain age estimates relies on contrasting growth patterns during rich versus poor feeding conditions, and is therefore related to variation in fish growth rate as well as variation in otolith deposition. Rich food conditions, that usually occur in summer, correlate with faster growth and accreting otoliths; while poor food conditions, that usually occur in winter, are related to slower somatic growth and little or no growth of otoliths (Panella *et al.*, 1971; Williams & Bedford, 1974; Koeda *et al.*, 2016; Uriarte *et al.*, 2016; but see Jones & Hynes, 1950; Greenbank & Nelson, 1959; Swan & Gordon, 2001). In the great majority of described fishes, faster growth of otoliths results in formation of an opaque ring that consists mainly of inorganic material, while slow growth produces a transparent ring which is dominated by organic materials (Panella, 1971). This opaque-transparent pattern repeats over the years, following the growth of the fish, and making it possible to use the resulting rings for age estimation. Although the term ‘otolith’ generally refers to three kinds of ear bone in the labyrinth system of the fish, the largest

otolith, i.e. the sagitta, is the most studied and used for age determination among researchers. In keeping with the wider literature, I use the term otolith to refer to the sagitta.

Here I review the use of otoliths to age three-spined stickleback *Gasterosteus aculeatus*, a small circumboreal fish of no significance to commercial fisheries, but which has increasingly become a model fish for the study of behaviour, ecology, parasitology, ecotoxicology, evolution and, recently and most prominently, evolutionary molecular genetics (Chan *et al.*, 2010; Roesti *et al.*, 2015; Magalhaes *et al.*, 2016). The increasing interest in this fish demands a careful approach to the ageing of wild-caught individuals if reliable conclusions are to be drawn, since many patterns of interest to researchers are age-related, including growth, senescence and other aspects of life history, as well as population dynamics, epidemiology and natural selection. To date, the main description of otolith reading in this species is Jones & Hynes (1950), but while still widely cited, their main conclusions are contradicted by the findings of later researchers. Their results match with those of Greenbank & Nelson (1959) and Swan & Gordon (2001), but are contradicted by Panella *et al.*, (1971; Williams & Bedford, 1974; Borland, 1986; Koeda *et al.*, 2016; Uriarte *et al.*, 2016). Most of the contrary studies are from different fish species, except for Borland (1986), but since otolith growth represents overall fish growth, the pattern of otolith growth among fishes from the same region (northern hemisphere) should be similar. Moreover, those studies that are conducted on a variety of fish species e.g. *Merluccius bilinearis* (Mitchill 1814), *Urophycis chuss* (Walbaum 1792), *Gadus morhua* (Linnaeus 1758), *Pempheris schwenkii* (Bleeker 1855) and *P. adusta* (Bleeker 1877), *Engraulis encrasicolus* (Linnaeus 1758) or even more species (Panella, 1971; Williams & Bedford, 1974; Koeda *et al.*, 2016; Uriarte *et al.*, 2016) show the same general pattern of otolith growth, which differs from Jones & Hynes (1950).

I summarise these contradictions into three main points. Firstly, the presence of a first transparent ring in the first summer of life i.e. June or early July in Jones & Hynes (1950) study does not occur in Borland's (1986; but see Blouw & Hagen, 1981) study. Secondly, the development of an opaque ring in the first autumn suggested by Jones & Hynes (1950) contradicts Borland (1986; Defaveri & Merila, 2013). Thirdly, the growth period of transparent and opaque rings in Jones & Hynes (1950) is opposite to the findings of most later researchers. Contrary to the general pattern of results from other fishes (e.g. Panella *et al.*, 1971; Williams & Bedford 1974; Uriarte *et al.*, 2016; but see Swan & Gordon 2001) which agree that opaque growth occurs in spring/summer and transparent growth in

autumn/winter, Jones & Hynes (1950) found that transparent rings in stickleback are formed mostly in summer i.e. June to September, while opaque rings are formed in other months.

In this study, I re-examined Jones & Hynes method by using consecutive sampling through the year in three different populations of wild stickleback, from Nottingham and North Uist. Our aims were to clarify the method of otolith reading in stickleback as well as to provide some discussions on this method that probably also applies to other fishes. I hypothesised that otolith growth in stickleback will follow either Jones & Hynes' (1950; Greenbank & Nelson 1959; Blouw & Hagen 1981) model or the opposite one from Borland (1986; Panella *et al.*, 1971; Williams & Bedford 1974; Uriarte *et al.*, 2016) (Table 2.1).

Table 2.1. Comparison of otolith growth in three-spined stickleback between Jones & Hynes' (1950) model and Borland's (1986)

No	Growth/Formation	Jones & Hynes (1950)	Borland (1986)
1	The first transparent ring	June and July of the year the fish were born	In the first autumn of the year fish were born and throughout their first winter
2	The second opaque ring	Directly after the growth of first transparent ring until the following June	Starting in the spring of the following year until the end of summer
3	Growth of the otolith through the year	Transparent growth is formed in June to September, while opaque growth in other months	Transparent ring is mostly formed during the coldest part of the year i.e. autumn and winter, while opaque growth occurs in other months

2.3 Methods

2.3.1 Populations and sampling

Five hundred and twenty two stickleback from three different populations were sampled in three sampling periods for each population during 2014-2015 (Table 1) in North Uist (57°36' N 7°20' W) and Nottingham (52°93N' 1°20'W). The sampling periods were designed only from the early spring until the late summer since both models proposed only a little transition of seasonal ring during autumn-winter. In addition, growth during autumn and winter can be tracked as it will be continuously opaque if following Jones & Hynes (1950) or completely transparent if following Borland (1986). However, due to easier access, sampling in Nottingham was more regular than in North Uist.

Two of these populations, Reiv and Bhar, are from North Uist, but they differed substantially in water chemistry of the lochs and in stickleback body size (MacColl et al 2013). Reiv stickleback are large and the loch they live in is shallow and resource-rich, while Bhar stickleback are very small, and live in a deep, resource-poor loch (MacColl *et al.*, 2013; Chapter 3). The third population, Tottle Brook in Nottingham was sampled because of its different latitude (800 km south of North Uist) and associated differences in seasonality. Stickleback in this stream are relatively large and long-lived, with good resources.

Stickleback were caught using unbaited minnow traps (Gee's traps, Dynamic Aqua, Vancouver, Canada) which were set overnight from the shore in water 0.3 - 3 m deep. The caught fish were sorted to ensure no other species of fish were sampled. Samples of the remaining fish were then haphazardly selected for analysis. Of these selected fish, I recorded individual standard length in millimetres. I also recorded the female reproductive status of those from Tottle Brook. Collection of reproductive status data was restricted to females, because of the difficulty of unambiguously assigning reproductive status to males. Female reproductive status was confirmed by dissection of the body cavity and observation of the ovaries. If the ovules were all small and the same size, then fish were recorded as immature. If any ovules were of larger size then the fish was recorded as maturing/mature. Fish were then preserved in 70% ethanol until their otoliths were extracted. To avoid the fragility of otoliths identified by Borland (1986), all of the otoliths were processed no later than six months after fish were preserved.

2.3.2 Otolith processing and reading

The otoliths were extracted from the left side of the fish following Jones & Hynes (1950) methods under a x10 dissecting microscope. In some cases where left otoliths could not be found, the right was taken out instead. The extraction was conducted from one side only because both Jones & Hynes (1950) and Borland (1986) found more than 90% consistency on ring pattern between left and right otoliths of stickleback. The extracted otoliths were then put into a dish containing several drops of water and were cleaned of the remnants of their sacculus. Cleaned otoliths were stored in an individual tube with 70% ethanol until they were mounted on microscope slides using Sigma Aldrich DPX.

Each mounted otolith was examined twice using two different methods: direct reading under a microscope and reading of a photograph, blind to information about fish size. Microscope reading was conducted under a compound microscope at x40 magnification using reflected light and dark backgrounds. To clarify the microscope reading, a photograph reading was carried out by analysing the pictures of each otolith which were obtained with a Panasonic Lumix L10 camera under reflected light and with a dark background. Photograph reading was also carried out blindly and independently without reference to the result from the previous method (“microscope reading”). Any results that differed between microscope and photograph reading were clarified by re-analysing the photographs and/or the slide under the microscope. Based on the results of the two readings, otoliths were categorised as ‘unreadable’, ‘in doubt’ or ‘clear’.

Otoliths were labelled as unreadable when no clear image could be captured due to rupture, lack of clarity or otoliths having been mounted perpendicular to the slides. Ruptured otoliths were mainly triggered by too hard handling when moving them from their individual tubes to the slides, while the unclear ones were mostly caused by imperfect extraction of sacculus remnants. Perpendicular mounting resulted in a vertical, not horizontal, view of the otoliths and therefore made reading impossible. The ‘in doubt’ category was otoliths with vibrant images but their ring/s were unable to be determined due to indistinct border of seasonal ring and/or presence of checks. Unreadable otoliths were excluded directly, while in doubt otoliths were re-checked at least twice by each reading method before being excluded from the analysis if they proved uninterpretable.

Edge pattern, number and type of seasonal rings, and the presence and type of checks on each otolith were recorded. Edge and type of seasonal rings were categorised as either opaque or transparent, while number of these rings were coded following Jones & Hynes (1950): S- for

an otolith with an opaque ring only, S for two seasonal ring (one opaque, one clear), S+ for two opaque rings flanking a transparent ring, 2S for two sets of seasonal rings and so on. Identification of checks was done by referring to Williams & Bedford (1974; Swan & Gordon, 2001; Waldron & Kerstan, 2001; Uriarte *et al.*, 2016) (Fig. 2.1).

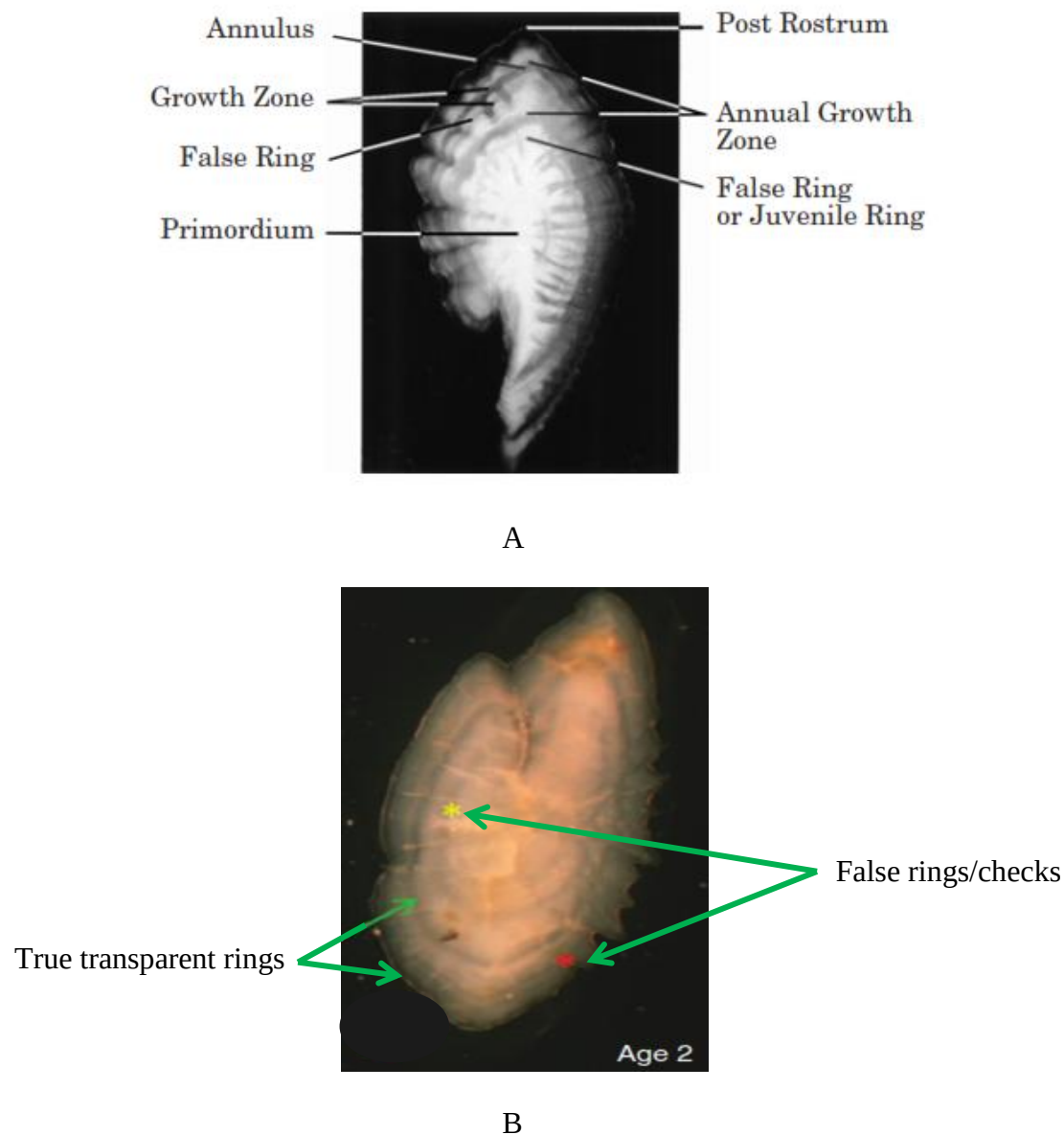


Figure 2.1. The differences between the true transparent rings and checks in the otolith of horse mackerel (A, Waldron & Kerstan 2001) and European anchovy (B, Uriarte *et al.* 2016).

The difference between true transparent rings and checks was mainly based on the wholeness and the clarity of the rings. Most of the checks were incomplete/interrupted, obviously narrower and/or less clear in comparison to true rings. All of the otoliths with complete and

clear transparent rings but in short unusual distance as acknowledged by Williams & Bedford, (1974) were also categorised as checks and were excluded from the analysis.

For those asymmetrical otoliths that could be interpreted differently depending on which sector was examined (Blouw & Hagen, 1981), the reading was made from the larger side since there is a possibility that otolith materials are not distributed uniformly during growth (Williams & Bedford 1974). Otolith pictures were then analysed with ImageJ (Schindelin *et al.*, 2012) to obtain the following data in micrometre (μm) units: maximum radius of each otolith, the width of opaque and transparent rings of one year old stickleback from April sampling and the width of transparent rings of young of the year from August sampling.

2.3.3 Data analysis

Edge patterns, growth of otoliths through the year and types of checks were analysed descriptively. Number of individuals as well as number of opaque or transparent edges in each age class were summarised as the percentage of total number of samples for each sampling period for each population. Generalised linear models (GLMs) with normal error distributions and identity link functions were used to test the differences in transparent width of S type otolith between August and April sampling in Tottle Brook, the differences of ring width of one year olds during April sampling among populations, and to analyse the relationship of length of the fish and otolith radius, age of fish and otolith radius, and length and age of the fish. In comparison of edge patterns between different reproductive statuses, length and age, edge was coded as a binomial variable (transparent or opaque), and the data were analysed by GLM with binomial error distribution and a logit link function.

2.4 Results

Of 522 samples, 484 (93%) were successfully analysed, while 38 others were unreadable (30) or were lost during processing. Of the 484, 451 (93%) of them were categorised as clear, with the remainder as 'in doubt'. The highest percentage of 'in doubt' was found in Bhar (14% of the Bhar samples or equal to 3.2% of the total sample), while the lowest was in Reiv (4%, less than 1% of the total sample). The two different reading methods, microscope and photograph, gave coherent results with more than 92% similarity indicated that both of these methods are recognised for otolith reading.

2.4.1 The pattern of otolith edges through the year

Most of the otoliths (90%) from fish sampled in April were transparent at the edge, and this was also true (98%) of fish sampled late in the year (October) on North Uist, consistent with transparent rings being laid down during the colder part of the year (autumn to spring on North Uist, where the summer season is short) (Table 2.2). The only exceptions to this pattern were fifteen fish (10%) from Tottle Brook sampled in April, when it is likely that growth had already begun at this latitude, and one fish from Reiv (2%) sampled in October. The proportion of opaque edges increased in all populations during sampling in May-June, with up to 60% in the Reiv population, but only 28% in Bhar and 42% in Tottle Brook. This percentage climbed to 65% in Tottle Brook in August sampling (Bhar and Reiv were not sampled at this time). The opaque edges were then almost completely replaced by transparent edges in autumn sampling in Bhar and Reiv with only one opaque edge in the Reiv population. When sampling period was controlled for Tottle Brook fish, transparent edges of otoliths were significantly more common in larger (Wald $F_{1,271}=20.36$, $P<0.001$) and mature (Wald $F_{1,183}=4.01$, $P=0.045$) fish.

Table 2.2. Indices of samples, and the pattern of otolith's edge and types among three populations three-spined stickleback from North Uist and Nottingham, Great Britain

Populations	Sampling date	No of sample					Edge		Otolith types				
		Total	Unreadable	Doubt	Clear	Opaque	Transparent	S-	S	S+	2S	2+	3S
Bhar	15-04-14	42	10	6	25	0	100		24		1		
Bhar	25-05-14	45		2	43	28	72		27	12	4		
Bhar	13-10-14	26			26	0	100		21		5		
Reiv	16-04-14	41	8		33	0	100		20		13		
Reiv	21-05-14	32	4		28	60	40		7	16	4	1	
Reiv	11-10-14	21	2		19	5	95	1	15		3		
Tottle Brook	02-04-15	110	3	7	97	15	85		68	13	14	2	
Tottle Brook	16-06-15	102	2	9	89	42	58		24	23	22	14	6
Tottle Brook	28-08-15	103	1	9	91	65	35	45	16	13	11	1	5

Data are actual number of samples, except for edges are in percentages.

2.4.2 Growth patterns of otolith through the year

The simplest otolith pattern (S-) (opaque with no clear transparent ring (Fig. 2.2: 1a and 2a)) was dominant (49%) in the August sample from Tottle Brook, suggesting that these fish are young-of-the-year. Size of fish with this type of otolith in Tottle Brook ranged from 28.90-57.10 mm (mean 38.53 ± 0.14). In the October sample from North Uist there was only one S-fish, caught in Reiv (Table 2.2). This is consistent with the later date of sampling on North Uist, where 80% of fish from Bhar and Reiv had S type otoliths (Table 2.2), the next most complex pattern with an opaque centre surrounded by a transparent ring at the edge (Fig. 2.2: 2b, 3a and 3b). These were also common (18%) in Tottle Brook in August samples, consistent with the first development of the transparent edge towards the end of the growing season in late summer or autumn.

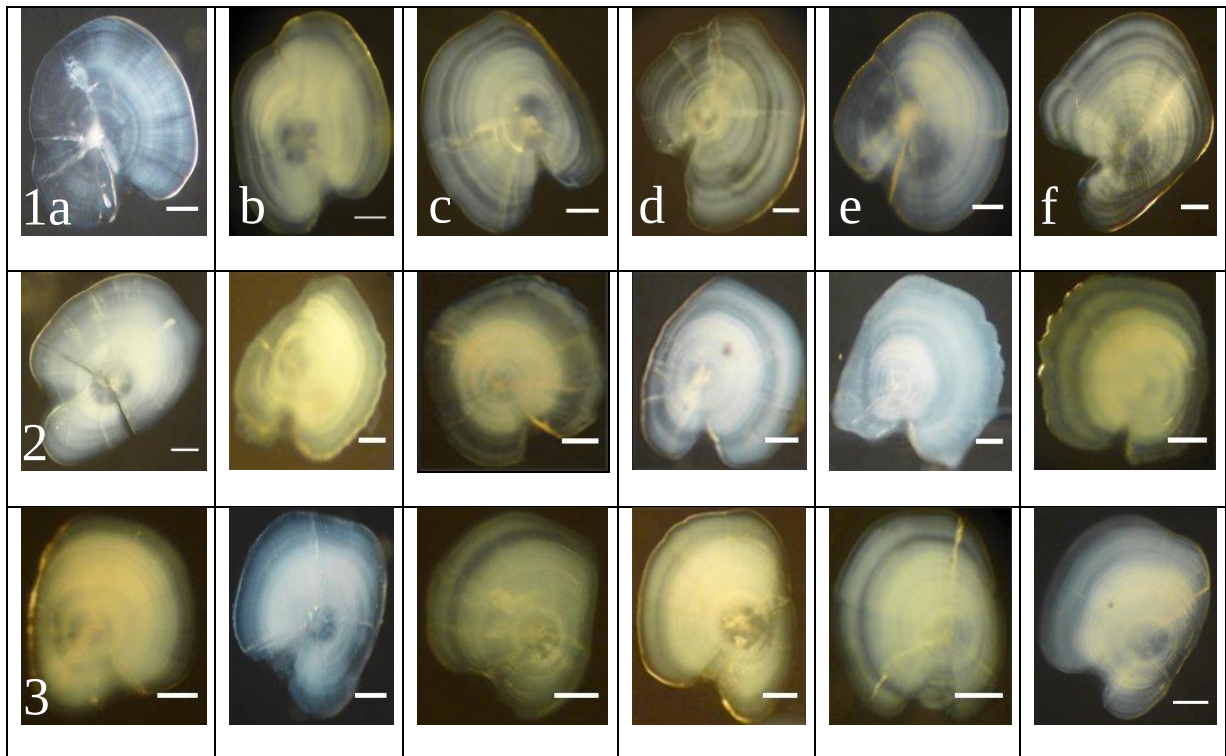


Figure 2.2. The pattern of otolith growth through the year in three populations of three-spined stickleback. 1st row=Tottle Brook, 2nd=Reiv, 3rd= Bhar. Contrasting otolith patterns were chosen for display. All sampling was conducted in the same year. Tottle Brook pictures are ordered as a) August, b) April, c) June, d) August, e) April, and f) June sampling; Reiv and Bhar: a) October, b) April, c) May, d) October, e-f) May sampling. Otoliths were pictured under reflected light with a dark background that results in opaque rings appearing cloudy white colour, while transparent rings are black. Scale bar = 100 μ m. See text for details.

Fish with a transparent edge (S type) were significantly larger (Wald $F_{1,60}=13.29$, $P<0.001$) than their counterparts with opaque edges (S-) potentially indicating that they had faster growth, or had hatched earlier in the season. Possession of this S type otolith during August sampling was significantly more prevalent in matured fish (Wald $F_{1,23}=6.85$, $P=0.009$), but was unrelated to the number of their checks (Wald $F_{1,59}=0.86$, $P=0.35$).

April sampling showed that the majority of fish (72%) in all populations still have otoliths of the S type. As this is before the start of, very early in, the breeding season, and none of the fish sampled were less than 20 mm, it seems most likely that these fish with S type otoliths are approximately one year old (0+ age class). The only difference from August/October samples is that the transparent ring becomes larger (Wald $F_{1,53}=10.72$, $P=0.002$) and more contrasting (Fig. 2.2, 3a versus 3b) suggesting the rings from August/October have not completed formation. Thus the evidence suggests that the transparent ring is laid down in the autumn and winter (non-growing season). Comparison within a single population, Tottle Brook, showed that fish with S otoliths in April were significantly larger than those with S-/S otoliths in August (Wald $F_{1,151}=13.83$, $P<0.001$).

April samples showed that new opaque growth had begun to form on the otoliths of some fish from Tottle Brook (for both 1S+ (Fig. 2.2, 1b) or 2S+ (Fig. 2.2, 1e)), but not in Bhar and Reiv, where the spring comes later. By May, new opaque growth had begun to occur in Reiv (Fig. 2.2, 2c) and Bhar (Fig. 2.2, 3c). The presence of a new opaque edge in all populations during May sampling suggests that new opaque growth had just been started. In Tottle Brook, this second ring of opaque growth continues to be formed until at least August when it still appeared in 14% out of the total sample (Table 2.2). The average size of these S+ fish in August was 43% larger (55.22 ± 0.21 mm versus 38.53 ± 0.06 mm) than the average of S- fish indicating it was unlikely that these two opaque zones could have been grown in the same year (i.e. implying that S+ fish are a year older than S- fish). At the same time, the otoliths of some stickleback were starting transparent growth (Fig. 2.2, 1d) and this became more evident during autumn sampling (Fig. 2.2, 2d and 3d). The fact that 95% of the autumn samples had transparent edges, either 1S or 2S (Table 2.2), provides a clear sign that the transparent ring was starting to be formed in autumn at the latest.

The otolith growth pattern (opaque rings in summer, clear in winter) seems consistent from year to year as some samples from Tottle Brook, where spring (and growth) begin earlier, also had second opaque growth (S+) in April sampling (Fig. 2.2, 1e), but this was absent in Reiv (Fig. 2.2, 2e) and Bhar (Fig. 2.2, 3e). The most complex otolith from Tottle Brook

showed three sets of growth zones (3S) (Fig. 2.2, 1f), while in Reiv the most complex otolith from this sampling had two sets of growth zones plus an opaque edge (i.e. 2S+, Fig. 2.2, 2f). A fish with a 2S+ otolith was also found in Bhar (Fig. 2.2, 3f), but its opaque edge was not as clear as the fish from Reiv (Fig. 2.2, 2f), suggesting that it had not begun to grow in the year it was caught.

2.4.3 Checks and doubts

The commonest check in my samples was the presence of a ‘fake’ transparent ring before the first true transparent ring. This fake ring can be either in the form of an incomplete smear ring that can be easy to determine (Fig. 2.3a), or a complete (but often narrow) clear ring (Fig. 2.3b and 2.3c) that can lead to doubt. The latter were assumed to be fake rings based on their thinner size (Fig. 2.3b), small radius and/or low clarity (Fig. 2.3c) compared to true transparent rings. These three types of check (Fig 2.3a, b and c) are more common in the Bhar population (Fig. 2.4), and may be related to poor resource conditions. Another type of check that was found is where a narrow opaque ring splits the transparent ring (Fig. 2.3d) or vice versa (Fig. 2.3e). A sixth type is when otoliths lack clear transparent or opaque rings (2.3f, g). The last type, that quite often led to doubt, is the persistence of nice regular, but very closely spaced rings (Fig. 2.3h). The occurrence of each type of check is shown in Figure 2.4.

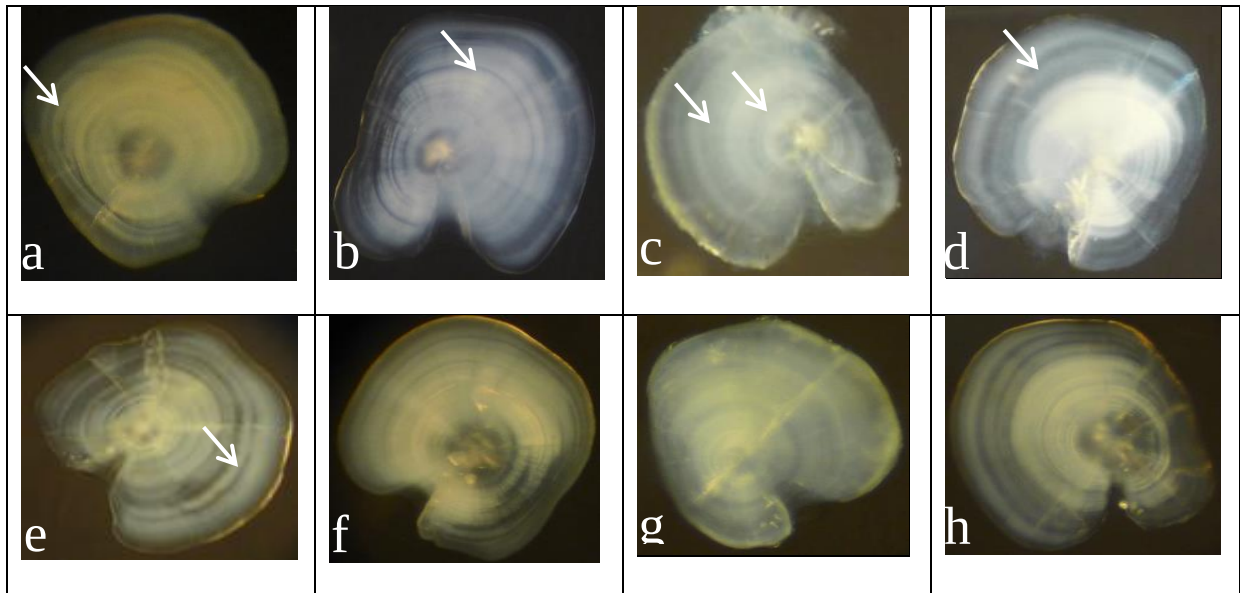


Figure 2.3. Types of common checks (highlighted with white arrows) in the otoliths of three-spined stickleback from North Uist and Nottingham, Great Britain. Otoliths were pictured under reflected light with a dark background that results in opaque rings appearing white and transparent ones as black. See text for details.

Most of the otoliths with checks in pictures 2.3 (except 2.3f and 2.3h) were likely only have one true transparent ring, either S type otolith for 2.3a, c and g or S+ for 2.3b, d, and e. Of pictures 2.3a and 2.3c, the true transparent ring was likely only the outermost. Other transparent rings in 2.3a can be categorised as checks due to their lower clarity and thinner size, compared to true transparent ones. A similar case applied to 2.3c; two of the transparent rings in the inner side seemed less clear compared to the outermost, and therefore the true transparent ring in this otolith was only one at the edge. Although the otolith in 2.3g does not show any clear opaque ring, this fish can be assumed to have at least one true transparent ring as it originated from Bhar population sampled in April. This type of otolith was typical for Bhar and the absence of an opaque ring in this type maybe related to poor conditions in this loch.

The true transparent rings in 2.3b, d and e were also counted only once because the ring with the arrow in 2.3b was assumed as a check, while multiple transparent rings in 2.3d and e were thought as a split form of the first transparent ring. Although the “arrowed ring” in 2.3b seemed too hard to judge, this ring could be categorised as check. The main reason for this judgement is its narrower and lower clarity comparing to the second transparent ring. Indeed, there was a new growth of transparent ring in 2.3b, d and e around the edge, but they were not in fully completed rings and therefore they were not counted as a ring.

Both otoliths in 2.3f and 2.3h could have three intact transparent rings. The problem with the 2.3f transparent rings was those rings looked too thin compared to their counterpart in 2S+ or 3S types from the same population. The transparent rings of 2.3h were firmer, but very closely spaced.

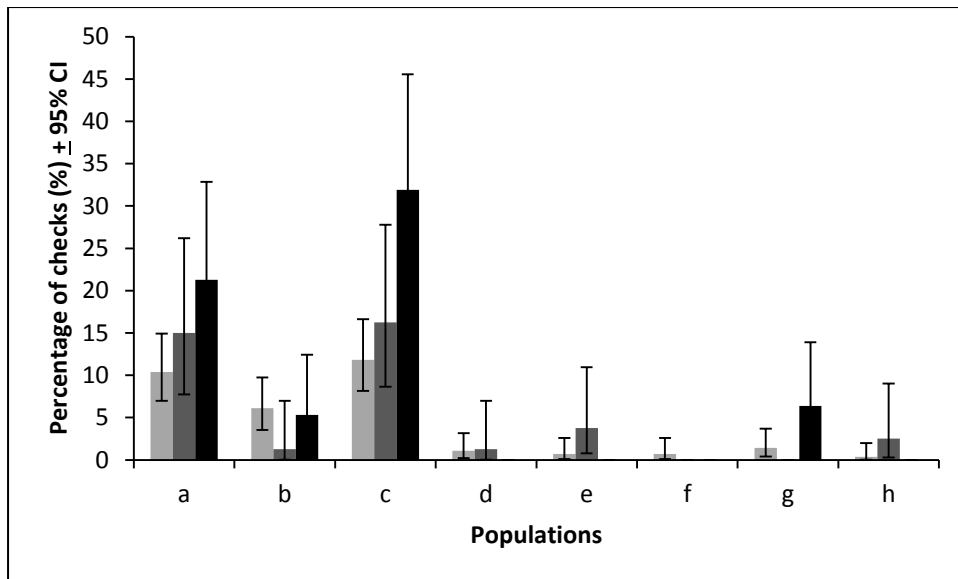


Figure 2.4. Percentage of fish with the occurrence each type of check ($\pm 95\%$ CI) among three populations of three-spined stickleback from North Uist and Nottingham. Light grey=Tottle Brook, dark grey=Reiv, solid black=Bhar. See text (Fig. 2.3) for the details of checks.

2.4.4 Age structure of the populations

Based on the pattern of the development of edges and the growth of stickleback described above, age class determinations were made by combining number of ring/s with periods of sampling. Briefly, fish with opaque otoliths (caught in summer) (S-) were counted as young of the year while those with an opaque centre and transparent ring (S) were assumed to be young of the year when caught in the autumn, and one year old when caught in the spring. Any samples with two opaque rings flanking a transparent ring (S+) were also described as yearlings. Samples with two pairs of rings (2S) were assumed to be ~ 1.5 years when caught in the autumn, and two years old when caught in the spring. Fish with two pairs of rings plus an opaque edge (2S+) were counted as two years old, and so on.

Individuals with S type otoliths in August sampling (18%) were counted as young of the year, not yearlings, as the presence of a first transparent ring in this group was more likely from the current year, not from the previous winter. This is because the formation of this ring seemed just to start in late summer (26% of young of the year by August sampling) and continued to develop through the coldest part of the year (98% of young of the year by October and 90% in April sampling). Moreover, as this S otolith in late summer was more common in mature fish, it can be assumed its earlier presence was due to the effect of early maturation in some individuals. Indeed, there is a possibility that fish with S otoliths during August sampling

were slow growing 1.5 year old stickleback. However, this option was less likely since the widths of these rings were significantly narrower and their appearances less contrasting in comparison to the April ones. If they were 1.5 years stickleback, they should have a wider and a more contrasting transparent ring than the April ones, as they had a longer period of slow/no growth.

In contrast to the fish with S otoliths in August sampling, coherent fish in June sampling (51% of S+(S+) fish) were assumed to be yearlings, not young of the year, as their first transparent rings was likely from the previous winter. The first reason for this assumption is the width of this ring was similar to the transparent ring of April sampling. While the second is, based on the age structure of a wild population, it is almost impossible that young of the year have the same percentage as yearlings (24 versus 23 individuals). The absence/delaying of a second opaque ring in this group was thought mostly due to the cost of reproductive investment.

Any transparent edges in older classes (2S and 3S) during June and August sampling were also assumed to be from the previous winter, therefore their age was counted normally. This judgement was based on the assumption that formation of opaque edges in older ages usually come later. Indeed, age of these individuals could be overestimated if their outermost rings are part of maturation rings and/or have been formed in the current year. However, as this type of case was only a minority of the total samples (10.6%), it probably will not affect the overall age estimation.

Following the ageing method above, I found that more than half of the samples (59%) belonged to one year old fish, while 22% were young of the year, 18% were two years old and only six individuals (1%) were in their third year. All of these three years old were from the Tottle Brook population. One year old fish were dominant in spring (April-May-June) sampling (70%), while young of the year were dominant in late summer-autumn (August-Oct-Nov) sampling (71%) (Fig. 2.5).

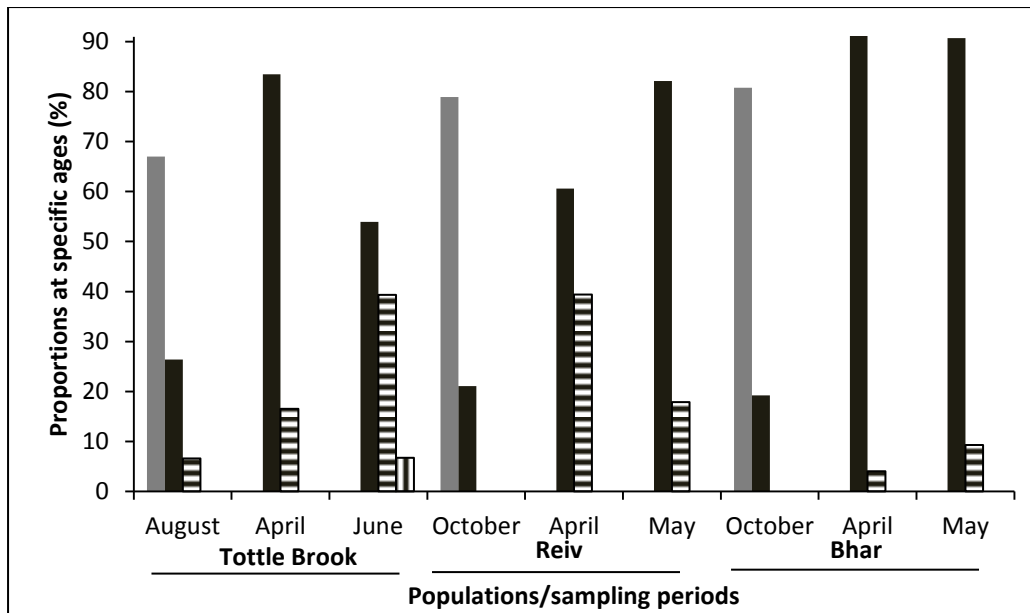


Figure 2.5. Populations structure of North Uist and Nottingham three-spined stickleback in each sampling period. Solid grey=young of the year, solid black=1 year old, stripe horizontal=2 year old and stripe vertical =3 year old.

When population and sampling time were controlled for, older age of stickleback was significantly correlated with larger size (Wald $F_{1,446}=67.30$, $P<0.001$; Fig.2.6) and larger otolith size (Wald $F_{1,446}=61.56$, $P<0.001$), and therefore, larger stickleback also had larger otoliths (Wald $F_{1,446}=559.95$, $P<0.001$). As older fish were larger and there was a positive relationship between fish size and the presence of transparent edges on their otoliths, younger fish were more likely to have otoliths with opaque edges (Wald $F_{1,447}=18.77$, $P<0.001$; Fig.2.7). A higher percentage of opaque edges in younger age classes suggested that younger stickleback started their otolith growth earlier in the year. The unusual pattern in the relationship of size and age of two years old stickleback in the Bhar population (Fig. 2.7) may indicate that there was an overestimating of stickleback age in this population. Alternatively, it may be that these are the most slowly growing or latest hatched fish that have delayed maturity until two years of age.

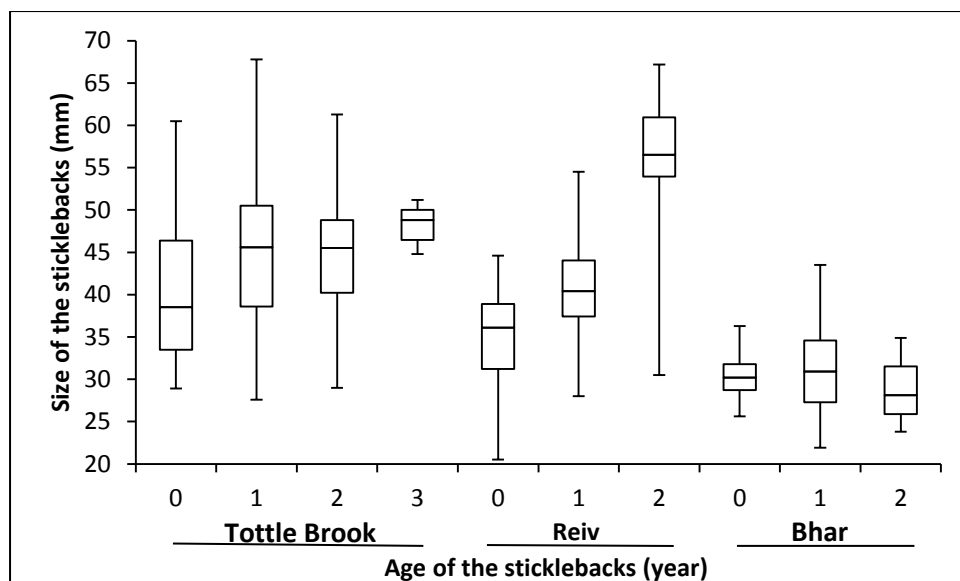


Figure 2.6. The relationship of age and size of North Uist and Nottingham three-spined stickleback.

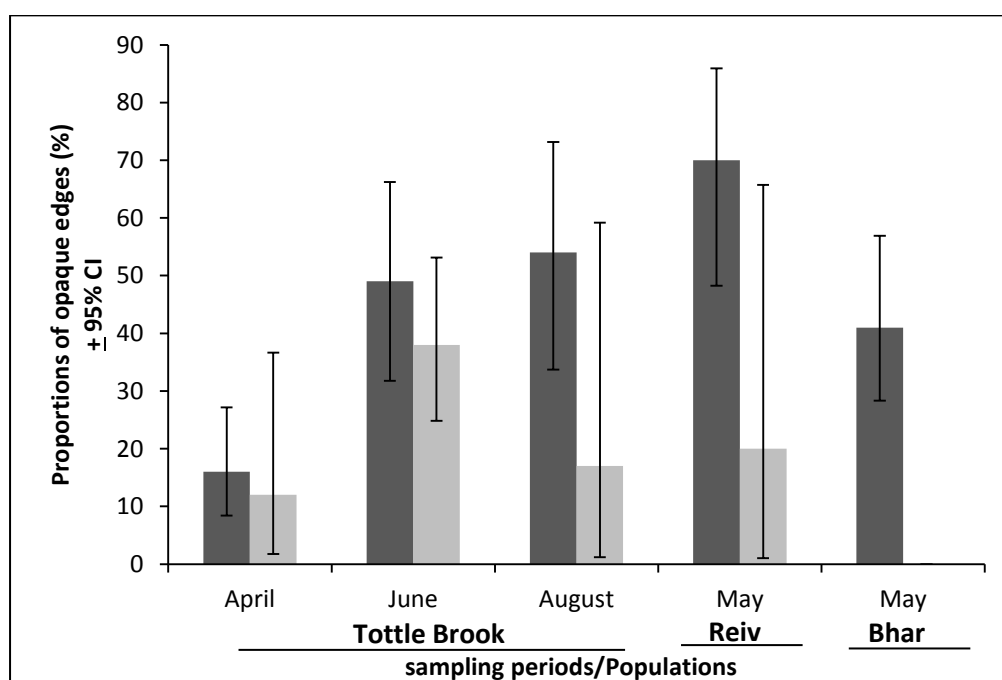


Figure 2.7. The proportion of otoliths with opaque edges ($\pm 95\%$ CI) of three-spined stickleback from North Uist and Nottingham in each sampling period. Dark grey bar=one year old and light grey bar=two year old. CI=confidence interval. There were no opaque edges in other sampling periods.

Among one-year old stickleback that were sampled during April, the width of opaque rings differed significantly between populations (Wald $F_{2,54}=26.25$, $P<0.001$) even between the two

North Uist populations (Wald $F_{1,37}=26.59$, $P<0.001$; Fig. 2.8). However, the transparent ring only differed between regions, (Nottingham vs. North Uist, Wald $F_{1,55}=51.16$, $P<0.001$), and not between Bhar and Reiv (Wald $F_{1,37}=3.36$, $P=0.075$). This is consistent with longer growing seasons, and shorter winters in more southerly (Tottle Brook) and better resource (Reiv) conditions.

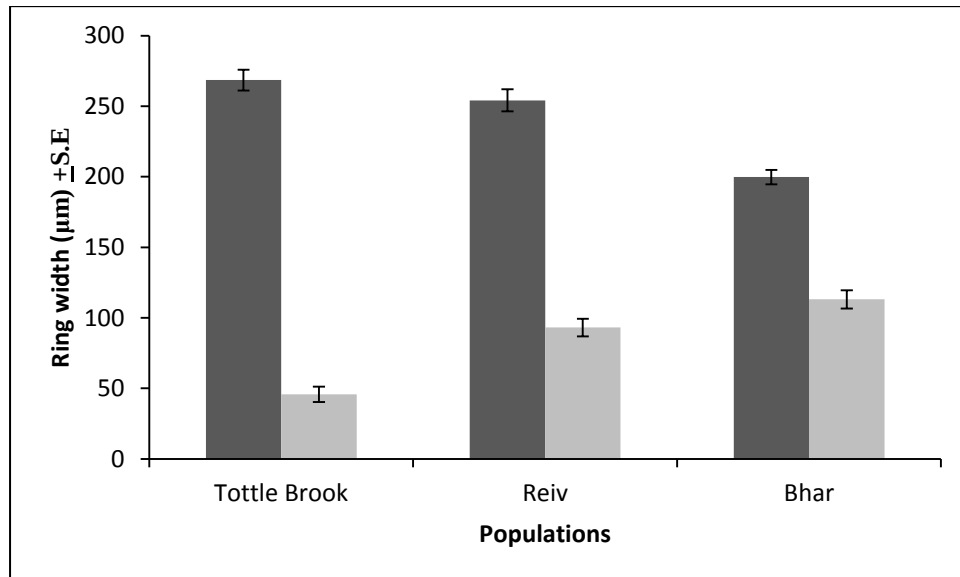


Figure 2.8. The width of opaque and transparent otolith rings ($\pm$ S.E) among one year old three-spined stickleback in three different populations. Tottle Brook is from Nottingham, Reiv and Bhar are from North Uist. Dark grey bar=opaque ring, light grey bar=transparent ring.

2.5 Discussion

My results on the otolith development pattern of the three-spined stickleback are more compatible with Borland (1986) and studies of many other fishes (e.g. Panella *et al.*, 1971; Williamns & Bedford, 1974; Uriarte *et al.*, 2016), than with Jones & Hynes (1950; Greenbank & Nelson, 1959). In keeping with Borland (1986), my study showed that it is more likely that the first transparent ring is formed after juvenile stickleback stop growing at the end of their first summer, rather than during that first summer i.e. in June or early July. Samples from the end of August showed that most young of the year from Nottingham still have opaque edges, suggesting continued growth, while the presence of a transparent edge in the remainder is probably an indication that growth had ceased, and the formation of the transparent ring was just starting. The appearance of these rings, the majority of which are

thinner in size and lighter in colour than those from April supported this assumption. Moreover, the great majority of stickleback sampled in April had transparent edges, suggesting that the formation of transparent growth was still continuing.

My results also show that there is no sign of a second period of opaque growth in the otolith of stickleback before their winter. August sampling shows that only fourteen percent of the samples displayed a second period of opaque growth, which matches with the results of Jones & Hynes (1950). However, this fourteen percent of opaque edges is more likely growth from April that indicates they are in their second summer, not young of the year. This assumption was supported by the fact that the average size of the fish in the S+ category was substantially larger than the average size of the fish in the S- category.

In striking contrast to the findings of Jones and Hynes, the fact that there were no juveniles with opaque edges in autumn samples, and only 10% in the April samples provides clear evidence that juvenile do not develop a second opaque ring before their first winter. The presence of a small proportion of fish in spring with opaque edges is more likely the result of the early onset of growth in the southerly Tottle Brook population, and the formation of these edges after, not before, the winter. Williams & Bedford's (1974) claimed that, in the northern hemisphere, the growth of opaque otolith rings usually started earlier in more southerly populations.

The growth pattern of stickleback otoliths through the year clearly shows that the opaque zone is formed during spring and through the summer. There is no indication of opaque growth during autumn or winter. In contrast, most transparent zones were formed during autumn and/or winter. However, formation of transparent zone seems more widespread through the year since it was detected in August samples and even during June. Again, this result differs from that of Jones & Hynes (1950; Greenbank & Nelson, 1959) which suggested that transparent rings should be formed in June to September while opaque rings should be formed in other months. However, it is consistent with other studies either in stickleback (Allen & Wootton, 1982; Borland, 1986) or other fishes (Jones, 1992; Uriarte *et al.*, 2016).

Jones & Hynes assessment of the development of otoliths was based on sequential sampling from the Birket, a small shallow stream on the Wirral peninsula, west of Liverpool U.K. According to Hynes (1950), this stream experiences reduced flow in summer, with “often no visible current”, high temperatures (at least as high as 18°C when they sampled), and is

choked by vegetation. In addition, there was a conspicuous shortage of large prey items in stickleback stomachs during June to September (Hynes, 1950, Table 8). It therefore seems likely that for stickleback, growth conditions in the Birket are at their poorest in the summer, and improve in the winter. This may help to explain the differences between Jones & Hynes findings about the development of otoliths, and those described here. Therefore other researchers, when interpreting stickleback otoliths, should think carefully about the growth conditions that their fish are likely to have experienced.

This study supports the theory that broader opaque zones in otoliths reflect better resources (Baumann *et al.*, 2005), or possibly longer growing seasons. Stickleback from Reiv, which has better resources compared to Bhar (MacColl *et al.* 2013; Chapter 3), also had significantly wider opaque zones during their first year of growth. Similar widths of transparent zones in these two populations, which are at identical latitudes, suggest that during the winter, they experience little difference in growth. Stickleback from Tottle Brook had wider opaque and narrower transparent rings than the populations on North Uist, consistent with their location further south. In all populations, the ratios of width of opaque to clear rings during the first year are lower than those (~1:20) described by Panella *et al.*, (1971) for the early stages of fish. This lower value is not a peculiar result as the growth of otoliths depends greatly on the somatic growth of the fish, and this could vary greatly as a result of multiple factors such as species, heritability, and/or ecological conditions.

All types of checks that were found in this study have been reported by previous researchers (Williams & Bedford, 1974; Waldron & Kerstan, 2001; Uriarte *et al.*, 2016). The most common check found in this study was one before the first transparent ring that can appear as a complete but narrow ring or an incomplete smear. The former can be identified as a check either by their thinness or their poor clarity. This type of check was reported by Waldron & Kerstan, (2001) in mackerel (*Trachurus trachurus*) otoliths and by Uriarte *et al.*, (2016) in European anchovy (*Engraulis encrasicolus*). Uriarte *et al.*, (2016), claimed that this type of check occurred in juvenile anchovy juvenile because of changing temperatures and/or poor feeding conditions. The fact that this type of check is more prevalent in the Bhar population is consistent with this explanation, since it has a lower quality environment compared to Reiv (MacColl *et al.*, 2013; Chapter 3).

The presence of abrupt opaque growth in the middle of a transparent ring or vice versa is probably due to rapidly changing environmental conditions. This type of check occurs commonly in the first transparent ring of anchovy when slower growth is interrupted by

higher temperatures or better food conditions (Uriarte *et al.*, 2016). An interruption of an opaque zone could be due to spawning (Uriarte *et al.*, 2016). A complete and clear ring within a short distance of a previous one could be a true ring in a slow growing fish, but also could be a check splitting a single transparent ring (Williams & Bedford, 1974). It is probably most appropriate to exclude this type of sample when analysing stickleback otoliths.

The size of the stickleback in this study was tightly correlated with their inferred age, and both size and age correlated with larger otolith size, supporting my inferences about the relationship between otolith development and age. Indeed, I found there was a small bias in the relationship between age and size of stickleback in this study with size of two years old Bhar fish smaller than yearlings. This bias could be an indication that age of those fish was overestimated. As stickleback in this population are annual and mostly matured in their second spring (Chapter 3 and 4), the presence of second transparent ring during May sampling in Bhar population may be a part of a maturation ring, not a seasonal ring. Alternatively, those two years old may be slow growing or late hatched fish that have delayed maturation.

The positive relationship of age, body size and otolith size in fishes have been reported widely by previous researchers (e.g. Williams & Bedford, 1974; Harvey *et al.*, 2000; Koeda *et al.*, 2016). A test by Harvey *et al.*, (2000) on 63 species of fishes found that 45 of them exhibited a linear relationship between body size and otolith size. Since size of fish also correlates positively with their age (e.g. Koeda *et al.*, 2016), it is reasonable to expect a linear correlation between age and otolith size.

In conclusion, my results clearly differ from Jones & Hynes (1950) in three main ways: i) there is no development of a first transparent ring among juvenile stickleback during their first summer i.e. June/July. The formation time of transparent rings does not differ greatly between juvenile and adult fish. They are formed during late summer and through the winter. ii). There is no formation of a second opaque growth band in the otoliths of juvenile stickleback until they pass their first winter. iii) Formation of the opaque ring in stickleback mostly occurs in spring and summer, with younger fish starting earlier. In contrast, the formation of transparent rings is mostly during autumn and winter, but can be more widespread since it was detected in late August, and can apparently still occur until the middle of June for some individuals. This conclusion is exactly the same as Borland's (1986).

Chapter 3. Role of body size and ecological factors on maturation event of freshwater stickleback

3.1 Abstract

Despite strong theoretical predictions, the relative importance of different selective agents in driving life history evolution is poorly understood. Using a large dataset from an adaptive radiation of freshwater three-spined stickleback from the Scottish island of North Uist, I examined the effects of variation in resources and predation on maturation time, a critical life history variable. I tested three theoretical predictions: (i) better resources and greater interspecific competition should select for larger body size, thus larger size at maturation, while the effect of density and predation should have a contrasting effect. (ii) Larger body size should correlate positively with earlier maturation. (iii) Individuals which living in rich resources should have a wider range in size at maturation. My results show that interspecific competition and better resources are strongly associated with larger body size and thus larger size at maturation. The strong effect of these two factors may outweigh or make difficult to detect, a negative impact of density and predation. Contrary to my second prediction, but consistent with my third, populations with larger mean body size which also live in richer resource conditions, tend to delay their maturation and have a wider range in size at maturation. In addition, my results confirm that a negative effect of predation on life expectancy can be compensated by an increase in resources.

Keywords: resources, predation, competition, body size, maturation, life expectancy, three-spined stickleback

3.2 Introduction

The relationship between age and size at maturity, and its underlying association with ecological factors has long been of interest to ecologists. Established models (Stearns & Koella, 1986; Reznick *et al.*, 1990; Roff, 1992; Heino *et al.*, 2002) predict that larger body size will be accompanied by earlier maturation, and smaller body size with delayed maturation. When a minimum size is attained, it is generally better to mature sooner to reduce generation time and avoid being killed before reproduction (Roff, 1992; Stearns, 1992). However, if growth is still possible, delaying maturation can increase fecundity and therefore be advantageous. Maturing later can also be advantageous for future growth and life expectancy since there is often a negative relationship between early maturation and future growth (Reznick, 1983) or life span (Stearns, 1992).

Although most of the available data fit with these established models, contradictory results from complex environments have also been prominent for some decades (Baltz & Moyle, 1982; Fox, 1994). Instead of maturing earlier, larger individuals (or those from populations where average size is large) may delay their maturation while, in contrast, smaller individuals mature at younger age. Low juvenile survival rate has been thought of as the most likely reason for delaying maturation (Stearns & Koella 1986; Roff 1992). When juvenile survival is low, organisms tend to delay their maturation in order to increase fecundity, and thus produce more or better offspring. This means that delaying maturity is only beneficial when there is a significant increase in size.

On the other hand, early maturation for populations with smaller body size is usually due to high adult predation or poor resources in the maternal environment. It is established that high adult mortality rate will force populations to mature earlier and at smaller size (Reznick *et al.*, 1990; Heino & Godo, 2002; Hunter *et al.*, 2015). Poor resource conditions can also cause organisms to mature swiftly, even at smaller size, as long as some minimum size is attained (Wilbur & Collins, 1973; Plaistow *et al.*, 2004). Fox (1994) emphasised that early maturation when growth is slow can be explained by a positive relationship between body size and survival rate. He found that if slower growth of pumpkinseed sunfish is associated with a lower adult survival rate, then maturing earlier with even smaller size can result in higher fitness. He also underlined that mortality rate is more important than growth in shaping maturation time.

Maturation time of poor- and rich-resource populations also could differ in the interval between initiation and completion of maturation. A model from Wilbur & Collins (1973), pointed out that populations which are living in poor resources tend to prioritise minimum size, not age, for maturation. Therefore, these populations should have a smaller range of size and age at maturation. In contrast, resource-rich populations tend to have a longer interval of completing maturation, and therefore they will have a wider range in their age and size at maturation. This model was formalised by Reznick (1990), though later studies showed mixed results that led some modifications to this theory by recent authors (Day & Rowe, 2002).

Another ecological aspect that can influence maturation time is competition. Interspecific competition can select for higher growth rate if this results in niche domination or differentiation in the presence of asymmetric competition (Arendt, 1997), and thus should favour early maturation. However, competition also can lead to lower growth rate due to the limitation of resources (Mittelbach, 1988), and thus result in later maturation. In contrast with interspecific competition, higher intraspecific competition (population density) should favour delaying maturation due to high competition for resources.

While the role of environmental factors may seem clear when they are considered individually, in the real environment, factors are correlated. This can have consequences for the evolution of life histories. Fox (1994) found that instead of delaying maturation, higher population density of pumpkinseed fish is associated with early maturation. Research by Arendt & Reznick (2005) on Trinidadian guppies showed that high resources can reduce the effect of predation on body size. However, a contrasting pattern was found by Martin et al. (2011) for the growth rate of broods of Passerine birds. They found that high predation risk can force individuals to grow faster even when they have lower resources.

Here, I test theories of maturation using a large dataset of wild populations of freshwater stickleback from the Scottish island of North Uist, where variation in environmental conditions has led to substantial divergence in life histories. As hypotheses, I predict that: (i) Better resources and interspecific competition should select for larger body size, thus larger size at maturation, while the effect of density and predation should have a contrasting pattern. (ii) Larger body size should correlate positively with earlier maturation. (iii) Individuals which are living in rich resources should have a wider range in size at maturation.

3.3 Methods

3.3.1 Sampling and collecting the environmental data

Eight hundred and seventy five freshwater stickleback from 24 different populations (Table 1) were sampled on North Uist (57°36' N 7°20' W) in April-June 2013. Fish sampling was carried out using unbaited minnow traps (Gee's traps, Dynamic Aqua, Vancouver, Canada) which were set overnight from the shore in water 0.3 - 3 m deep. The fish caught were counted to estimate density as the average of stickleback per trap per night, a measure of catch per unit effort ('Mean CPUE'). The relative densities of nine-spined stickleback (*Pungitius pungitius*), the main competitor of three-spined stickleback in these lochs, were also estimated from these catches, as the percentage of 100 haphazardly selected fish ('Per cent *Pungitius*'). Thirty five three-spined stickleback were then haphazardly selected for analysis. From these, I collected individual data for standard length in millimetres, weight in grams, and female reproductive status (maturing/mature or immature, recorded as a binary variable; Chapter 2). Fish were preserved in ethanol for later determination of the age of each fish by otolith analysis (Chapter 2).

Using the otolith data, I assigned fish to annual age classes (generally 1+, 2+, 3+, since fish were collected early in the breeding season, before recruitment of that year's offspring), and calculated the proportion of fish older than one year as an estimate of life expectancy (a majority of stickleback in these populations mature at about one year of age). I used the average size of individuals in each year class as an estimate of body size and the differences between age classes 1+ and 2+ as estimates of growth rate in each population. Size and age at maturation were estimated as the average of length and age of mature females from the same population.

Indices of variation in resource availability among lochs, based on water chemistry, were also collected at the same time. The following data were collected for each loch: pH, conductivity, and the concentration of sodium (Na^+) and calcium (Ca^{+2}) ions, and dissolved organic carbon (DOC). Measurements of pH and conductivity were conducted directly on the lochs (*in situ*) using a multi-probe digital meter (Semat International, Multi 340i), while the concentration of Na^+ , Ca^{+2} and DOC were measured at the University of Nottingham by quadrupole inductively coupled plasma mass spectrometry (detail in MacColl & Aucott (2014)). These factors were chosen because they are common measures of environmental variation in aquatic systems, and are known to vary greatly between North Uist lochs (Giles, 1981; MacColl *et al.*, 2013), where they also correlate with resource availability (Waterston *et al.*, 1979;

Chapter 4) and stickleback body size (MacColl 2013). In addition, I used published estimates of the density (catch per unit effort from angling competitions) of brown trout, *Salmo trutta*, which are probably the main predator of stickleback in these lakes (MacColl and Aucott, 2014).

3.3.2 Data analysis

Generalised linear models (GLMs) with binomial error distributions and logit link functions were used to analyse the probability of maturation at one year old and ‘life expectancy’ (proportion of individuals older than one year old) between populations. Age at maturation, with females classified as 1+ or older, was also analysed with a binomial error distribution and logit link function. GLMs with normal error distributions and identity links were used to analyse variation between populations in size at one year old and size at maturation.

Mean size at one year old, and age and size at maturation data for each population were then combined in a principal component (PC) analysis, using a correlation matrix approach, to identify the main axes of life history divergence between populations. Using the same method, the individual water chemistry data were reduced to principal components reflecting overall water chemistry variation. The relationship among life history traits, and between life history PCs and environmental factors (PC1 of water chemistry, trout catch rate, stickleback Mean CPUE and relative density of *Pungitius*) were then analysed using multiple linear regression. Relationships between variance of size and age at maturation within populations with PC1 of water chemistry were also tested using simple linear regression. All statistical analyses were conducted in GenStat 15th edition (VSN International, 2011).

3.4 Results

I collected the main ecological factors i.e. lochs' water chemistry, density, relative density of main competitor and intensity of their potential predator for 24 populations of North Uist stickleback. I also aged 813 stickleback in total for these 24 populations (Table 3.1).

Table 3.1 Indices of ecological factors, age structure, and females maturation for 24 populations freshwater stickleback from North Uist

Pop*	PC1 water chemistry	Trout catch rate	Mean CPUE	Percent <i>Pungitius</i>	Age (year)		Female at 1 year old		F1 total
					1+	2+	F0	F1	
AONG	-0.69	0.25	12.68	0	33	1	1	26	27
AROI	-1.02	N/A	0.79	3.92	31	3	7	7	10
BHAR	-2.04	0.26	2.02	0	30	4	7	15	15
BUAI	-0.05	0	9.76	0	34	0	2	21	21
CREI	-0.45	N/A	9.29	0	33	1	7	13	13
DUBH	-0.88	0.05	3.18	3.65	33	2	14	7	9
EISI	-1.23	0.34	3.19	0.00	29	5	6	8	10
FADA	-1.65	0.80	1.39	0.00	32	2	3	14	16
FEIT	4.22	0.17	7.12	4.11	27	8	1	12	14
FHAI	-1.26	0.31	10.03	1.24	28	6	4	22	26
GEIR	-1.71	0.12	6.65	0.00	29	6	1	16	19
GILL	-1.02	0.30	0.97	2.94	25	5	5	11	15
GROG	2.91	0.13	12.07	1.53	24	10	3	16	19
HOST	2.41	0.16	10.84	3.35	32	1	11	4	4
MAGA	1.37	0.20	1.84	4.17	29	5	1	15	19
MAIG	-0.79	0.57	1.65	1.09	34	0	3	19	19
MORA	-1.22	2.29	1.44	0.45	30	4	0	9	10
NAGE	0.36	N/A	2.79	0.46	29	3	2	9	10
REIV	3.64	0	42.79	2.98	24	9	6	4	7
SAND	3.19	0.20	12.28	2.04	19	15	2	14	28
SCAD	-1.89	1.29	30.43	1.65	29	5	6	14	17
STRU	-0.64	0.51	15.75	0.00	31	3	2	17	17
TORM	-0.70	0.25	16.88	0.00	35	0	0	8	8
TROF	-0.84	0.10	1.89	3.32	32	1	0	15	16

*Populations (lochs) names and coordinates are in MacColl et al., (2013), CPUE=catch per unit effort, F0= immature females, F1=mature females.

Of these 813 stickleback, 88% were one year old, suggesting that most freshwater stickleback populations on North Uist are annual. The great majority of females (77%) matured at one-year old, although this varied between populations (Wald $X^2_{23}=95.80$, $P<0.001$) with only 33% mature in Dubh but 100% mature in Mora, Torm and Trof (Table 1). Of two-year old females, 24% were found to be still immature on average, but there were no immature females at three years old, although this was based on only 12 females. The proportion of individuals that were older than one year old differed significantly between populations (Wald $X^2_{23}=80.52$, $P<0.001$) ranging from 0% in Buai and Torm to 44% in Sand (Table 3.1).

3.4.1 Variation in ecological factors among the sampled lochs

Principal component (PC) analysis summarised 71% (eigenvalue=3.53) of water chemistry factors in PC1 and 20% (eigenvalue=1.00) in PC2. Each of the measured water chemistry factors had a roughly equal contribution to PC1, except for DOC which strongly dominated the PC2 axes (Table 3.2). Higher scores in PC1 were correlated with higher pH, conductivity, and the concentrations of calcium and sodium ions, while higher values in PC2 were correlated with decreased DOC (Fig. 3.1). Lochs on the west side of the island clearly have better resources as indicated by higher scores on PC1 water chemistry (Fig. 3.1; Table 3.1).

Table 3.2. Contributions (loadings) of each (a) ecological factors and (b) life history traits in the main axes PCA of 24 freshwater stickleback populations on North Uist

(a) Factors	PC1 water chemistry	PC2 water chemistry	(b) Factors	PC1 life history	PC2 life history
Ca ⁺²	0.50	0.08	SL at 1 year old	0.60	0.42
Na ⁺	0.47	0.06	Size at maturity	0.63	0.26
pH	0.51	-0.06	Age at maturity	0.49	-0.87
Conductivity	0.52	0.04			
DOC	0.06	-0.99			

Table 3.3. Results of GLMs to estimate the relationship among ecological factors on 24 populations North Uist freshwater stickleback. The model fitted by using identity link function with normal errors.

Response	Fitted model	df	Wald F	p	<i>r</i>
Density	PC1 water chemistry	1,20	2.66	0.119	0.357
	Competition	1,19	0.85	0.368	-0.001
	Predation	1,18	0.04	0.836	-0.069
Competition	PC1 water chemistry	1,20	9.45	0.006	0.458
	Density	1,19	0.85	0.368	-0.001
	Predation	1,18	0.10	0.754	-0.278
Predation	PC1 water chemistry	1,20	2.96	0.102	-0.354
	Competition	1,19	0.15	0.707	-0.278
	Density	1,18	0.04	0.836	-0.069

3.4.2 Size at one year old, and age and size at maturation between populations

There was a significant difference in size at one year old, age at maturation and size at maturation between populations of stickleback on North Uist (Wald $F_{23,688}=18.99$, $P < 0.001$, for size at one year old; Wald $F_{23,345}=2.73$, $P < 0.001$ for age at maturation; Wald $F_{23,345}=14.75$, $P < 0.001$ for size at maturation). Variance in size at maturation within populations varied from 2.46 (mean = 47.68, $n = 4$) in Host to 83.91 (mean = 42.27, $n = 15$) in Gill.

Populations with larger size at one year old matured at significantly larger size (Wald $F_{1,22}=91.00$, $P < 0.001$) (Fig.3.2a) and older age (Wald $F_{1,22}=6.08$, $P=0.022$) (Fig 3.2b). Age at maturation was positively correlated with size at maturation (Wald $F_{1,22}=9.87$, $P=0.005$) suggesting that delaying maturation has a significant impact in increasing size at maturation. Populations with earlier maturation have a significantly lower proportion of older fish (Wald $F_{1,22}=33.12$, $P < 0.001$) (Fig. 3.3) supporting a positive relationship between age at maturation and life expectancy.

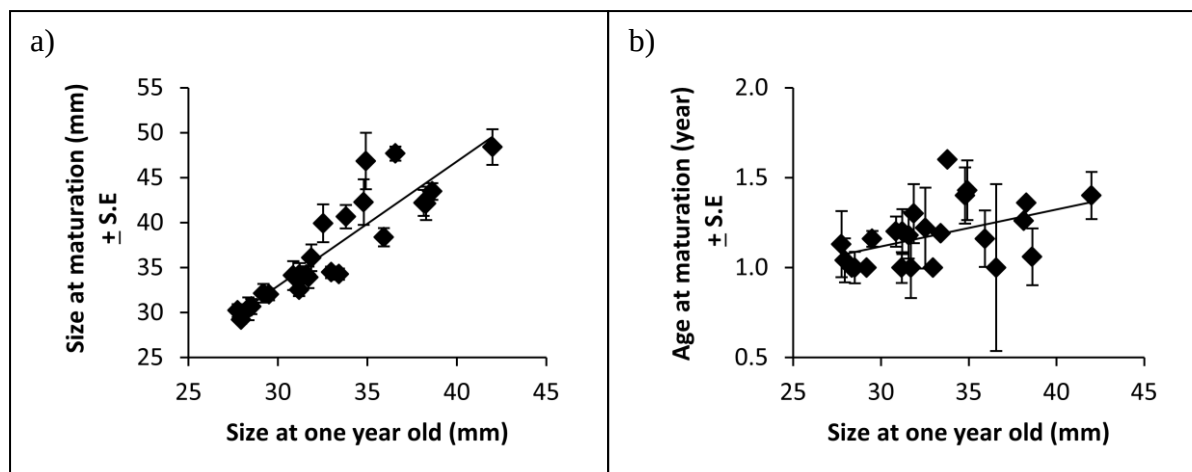


Figure 3.2. Relationship of size at one year old with size (a) and age (b) at maturation ($\pm$ S.E) among 24 populations of freshwater stickleback from the North Uist.

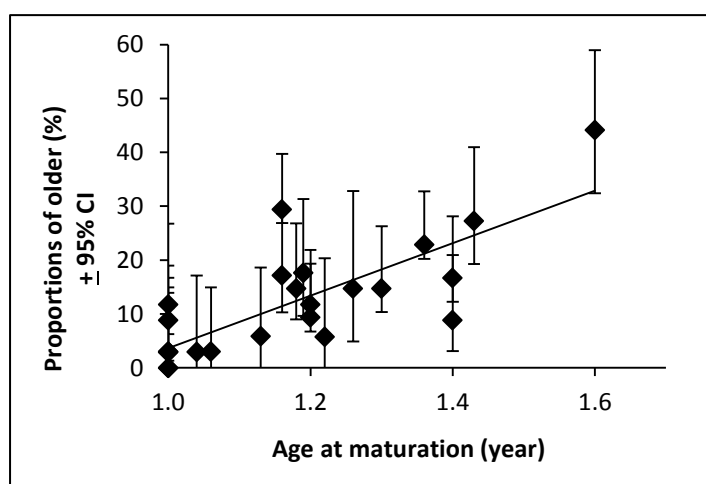


Figure 3.3. Relationship of age at maturation with proportions of older ($\pm$ 95% CI) among 24 populations of freshwater stickleback from the North Uist.

Principal component analysis of life history traits summarised 77% (eigenvalue=2.3) of measured traits in the first axes (PC1), while 20% (eigenvalue=0.60) was summarised in the second axes (PC2). Each of the measured life history traits tended to have a similar contribution (loading) on PC1, while PC2 was mostly affected by the loading of age at maturation (Table 3.2). The higher scores on PC1 were associated with larger size at one year old and larger size at maturation, while higher values on PC2 was associated with reduced age at maturation (Fig. 3.4). This finding suggests that most North Uist stickleback with larger size at one year old tend to delay their maturation, resulting in larger size at maturation, while fish with smaller body size tend to mature at younger age and smaller size. Eisi, Host, Sand, and Trof were the populations that do not adopt this common pattern, which also

means their PC scores were substantially higher in PC2 compared to PC1 (Fig. 3.4). Individuals in Eisi and Sand were characterised by smaller size at one year old, but tend to delay their maturation, resulting in larger size at maturation. In contrast, individuals in Host and Trof have larger size at one year old, but tend to mature earlier.

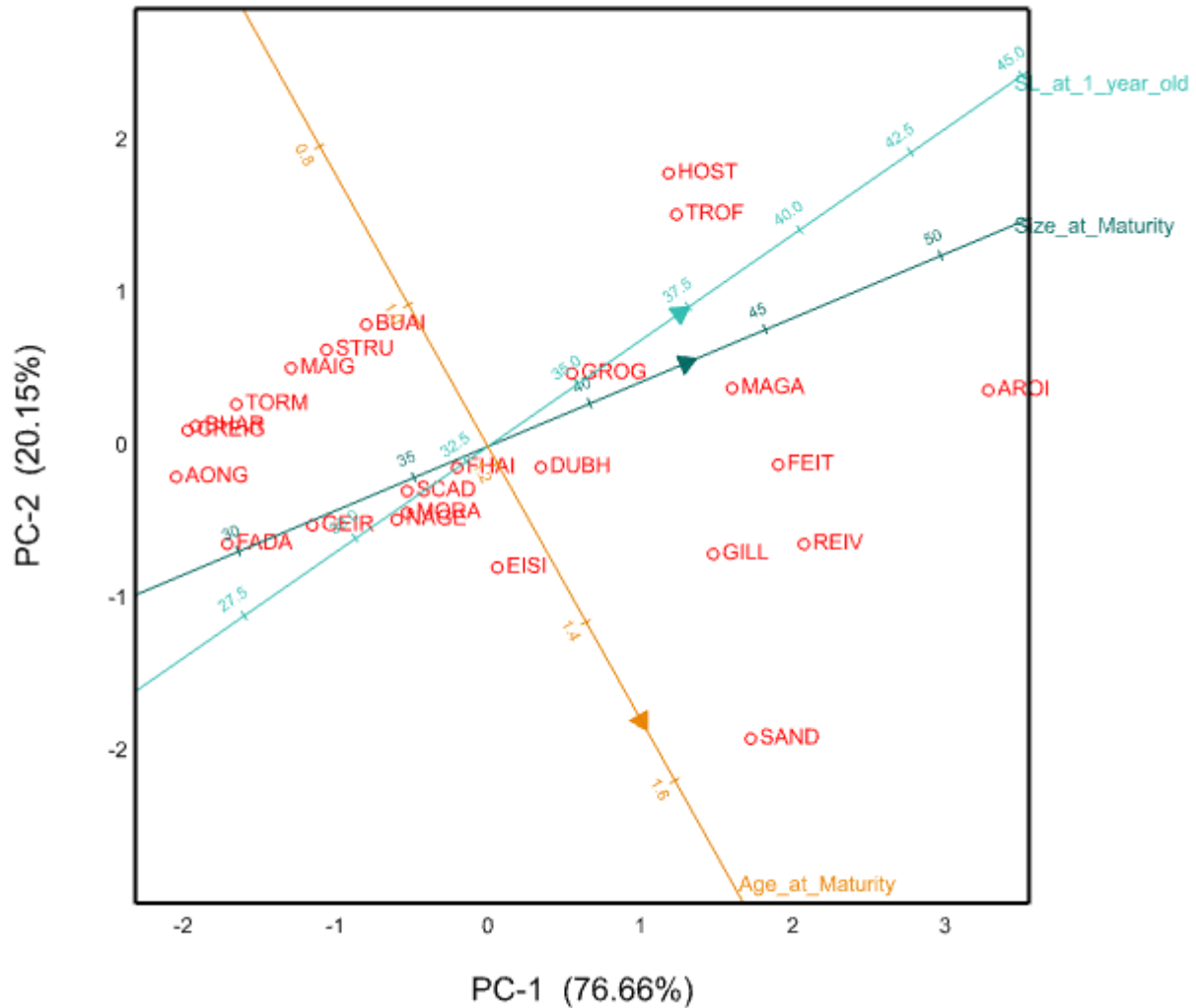


Figure 3.4. Biplot showing the position of 24 populations of North Uist's stickleback in the main PC axes of life history traits.

3.4.3 Ecological constraints in size at one year old, age and size at maturation and life expectancy

Among the ecological factors, the relative abundance of the competitor species, *P. pungitius*, was the most important factor affecting life history variation (Table 3.4) suggesting that the presence of a competitor selects for larger size at one year old and results in larger size at maturation (Fig. 3.5a). PC1 water chemistry was also significantly positively correlated with PC1 life history indicating that better resources also favour larger size at one year old, and larger and later maturation (Fig. 3.5b). The intensity of predator and the density of

stickleback did not have a significant relationship with PC1 life history (Table 4) (Fig. 3.5c and d), and none of the ecological factors correlated with PC2 life history.

Table 3.4. Results of GLMs to estimate the relationship between ecological factors with life history traits on 24 populations North Uist freshwater stickleback. The model fitted by using identity link function with normal errors.

Response	Fitted model	df	Wald F	p	<i>r</i>
PC1 life history	Competition	1,19	28.82	<0.001	0.874
	PC1 water chemistry	1,19	7.61	0.013	0.548
	Predation	1,18	0.11	0.740	-0.278
	Density	1,17	0.01	0.933	0.058
Proportions of older	PC1 water chemistry	1,20	9.35	0.006	0.659
	Predation	1,19	0.49	0.495	-0.087
	Density	1,18	0.16	0.692	0.292
	Competition	1,17	0.09	0.765	0.257

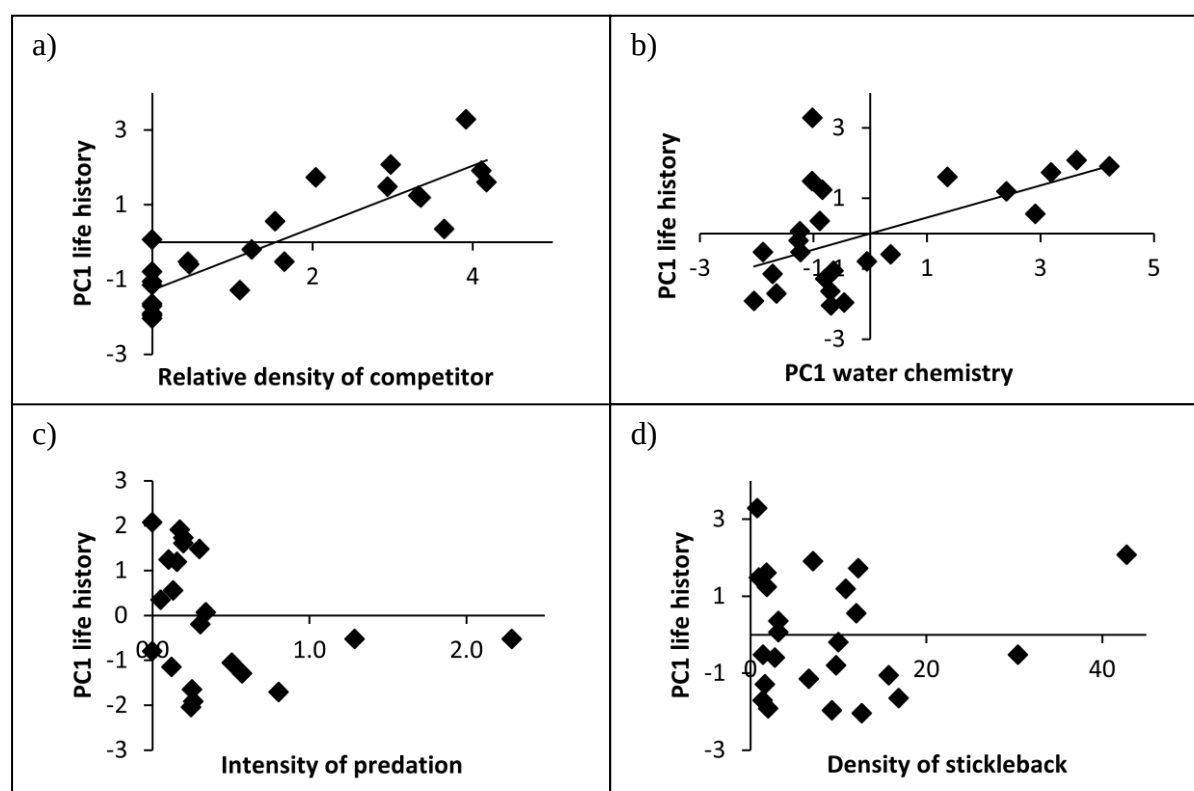


Figure 3.5. Relationship of ecological factors with life history traits among 24 populations freshwater stickleback in North Uist.

The proportion of older fish in populations was significantly lower where resources were less available (Table 3.4, Fig. 3.6), but unrelated to other factors. By conducting analysis within populations with representative sample size (Host is excluded due to really low sample size, making the estimate of variance unreliable (Table 1)), it is clear that richer-resources populations have a wider range in size (Wald $F_{1,21}=6.13$, $P=0.022$) and age (Wald $F_{1,21}=5.77$, $P=0.026$) at maturation (Fig. 3.7a and b).

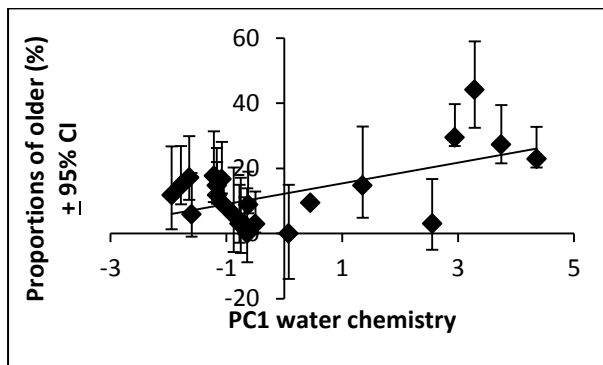


Figure 3.6. Relationship of PC1 water chemistry with proportions of older fish ($\pm 95\%$ CI) among 24 populations of freshwater three-spined stickleback in North Uist.

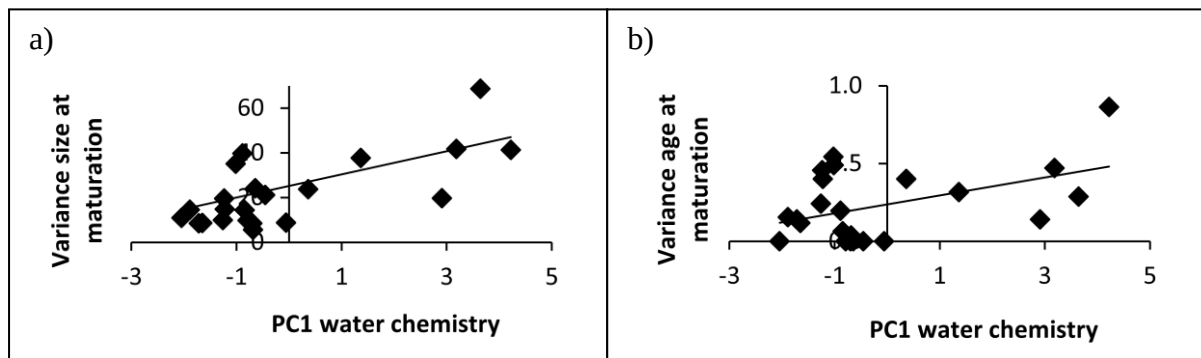


Figure 3.7. Relationship of PC1 water chemistry with variance in size (a) and age (b) at maturation among 24 populations of freshwater stickleback from the North Uist.

3.5 Discussion

My results suggest that the evolution of body size and maturation time among North Uist stickleback is mainly driven by the combination of interspecific competition and resource availability. Both of these factors are strongly associated with larger body size, and this probably acts as the primary factor contributing to larger size at maturation. The effect of competition in my results is consistent with the findings of Arendt (1997), but not those of Mittelbach (1988). Arendt (1997) highlighted that competition favours rapid growth and thus larger body size, because body size is positively correlated with competitive ability and it can be advantageous in escape from competition by shifting or expanding the niche.

A lab based experiment by Hart (2003) found that stickleback and *Pungitius* clearly overlapped in their dietary preferences, but differed in habitat use and foraging behaviour. Thus, larger, fast-growing stickleback in this study may be selected for enlarging and/or changing hunting area when in competition with *Pungitius*, and when supported by better resources. The positive effect of resources on density of *Pungitius* also fits with the prediction that density of organisms correlates positively with resource availability (Skogland, 1985). This suggests that the effect of competition on resource limitation as stated by Mittelbach (1988) may not apply when resources are abundant, although this may still be important in resource-poor conditions. The strong positive impact of resources and interspecific competition on body size of North Uist stickleback may have meant that the negative effect of predation and density became unclear.

My results contradict the second prediction of a negative relationship between body size and age at maturity. Instead of maturing earlier, individuals in populations with larger size at one year old matured later than their counterparts in populations with smaller body size. This result is compatible with Baltz & Moyle (1982; Fox & Keast, 1991a) and those acknowledged by Stearns and Koella (1986), but is not consistent with established models (Stearns & Koella, 1986; Reznick, 1990; Roff, 1992; Heino *et al.*, 2002).

Higher juvenile mortality rate seems the most likely reason for the delay in maturation in resource-rich populations. Odonata larvae, which are known to prey upon juvenile stickleback are common in the littoral invertebrate communities of resource-rich lochs, especially those where larger salmonid predators are absent (e.g. Reiv and Maga) but rare or absent in those of resource-poor lochs (e.g. Bhar, Fada and Torm) (Chapter 4). Stickleback in resource-rich populations also produce a larger number of eggs than those in resource-poor

populations (Chapter 4) consistent with a presumably higher juvenile mortality rate in these populations.

On the other hand, early maturation in populations with slower growth is probably due to limitation of resources. Consistent with Fox's (1994) results for pumpkinseed sunfish (*Lepomis gibbosus*) I also found a strong relationship of resources with life expectancy as judged by the proportion of older fish in populations. Populations in resource-poor environments thus have a lower (adult) survival rate. Therefore earlier maturation is likely to increase fitness in these populations, even though it occurs at smaller size. It emphasizes Fox's (1994) statement that mortality rate, not growth, is more important in shaping the maturation time. Other factors identified by previous studies, such as temperature (Jonsson *et al.*, 2012), chemicals and pollution (Kraak 2007) and latitude (Trip *et al.*, 2014; de Lira *et al.*, 2015) do not seem important in my study. MacColl *et al.* (2013) found that temperature does not vary strongly among North Uist lochs, nor is there obvious pollution in these lakes, which are all at essentially the same latitude.

My findings match my third prediction, that richer resources correlate positively with a wider range of size at maturation, while resource-poor populations show a contrasting pattern. As in Reznick's (1990; Day & Rowe, 2002) model, I also found that in resource-rich populations body size at the attainment of maturity is more variable, perhaps suggesting a clear maturation threshold in poor resource conditions. In support, lower size variation at maturation in resource-poor populations indicate that stickleback in these populations will mature as soon as their minimum size of maturation is attained. My results also showed that range of age at maturation was more variable in rich-resources populations. This result was exactly compatible with Wilbur & Collins (1973; Reznick, 1990).

In addition to the results above, I found no relationship of trout catch rate with life expectancy of North Uist stickleback as judged from the proportions of older fish. Since theory predicts that predation should have a negative impact on life expectancy, this result is perhaps unusual. There are two potential explanations for this (see also MacColl & Aucott, 2014). First, and most likely, is that life expectancy is shaped mostly by resources, not by predation. This assumption was supported by a clear relationship between higher survival rates and increasing resources. Increasing resources also tend to correlate positively with the density of stickleback, suggesting that resources may have acted as a limiting factor for population size. Arendt & Reznick (2005) found a similar pattern in Trinidadian guppy

populations. By comparing the effect of predator regime and resource level on juvenile growth rate, they found that growth rate was primarily determined by resource level.

Secondly, the number and size of predators may be relatively small compared to the number and size of stickleback and hence predation risk may be low. However, this seems unlikely, since a comparison of lochs with and without trout (but otherwise ecologically matched) showed that age at maturation and life expectancy (measured by the proportion of older stickleback) were lower in the presence of trout (Chapter 6).

As a conclusion, my results show that the presence of the competitor, *P. pungitius*, and better resources were associated with larger body size and a 'slower' life history of stickleback on North Uist. The negative effect of population density and predation on body size, age and size at maturation may be undetected due to a strong effect of interspecific competition and resources. Populations with larger body size tend to mature later and at older age. Populations living in rich resources were also more varied in their age and size at maturation. Finally, even though there is obvious evidence that growth rate, maturation and life expectancy are induced by competition and resource factors, I consider that part of these results are linked to genetic factors. Further research which examines the effect of genetics component on growth and maturation of these populations is required (Chapter 5).

Chapter 4. Ecological correlates of reproductive strategies of freshwater three-spined stickleback

4.1 Abstract

Although maternal investment theories have been established for several decades, contrasting empirical patterns continue to be described. Here, I tested some of those maternal investment theories using a large and long-term data set of wild populations of three-spined stickleback (*Gasterosteus aculeatus*) (hereafter stickleback) from the Scottish island of North Uist. As hypotheses, I predicted: i) Poor resource conditions should correlate with a lower maternal investment; ii) Low-quality environments should select for a larger investment per offspring; iii) There should be a trade-off between number and size of eggs; and iv) Maternal size should correlate negatively with egg size. My results showed that the first hypotheses was rejected, the second one was partially supported, while the third and the fourth were fully accepted. The degree of maternal investment in these populations was not correlated with the resources availability, but was negatively related to the relative density of their main competitor, *Pungitius pungitius*. The offspring (egg) size was positively correlated with the density of a potential predator, the brown trout *Salmo trutta*, but unrelated with resource conditions. I also found that there was no significant effect of maternal size or quality of the environment on the eggs size-number trade-off.

Keywords: ecological constraints, maternal investment, egg size, eggs number, three-spined stickleback

4.2 Introduction

Variation in maternal investment is central to our understanding of life history evolution, but although very widely studied, its relation to complex environmental variation remains surprisingly poorly understood. Maternal investment theories depend on the basic concept of optimal use of available resources to maximise the fitness of mothers and their reproductive output (Räsänen *et al.*, 2008). This concept has been widely expected to involve a trade-off between fitness of the mother and her offspring, and between the offspring themselves. Investment in offspring will cost the mother if there is a trade-off between the mother's growth or maintenance, and her reproductive effort (Stearns, 1992), while increasing investment per offspring should reduce the fitness of siblings (Smith & Fretwell, 1974). As the priority in this trade-off is maternal fitness, not the offspring's (Marshall & Uller, 2007), maternal investment theories (Morris, 1987; Erikstad *et al.*, 1998) predict that mothers will reduce their investment in low quality environments, for example where there are poor resources and/or high competition.

Maternal investment theories also predict that low quality environments should favour increased investment per offspring, including larger offspring size (Parker & Begon, 1986; McGinley *et al.*, 1987), even at a cost of lower brood size. Larger progeny are thought to achieve higher fitness (Smith & Fretwell, 1974), including a higher probability of survival (Hendry *et al.*, 2001; Rollinson & Hutchings, 2013b). This increased fitness of larger progeny could be due to their larger initial size (Stearns, 1992), faster growth (Einum & Fleming, 1999; Murphy *et al.*, 2014) and/or a lower risk of predation (Biro *et al.*, 2006; Bell *et al.*, 2011) than smaller siblings. However, a larger investment per offspring often results in lower fecundity because of the trade-off between size and number of offspring in a fixed investment (Smith & Fretwell, 1974).

Although most empirical studies fit with maternal investment theories (Sih & Moore, 1993; Räsänen *et al.*, 2008; Leips *et al.*, 2009; Schrader & Travis, 2012; Rollinson & Hutchings, 2013a; McBride *et al.*, 2015), contrasting results have also been prominent for many decades (e.g. Fox & Keast, 1991; Litvak & Leggett, 1992; Vøllestad *et al.*, 2004; Murphy *et al.*, 2014). A study by Fox & Keast (1991) on Canadian pumpkinseed (*Lepomis gibbosus*) fish found that winter-kill populations, those with high over-winter mortality of offspring, have higher gonadal investment than non-winter-kill. They argued that higher investment in winter-kill populations is because the mothers tend to maximise offspring fitness to avoid the early risk of mortality. In another study Morrongiello *et al.*, (2012) found that Australian perch

(*Nannoperca australis*) produce a larger number of smaller eggs as environmental quality decreased. They claimed that rather than increasing investment per offspring, diversification of offspring size is more valuable in harsh environments.

Despite these unpredictable results, maternal investment studies have also been widely criticised because little attention has been paid to the variation among individuals within population (e.g. Hendry *et al.*, 2001; Rollinson & Rowe, 2015). Hendry *et al.*, (2001) argued that maternal investment studies should consider the effect of maternal size, as it generally influences reproductive output i.e. gonad size, fecundity and offspring size, and may also influence the quality of parental care. Larger mothers are generally expected to have larger gonad size and greater fecundity (Stearns, 1992; Hendry *et al.*, 2001), but expectations about egg size are less clear (Hendry *et al.*, 2001). Hendry *et al.*, (2001) predicted a negative correlation of maternal size with egg size if the fitness of offspring is negatively related with offspring density, but a positive correlation if maternal size is positively correlated with quality of parental care of the offspring. This pattern of relationships could be a part of the explanation for why some studies have found contradictions with Smith & Fretwell's (1974) model of the trade-off between number of offspring and offspring size.

In this study, I revisited those maternal investment theories using a large and long-term data set of 18 wild populations of freshwater stickleback (*Gasterosteus aculeatus*) (hereafter stickleback) from the Scottish island of North Uist. Lochs on North Uist are characterised by poor resources, few competitors and a high density of small predators (brown trout, *Salmo trutta*) on the east side, but rich resources, more competitors and a low density of larger brown trout on the west side (MacColl *et al.*, 2013; MacColl & Aucott, 2014; Chapter 3). As hypotheses, I predicted that: i). Stickleback in resource-rich populations should have larger reproductive investment; ii). Stickleback in low quality lochs should have larger eggs. As low quality lochs on North Uist are characterised by poor resources and a higher density of potential predators, I expected that this two factors will correlate positively with larger egg size; iii). Egg size should increase with decreasing number of eggs; and iv). Maternal size should correlate negatively with egg size of stickleback. A negative correlation of maternal size with their egg size (Hendry's *et al.*, 2001) was expected because stickleback have paternal care (Wootton, 1984), and therefore there is unlikely to be an effect of maternal size on the habitat quality of their offspring.

4.3 Methods

4.3.1 The populations and ecological variables

This study involved 18 populations (lochs) of North Uist freshwater stickleback (Table 4.1). These populations were chosen due to the variety in their ecology i.e. resource availability, predation intensity, the density of their main competitor and the density of stickleback themselves, and in their life history traits i.e. size at one year old, maturation time and life expectancy (Chapter 3).

Most of the ecological variables of the lochs that were used in this chapter i.e. resource index, the density of stickleback as well as the density of their main competitor and main predator were from chapter three of this thesis. Indices of resources were quantified as the first principal component axis (PC1) of water chemistry: pH, conductivity, dissolved organic carbon (DOC), and the concentration of sodium (Na^+) and calcium (Ca^{+2}) ions of the lochs. The density of stickleback was counted as the number of stickleback caught per trap per night (“MEAN CPUE”), while interspecific competition was estimated from the percentage of their main competitor, the nine spined stickleback *Pungitius pungitius* (hereafter *Pungitius*) in 100 haphazardly selected fish. Indices of predation were estimated from the number of brown trout, *Salmo trutta* caught by anglers in angling competitions (“trout catch rate”). Details of lochs are in Chapter one, while detailed procedures for environmental measurements and the ecological factors of these populations are in chapter three and in MacColl *et al.*, (2013; MacColl & Aucott, 2014).

In addition to those data, I sampled the abundance and richness of aquatic invertebrates for 11 lochs and measured the protein content of the stickleback’s stomachs in eight populations (Table 4.1). Collection of these invertebrate communities is expected to give a better estimate of the resource availability for each loch, albeit at a single time-point, while analysis of the stomach contents aimed to clarify the relationship of resources availability in the loch with the nutritional intake of the stickleback.

4.3.2 Invertebrate community sampling

‘Three minute sampling’ of invertebrates communities was conducted following Biggs *et al.*, (1998). Briefly, each loch was surveyed to explore and define the different types of potential habitat (mesohabitats) for invertebrate communities. The common mesohabitats in this study were littoral areas with aquatic plants and shallow benthos without plants (less than 0.5m in depth). After defining the mesohabitats, a total of two minutes was spent collecting

invertebrates in all of the mesohabitats. Collecting was conducted by combining kick sampling in the benthos and/or net sampling around aquatic plants. The one minute remaining was spent searching for invertebrates that were attached to rock, logs or other big materials in the environment.

Collected samples were then sorted roughly to eliminate non-invertebrate material e.g. plants, rock, sand or others. Sorted samples were preserved in 10% buffered formalin and brought to the laboratory for further analysis. In the laboratory, samples were re-sorted thoroughly leaving only invertebrates or presumed invertebrates as clean samples. These clean samples were preserved in ethanol 70% and subsequently examined under a binocular microscope to allow taxonomic identification to at least family level following Dobson *et al.*, (2012). These data were used to estimate richness and abundance at the family level. For those lochs with very high invertebrate abundance i.e. Host and Reiv, sub-sampling was used.

The abundance of invertebrates was split into two categories based on their body sizes: small and big invertebrates. The small category included small leeches (*Glossiphoniidae*), water spider (*Arrenuridae*) and all insect larvae or pupae with the exception of Odonata and Coleoptera larvae, while the big category included all other invertebrates not in the small category (e.g. Crustaceans, Molluscs, Annelids, and big bugs i.e. Corixidae and Notonectidae). This splitting was carried out because it was thought likely that the body size of the invertebrates would affect their availability to stickleback. Invertebrate richness was calculated as the total number of families identified.

4.3.3 Stomach content analysis

Fifteen stickleback were collected haphazardly from each population (procedures for stickleback sampling are in Chapter two). After measuring their body size i.e. length and weight (in some populations), their full digestive tract was extracted into a labelled 1.5ml tube and stored at -20°C. In the laboratory, the content of their digestive tract was weighed (wet mass) and then dried overnight using a vacuum freeze drier to obtain a dry weight.

All of the dried stomach contents (hereafter stomach content (mg)) were then diluted with 200µl lysis buffer (1% Triton 114 + 1% protease inhibitor in 1% PBS) and were then homogenised using a hand homogeniser. After homogenising, the samples were left on ice for 30 minutes to allow reaction with the lysis buffer. Centrifugation (10,000 rpm) at 4°C for 10 minutes produced a supernatant which was transferred to a new tube. Dilutions with PBS to following concentration of the original supernatant were then made for absorbance reading: 1:5, 1:10, 1:50 and 1:100. These dilutions were processed according to the Bio-Rad

protocol (http://www.bio-rad.com/LifeScience/pdf/Bulletin_9004.pdf) and the absorbance was read on a Bio-Rad 680 machine. Each run included five different standard solutions (S1 to S5). Calculation of total protein content (mg) in samples was estimated by comparison to standard solutions. Total protein was then standardised by dividing by the weight of stomach content. Fish that were found with empty stomachs were still included in the analysis, for calculation of population mean values.

4.3.4 The fecundity data

Sampling for this fecundity study was conducted over 9 years (2007-2015) and aimed to get at least 10 fully gravid stickleback from each of population (Table 1) (details of the procedures for stickleback sampling are in Chapter two). Samples were limited to fully gravid females at the well-developed ripening and/or ripe stage (MR and RE) following Baker *et al.*, (1998), to reduce the bias in the calculation of gonadosomatic index and eggs size from unripe eggs. I took length (mm) and weight (g) data for each collected female.

Fecundity was measured in four ways: ovary weight (gr), gonadosomatic index (GSI), the number of eggs and mean of egg diameter (mm). GSI was calculated as the ratio of gonad weight to the remaining fish weight i.e. $100 \times \text{ovary weight} / (\text{fish weight} - \text{ovary weight})$. Mature eggs were counted manually, and 5 to 10 eggs were haphazardly selected for measurement of diameter. Egg diameter was measured with a graticule under a 4 x 10 inverted microscope and measurements converted to millimetres (mm). Fecundity data were collected at an individual level, and then was used to calculate population means.

4.3.5 Data analysis

The diversity of invertebrate abundance was measured using the J Pileau index (Smith & Wilson, 1996), while the species richness was simplified as Whittaker index modified by Harrison (Magurran, 2004). Generalised linear models (GLMs) with normal errors and identity link function was used to analyse variation in measures of fecundity and stomach contents within and among populations, the relationships between fecundity and ecological variables: PC1 water chemistry, invertebrate communities and stomach content data, and between different measures of fecundity and fish size. All statistical analyses were conducted in Genstat 15th edition (VSN International, 2011).

4.4 Results

I analysed 218 gravid stickleback from 18 different populations for fecundity parameters and 119 stickleback from 8 populations for the stomach content assay (Table 4.1). In addition to this, 11 lochs were surveyed for the abundance and the richness of their invertebrate communities.

Table 4.1. Samples of fecundity data, indices of invertebrate communities of the lochs and the stomach content among populations of freshwater stickleback in North Uist

	Loch	N*	Aquatic invertebrates			Stomach content analysis			
			Abundance		Family richness	Empty guts (%)	Dry weight (mg)	Protein total (mg)	Protein/mg sample (mg)
			Big	Small					
1	Dubh	6	39	9	7	N/A	N/A	N/A	N/A
2	Iala	14	65	6	13	N/A	N/A	N/A	N/A
3	Mora	5	1	58	4	N/A	N/A	N/A	N/A
4	Bhar	11	26	13	7	27	9.20±3.31	2.49±0.65	0.44±0.10
5	Chru	11	262	9	14	20	35.42±11.04	4.96±1.24	0.17±0.03
6	Daim	13	14	20	6	27	9.82±1.95	1.90±0.26	0.18±0.04
7	Host	25	118	1021	9	27	22.50±3.93	3.99±0.89	0.21±0.03
8	Maga	16	192	40	15	20	38.17±7.40	5.99±1.06	0.21±0.04
9	Reiv	14	539	164	12	7	45.57±12.76	6.16±1.45	0.19±0.03
10	Stru	8	150	58	12	60	6.50±1.17	1.67±0.21	0.40±0.07
11	Torm	22	9	15	8	47	5.25±0.78	0.99±0.22	0.27±0.05

*N=number of gravid stickleback that were used for fecundity analysis. Others lochs that were included for fecundity analysis, with number of samples for each loch in bracket, are Aroi (2), Buai (17), Eisi (3), Fhai (12), Gill (9), Maig (12) and Scad (18). Details of the lochs are in chapter one. Stomachs content were the average of individuals (±S.E) in each population. N/A=not available.

There was substantial variation among populations in the number of gravid females collected (mean=12.1± S.E=1.45) (Table 4.1). These small sample sizes were mainly due to the low density of stickleback in some populations, compounded by short breeding seasons. Almost a

third (29%) of the stickleback for the stomach contents assay was found with empty stomachs. The highest percentage of these empty guts was from Stru (60%) followed by Torm (47%) (Table 4.1).

4.4.1 Invertebrate communities, stickleback' stomach contents and their relationship with other environmental factors of the North Uist lochs

Lochs on North Uist differed significantly in the abundances of both small (J Pielou index=0.33) and big invertebrates (J Pielou index=0.34), but were similar in their taxonomic richness (β_{H1} =16%) (Table 1). Maga had the highest richness of invertebrates with 15 out of the 26 (58%) invertebrate families present on North Uist found in this loch, while Mora had the lowest with only 4 out of 26 (15%). Host had the highest invertebrate abundance, containing more than 40% of the total invertebrates counted, while Torm was the poorest with less than 1% of the total (Table 4.1). Corixidae, Limnephilidae and Physidae were the most common families in North Uist lochs, present in 10 out of 11 lochs sampled.

A similar result was found in the stomach content analysis. Different populations of stickleback varied significantly in their stomach contents (Wald $F_{7,111}=4.69$, $P<0.001$). Populations also differed significantly in their total protein content (Wald $F_{7,111}=5.69$, $P<0.001$), but not in their protein per milligramme sample (Wald $F_{7,111}=1.28$, $P=0.27$) (Table 4.1).

Lochs with better resources as judged by higher scores on the water chemistry first principal component had higher abundance of both big and small invertebrates (Wald $F_{1,9}=8.43$, $P=0.017$ for big category; Wald $F_{1,9}=5.49$, $P=0.044$ for small; Fig. 4.1), but there was no relationship between PC1 water chemistry and invertebrate taxonomic richness (Wald $F_{1,9}=0.92$, $P=0.36$). Larger mean stomach contents of stickleback were associated with greater abundance of big invertebrates in lochs (Wald $F_{1,6}=15.83$, $P=0.03$; Fig. 4.2), but not with the density of (three-spined) stickleback themselves (Wald $F_{1,5}=4.03$, $P=0.12$), the relative density of *Pungitius* (Wald $F_{1,4}=3.79$, $P=0.15$) or density of trout (Wald $F_{1,3}=5.31$, $P=0.15$).

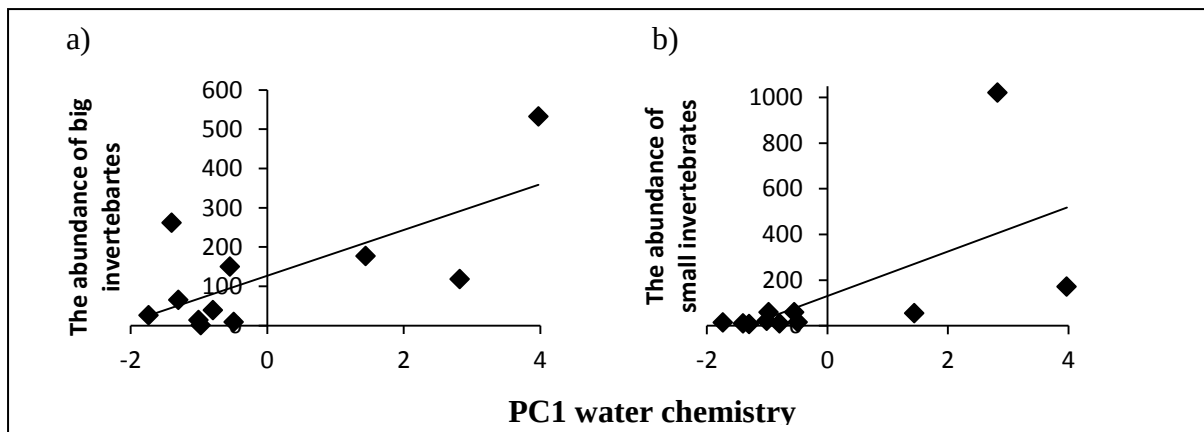


Figure 4.1. The relationship of PC1 water chemistry with the abundance of invertebrates among 11 lochs in North Uist.

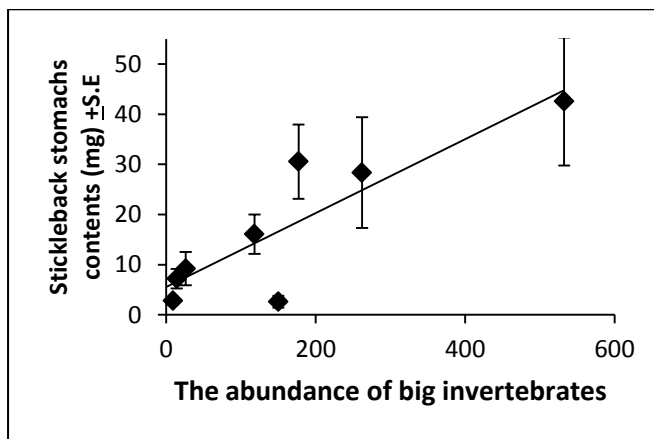


Figure 4.2. The relationship between the abundance of big invertebrates in lochs and the stomachs contents ($\pm$ S.E) of the stickleback among eight populations of freshwater stickleback in North Uist.

4.4.2 Fecundity between and within populations

Populations differed significantly in ovary weight, gonadosomatic index (GSI), number of eggs and mean egg diameter (Table 4.2). Controlling for population, larger females had larger ovaries and larger numbers of eggs, but with smaller mean egg diameters. Across populations, when the weight of females was controlled, mean egg diameter was negatively associated with number of eggs (Table 4.2). The pattern of results was very similar if length was substituted for weight in these analyses.

Table 4.2. Results of GLMs modelling the relationships between fecundity parameters, and population and body size in 18 populations of North Uist freshwater stickleback. The models were fitted by using identity link function with normal errors.

Dependent variable	Model	df	Wald F	p	r
Ovary weight	Population	17,199	3.64	<0.001	NA
	Female weight	1,199	1381.70	<0.001	0.941
	Population x female weight	17,182	1.36	0.163	NA
GSI	Population	17,200	6.28	<0.001	NA
Number of eggs	Population	17,199	5.71	<0.001	N/A
	Female weight	1,199	815.05	<0.001	0.900
	Population x Female weight	17,182	1.99	0.014	N/A
Mean egg diameter	Population	17,198	10.74	<0.001	N/A
	Female weight	1,199	9.12	0.003	-0.068
	Number of eggs	1,199	10.78	0.001	-0.146
	Population x Number of eggs	17,181	0.00	1.000	N/A
	Female weight x egg number	1,197	1.96	0.163	N/A

*N/A=not available

4.4.3 Ecological constraints and life history trade-offs on fecundity

Among the ecological factors, the availability of resources as judged by PC1 water chemistry was the most important in shaping the ovary weight and number of eggs of North Uist stickleback (Table 4.3). Higher loading values on this PC were significantly correlated with larger ovaries and larger number of eggs (Fig. 4.3). On the other hand, higher trout catch rate was correlated with larger mean egg diameter (Table 4.3; Fig. 4.4).

Table 4.3. The results of GLMs examining the relationship of fecundity parameters with ecological factors across 18 populations of North Uist freshwater stickleback. The models were fitted using identity link function with normal errors.

Response	Fitted model	df	Wald F	p	r
Ovary weight	PC1 water chemistry	1,15	19.57	<0.001	0.342
	Competition	1,14	0.98	0.340	0.598
	Density	1,13	0.87	0.369	0.075
	Predation	1,12	0.35	0.566	-0.217
GSI	Predation	1,15	4.12	0.062	0.477
	Competition	1,14	3.31	0.092	-0.508
	PC1 water chemistry	1,13	1.16	0.303	-0.118
	Density	1,12	0.00	0.948	0.138
Number of eggs	PC1 water chemistry	1,15	16.84	0.001	0.739
	Predation	1,14	1.24	0.286	-0.405
	Competition	1,13	0.54	0.476	0.538
	Density	1,12	0.25	0.628	0.379
Mean egg diameter	Predation	1,15	5.03	0.042	0.514
	PC1 water chemistry	1,14	1.45	0.250	0.111
	Density	1,13	0.81	0.385	-0.084
	Competition	1,12	0.01	0.913	0.135

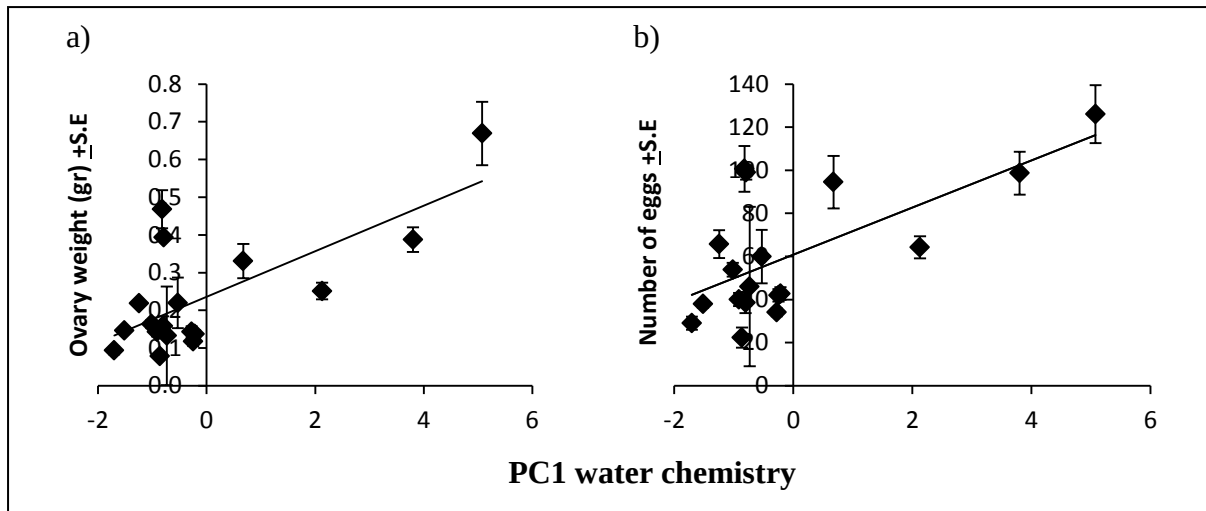


Figure 4.3. The relationship between PC1 water chemistry and mean ovary weight (a) and number of eggs (b) ($\pm$ S.E) among 18 populations of freshwater stickleback in North Uist.

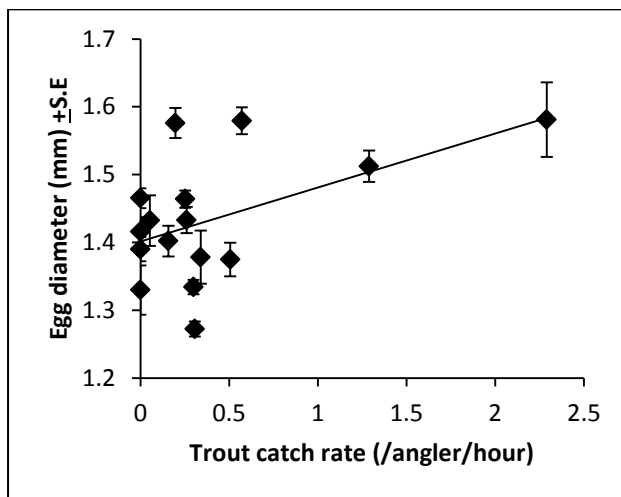


Figure 4.4. The relationship between predation, trout catch rate, and mean egg diameter ($\pm$ S.E) among 16 populations of freshwater stickleback in North Uist.

None of the single factors had a significant correlation with GSI. However, by constraining the analysis to populations with representative sample sizes, i.e. more than ten samples for each population, it appeared that a higher relative density of *Pungitius* was significantly associated with a lower GSI in North Uist stickleback (Wald $F_{1,10}=5.43$, $P=0.045$). There is also clearly a strong negative correlation of GSI with the relative density of *Pungitius* in North Uist when only those lochs with *Pungitius* are considered (Wald $F_{1,9}=32.59$, $P<0.001$).

The combination of trout catch rate and the relative density of *Pungitius*, when included together as main effects, also predicted the GSI of North Uist stickleback (Wald $F_{2,13}=4.08$, $P=0.042$). Higher trout catch rate tended to correlate with higher investment, while the relative density of *Pungitius* had the opposite effect (Figure 4.5). Nevertheless, there was a large effect of the Mora population on this relationship. Excluding Mora from the analysis rendered these two factors insignificant (Wald $F_{2,12}=1.62$, $P=0.24$).

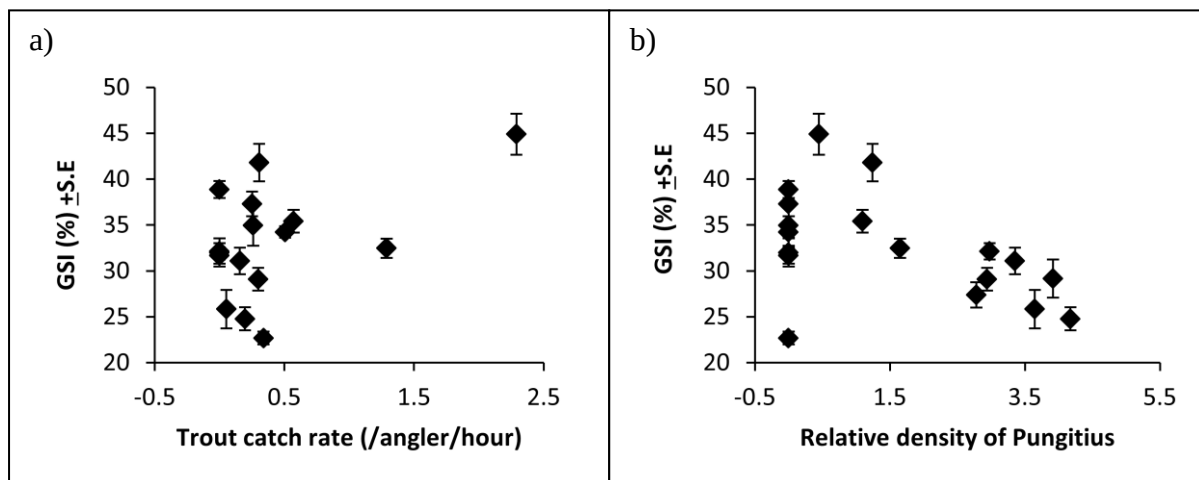


Figure 4.5. Relationship between predation, trout catch rate, (a) and the relative density of competitor, *Pungitius*, (b) with reproductive investment ($GSI \pm S.E.$) among populations of freshwater stickleback in North Uist.

A multivariate analysis of the relationship between ecological factors and all fecundity traits showed that PC1 water chemistry is the most prominent in determining the reproductive strategies of North Uist stickleback, while the density of predation is likely to be a second one (Table 4.4).

Table 4.4. The results of Manova analysis on the fecundity factors across 18 populations of North Uist freshwater stickleback. The models were fitted using identity link function with normal errors.

Ecological factors	WLambda	RaoF	p
PC1 water chemistry	0.2622	5.63	0.019
Predation	0.4094	2.89	0.094
Competition	0.6227	1.21	0.377
Density	0.9057	0.21	0.927

4.5 Discussion

I have demonstrated substantial variation in maternal investment, both within and between populations on North Uist, and this variation is meaningfully associated with individual variation in fish, and with ecological variation between populations. The first hypothesis of a positive correlation between resources and maternal investment was rejected in this study, while the second one of a positive association of low-quality environments with egg size was partially supported. The other two hypotheses i.e. the trade-off of egg size and egg number, and a negative correlation of maternal size with egg size were fully accepted.

4.5.1. Ecological constraints on the fecundity of North Uist stickleback

My results reject the first hypothesis that predicted a positive relationship of resources with reproductive investment. Contrary to the prediction of a positive correlation of gonadosomatic index (GSI) with resources (Morris, 1987; Erikstad *et al.*, 1998), my data showed that GSI of North Uist stickleback was independent of resource variation across lochs. The GSI of North Uist stickleback were mostly driven by the effect of inter-specific competition, the relative density of *Pungitius*. A higher relative density of this main competitor was associated with lower GSI in North Uist stickleback. Indeed, across populations on North Uist, higher relative density of *Pungitius* was strongly correlated with rich-resources (Chapter 3), but eliminating *Pungitius* from the model still did not result in a significant correlation of resources with GSI.

A negative relationship between interspecific-competition and GSI in North Uist stickleback is probably explained by established theory on the trade-off between growth and reproduction. It is widely believed (Reznick, 1983; Jennings & Philipp, 1992; Stearns, 1992) that an increase in reproductive effort will bring a somatic cost. Since higher interspecific competition was highly correlated with faster growth rate in North Uist stickleback i.e. larger size at one year old (Chapter 3), it can be assumed they reduced their reproductive investment in order to grow faster. Indeed, part of this larger body size was correlated with better resources, but the effect of higher inter-specific competition was clearly stronger (Chapter 3).

A second hypothesis of a positive relationship between low-quality environment and egg size (Parker & Begon, 1986; McGinley *et al.*, 1987) was partially accepted in this study. My result showed that egg size of North Uist stickleback was positively correlated with the density of potential predators, brown trout, but unrelated to resource availability. As poor resources in North Uist lochs tend to be accompanied by higher density of trout (Chapter 3),

it could be that the absence of an effect of poor resources was confounded by the effect of trout. However, again, eliminating the trout catch rate from the model did not result in a significant effect of resources upon egg size of the stickleback.

As the positive correlation of poor resources with larger eggs has been widely reported (e.g. Einum & Fleming, 1999; Räsänen *et al.*, 2008; Oravec & Reimchen, 2013; but see Morrongiello *et al.*, 2012), and the growth rate and maturation time of these populations was more interrelated with the degree of resources rather than predation density (Chapter 3), my result is perhaps unusual. A possible explanation for this unexpected result is the effect of resources might be undetected due to a strong effect of predation on the offspring of the stickleback. Diversification of the offspring in low-quality environments as argued by Morrongiello *et al.*, (2012) may not apply in this study since the egg size of stickleback was significantly larger with increasing trout density.

The positive correlation of egg size and predation density in this study suggested that the effect of trout on North Uist stickleback was mostly on younger age classes. Established theories (Roff, 1992; Stearns, 1992) predict the relationship of egg size and predation will be positive when predators favour juveniles or predators are gape-size limited. As the proportion of older stickleback in these populations was unrelated to trout density (Chapter 3), it can be assumed that the impact of predation on adult age classes is less. A study by MacColl *et al.*, (2013) on these populations also suggested that the presence of small trout in poor resources lochs is more likely to act as competition than predation for adult stickleback.

4.5.2. Egg number-egg size trade-off

My result was consistent with the third hypothesis that predicted a negative correlation between number of eggs and egg size. Consistent with the Smith & Fretwell (1974) model, I found there was a trade-off between number of eggs and mean egg diameter for a fixed investment in North Uist stickleback. However, I did not find any correlation of this trade-off with maternal size or with the quality of environment as found by Räsänen *et al.*, (2008; Ford & Seigel, 2015). A study by Ford & Seigel, (2015) on viviparous garter snakes (*Thamnophis proximus*) found the egg number-egg size trade-off was more prominent in smaller females, while the study by Räsänen *et al.*, (2008) on moor frogs (*Rana arvalis*) found this trade-off was stronger in acid (poor resource) conditions.

4.5.3. Maternal size versus egg size

The fourth hypothesis predicting a negative relationship of maternal size with egg size was also supported in this study. As expected from Hendry *et al.*'s, (2001) first general model, I found that egg size decreased with increasing maternal size. Hendry *et al.*'s (2001), first general model is more suitable for the stickleback than their second model that projected a positive relationship of maternal size and offspring size, because there is no obvious relationship between maternal size and offspring environment in this species. Female stickleback are not involved in parental care during incubation and/or post hatching periods (Wootton, 1974). The main reason for this negative correlation is because, in aquatic environments, larger egg correlates with higher incubation cost. Therefore, when there is a chance for larger reproductive output, mothers tend to optimize (not maximise) their egg size and gain from a higher fecundity (Hendry *et al.*, 2001).

Although the negative correlation of maternal size with egg size fits with the prediction, this result contradicted previous studies on similar species (Snyder & Dingle, 1989; Baker, 1994; Ali & Wootton, 1999; Wootton & Fletcher, 2009; Oravec & Reimchen, 2013). In contrast to my results, the last three of those studies found a negative relationship of maternal size with egg size, while Baker (1994; Snyder & Dingle, 1989) documented no correlation of these two traits. As the studies by Ali & Wootton, (1999; Wootton & Fletcher, 2009) were not of fully wild environments, but rather of lab-raised fish, they may not be strictly comparable. The study by Oravec & Reimchen, (2013) on wild Canadian stickleback is more relevant, however; i) their samples were preserved in formalin for several years; ii) they argued their result was likely confounded by the effect of population. Apart from the impact of preservation as mentioned by Heins & Baker, (1988), the confounding effect of ecological factors among population in Oravec & Reimchen's (2013) study, could significantly contribute. Meanwhile, Baker (1994) and Snyder & Dingle (1989) did not provide explanations for their neutral relationships.

My result contradicts the statement by Rollinson & Rowe, (2015) that maternal size per se is not a good predictor of the maternal-offspring size relationship. They argued that rather than maternal size, maternal condition or maternal nutritional should be more appropriate for analysing maternal-offspring size relationships. As my result showed a clear correlation between weight or length of stickleback with their egg size, it can be assumed that maternal size is appropriate for analysis. However, a different test which included maternal condition and/or maternal nutrition is required to confirm this assumption.

In summary, my results on the reproductive strategies of North Uist stickleback showed that:

- i) Maternal investment, as judged from gonadosomatic index (GSI), was independent of resource factors, but significantly reduced in a lochs with higher inter-specific competition;
- ii). Mean egg diameter also was uncorrelated with resource availability, but was positively related with increase in predation density; iii). Larger maternal size and higher fecundity were negatively correlated with mean egg diameter. Moreover, I found that there was no significant effect of maternal size and quality of the environment on the trade-off between egg size and egg number.

Chapter 5. Heritability of growth in North Uist freshwater three-spined stickleback

5.1 Abstract

I have previously shown that freshwater three-spined stickleback (*Gasterosteus aculeatus*) on North Uist display significant variation in life history traits among populations. There is clear evidence that this variation is related to environmental factors, but this could arise as a result of genetic, evolutionary change over many generations or plasticity within each generation. The simplest way to estimate the relative importance of evolved, genetic versus immediate, environmental effects on life history is to estimate heritability in a common garden setting. In this study, I conducted a full-sib half-sib common garden experiment to estimate the heritability of growth rate in six populations of North Uist freshwater three-spined stickleback. Contrasting with the general finding in other studies, my results demonstrated high heritability of growth in these populations (0.66 ± 0.16 S.E.). This high value was unlikely to be confounded by maternal effects, individual or common garden environment. The contrast in environmental quality among the lochs was probably a significant factor favouring the evolution of this high heritability. In addition to the high degree of heritability of growth rate, I also found that mortality rates in the lab during the experiment were positively correlated with fecundity in the wild across populations. Furthermore, sizes of fish in the common garden were positively correlated with sizes in the corresponding wild populations.

Keywords: heritability, growth rate, common garden, three-spined stickleback

5.2 Introduction

Although research on disentangling the roles of heritability and plasticity in determining phenotypic variation is well established (Mousseau & Roff, 1987; Scheiner, 1993; Via *et al.*, 1995; Crozier & Hutchings, 2014), further work on this topic is still required. The most important reason for this continuous work is to determine whether current phenotypes have an evolutionary (genetic) basis or are simply immediate responses to environmental variation. Estimating the extent of genetic contributions will be useful in mapping the pattern of population divergence through time, while the presence of phenotypic plasticity could be an indication of the recent instability of the environment. Moreover, disentangling these two factors is also required to predict the fitness of organisms in facing environmental fluctuation (Oomen & Hutchings, 2016).

As heritable, genetic change requires long time periods, fitness of organisms in fluctuating habitats mostly depends on the degree of their plasticity (Via *et al.*, 1995; Dieckmann & Heino, 2007). This may be the reason why empirical studies show that the majority of phenotypic variation is the result of plasticity, and not genetically determined (Mousseau & Roff, 1987; Crozier & Hutchings, 2014). A study by Mousseau & Roff, (1987) on 1120 published data found that simple (additive) genetic effects only contribute in the region of 26%-46% of the variation in phenotypes. Of the four phenotype classes analysed i.e. morphological traits, behaviour, physiology and life history, morphological traits had the highest heritability (46%), while life history characters had the lowest (26%) (Mousseau & Roff, 1987; but see Houle, 1992). A consistent pattern was revealed by Crozier & Hutchings, (2014) who found that almost 90% of the phenotypic variation in published studies of fish was linked to plasticity. This strong impact of plasticity has led to a biased assumption that phenotypic variation is likely to be substantially plasticity unless there is evidence that it has a genetic basis (Merilä & Hendry, 2014).

Despite the likely importance of plasticity, the estimation of heritability in studies of phenotypic variation is crucial and remains a main challenge in evolutionary studies (Merilä & Hendry, 2014). Merilä & Hendry, (2014) pointed out that low estimates of heritability might not be because of the absence of the effect per se, but also could be due to inappropriate estimation methods. Moreover, although still in the minority, some current studies (e.g. Ariyomo *et al.*, 2013; Dahlke *et al.*, 2016; Magalhaes *et al.*, 2016; Nilsson *et al.*, 2016; Oomen & Hutchings, 2016; and those acknowledged by Crozier & Hutchings, 2014; Merilä & Hendry, 2014) have shown significant correlations of phenotypic with genetic

variation. A study by Dahlke *et al.*, (2016) found that up to 50% of hatching success in Atlantic Cod (*Gadus morhua*) was associated with sire effects. Changes in growth and survival in this species, in response to fluctuations in temperature, are also determined more by genetic factors than plasticity (Oomen & Hutchings 2016). Analysis of skin pigmentation by Nilsson *et al.*, (2016) in Swedish arctic charr (*Salvelinus alpinus*) estimated up to 76% of heritability. Variation in boldness and aggressiveness of laboratory zebra fish (*Danio rerio*) have also shown a high degree of heritability, 76% and 36% respectively (Ariyomo *et al.*, 2013).

My previous studies (Chapter 3 and 4) of North Uist freshwater stickleback found that these populations differed significantly in their growth rate as judged by their size at one year old, maturation time, reproductive investment, and number and size of their eggs. Most of these traits are related to body size. Despite evidence that some of these life history variations were related to environmental factors, the heritability of these traits has not been analysed. Magalhaes's *et al.*, (2016) study on an overlapping set of populations suggested an equal contribution of genetic and plastic factors to variations in body size, body shape and body armour of these stickleback.

In this study, I calculated the heritability of a measure of body size, early growth rate, of six populations of North Uist freshwater stickleback using full-sib half-sib analysis of a common garden experiment. I hypothesized that the growth rate of these populations should be heritable. I also tested some life history predictions of the relationship of number and size of offspring with mortality rate in this fish. As larger size of offspring is highly correlated with higher fitness and there is a trade-off between number and size of offspring on a fixed investment (Smith & Fretwell, 1974; Stearns, 1992), I assumed the mortality rate of fry should increase with higher fecundity but correlate negatively with larger egg size.

5.3 Methods

5.3.1 Fish population and sampling

Six populations of North Uist freshwater stickleback (Reiv, Host, Chru, Stru, Bhar and Torm (Table 1.1, Chapter 1)) were chosen for this common garden experiment. These six populations were chosen because of their apparent diversity in yearling (1+) size, maturation time and number and size of eggs (Chapter 3 and 4).

Fish in breeding condition from each of the six populations were trapped from the wild (see Chapter 2) for use in crossing (see below). Breeding males were identified by their breeding colouration, while breeding females were selected on the basis of appearing gravid. The breeding status of females was confirmed by observation of their eggs under a low power microscope following Baker *et al.*, (1998) and selecting only those at the ripe stage for crossing.

5.3.2 Common garden experiment

Fish were reciprocally crossed in a North Carolina II design (Comstock & Robinson, 1948; Fox & Rauter, 2003) in blocks of two males and two females i.e. each female in blocks was fertilised by two males and each male fertilised two females. As I aimed to get 12 families of each population above, six pairs of parents (six males and six females) were selected for each population. Crossing fish and rearing of resultant offspring followed De Roij *et al.*, (2011). Briefly, fertilisation was conducted by mixing half the eggs stripped from a female with the minced testis of one male, in a dish containing embryo medium (1 ppt marine salt). Fertilisation was confirmed by observation of initial cleavage under a low power microscope. Fertilised eggs were transferred to the University of Nottingham and were randomised into separate tank compartments for each family until they hatched. Hatched fry were kept in their original tank and were fed with *Paramecium sp* for one week and then with newly hatched brineshrimp (*Artemia salina*) until 64 days old. From 65 days old fry were fed with *Chironomid* larvae (defrosted bloodworm) for the remainder of the experiment. The number of individuals in each family was standardised to 12 at 30 days old, and then to seven at 60 days old.

In order to estimate growth data, the largest fry of each family was photographed on the following ages (days): 10, 17, 24, 31, 38, 45, 59, 73, 87, 120, 150, 180 and 270 with a Panasonic Lumix L10 camera. Photography of the fry was conducted in a small glass dish (50 mm in diameter) with 1 mm graph paper as a scale. This dish was filled with the minimal

amount of water to cover the fry, in order to keep them as close to the graph paper at the bottom of the dish as possible. The size of fry were obtained by measuring their pictures in ImageJ (Schindelin *et al.*, 2012). Starting at 30 days old, after the number of fry was standardised to 12 per family, the number and date of dead fry were recorded to obtain the mortality rate. Estimating the mortality rates before standardisation would have been very difficult because of the small size of fry and the large number of them in some families.

5.3.3 Data analysis

Estimation of heritability in this study was conducted following Blanc (2003) i.e. heritability (h^2)= V_A/V_P , with $V_P=V_A+V_D+V_M+V_{Ec}+V_{Ew}$. V_A is the value of additive genetic, V_D represents the dominance value, V_M corresponds to the maternal effect, while V_{Ec} and V_{Ew} denote for the effect of common and individual environment respectively. Heritability estimation was conducted in general linear mixed models (GLMMs) with sire and dam, and combination of sire and dam as the random factors. Due to small sample sizes in each population, heritability was only estimated on a total sample of all populations. Therefore, the presented value here was the degree of heritability for North Uist stickleback as one population.

Following the calculation of heritability generalised linear model (GLMs) with normal errors and identity link were used to estimate the differentiations of growth within and among populations. In comparison of mortality rate between populations, data (numbers of dead fish, with initial family size as the denominator) were analysed by GLM with binomial error distribution and a logit link function. GLMs with normal errors and identity link were also used to estimate the relationship of population average mortality rate in the lab and life history traits in the wild i.e. fecundity, egg size (Chapter 4), and yearling size in the wild (Chapter 3), and between size at 270 days old in the lab and yearling size in the wild. Data on mortality rate and size at 270 days old were collected at the family level, and were then used to calculate population means. All statistical analyses were conducted in Genstat 15th edition (VSN International, 2011).

5.4 Results

Seventy two families of fertilised eggs were made from 18 blocks of parents. Only four families were made from Host because of the difficulty of finding wild fish in breeding condition. However, 4 families were added for each of Stru and Torm (8 total) as these populations had very low egg numbers but there was a surplus of fish in breeding condition. Of the 72 families, only 58 (81%) had surviving members at the end of experiment (270 days) and were included in the data analysis (Table 5.1). Eight of the original 72 families (11.11%) did not hatch, while six others (10%) hatched but in poor condition. The maximum age attained by fry in these families was only 17 days.

5.4.1 Heritability aspect, growth and mortality rates among North Uist stickleback

Additive genetic effects (estimated from the variance between sires) contributed 66 ± 16 % of the total phenotypic variation in growth among the six North Uist stickleback populations analysed. The estimate of the maternal effect was negative ($V_M = -1.96 \times 10^{-7}$) suggesting that its importance was of small magnitude compared to the additive ($V_A = 2.04 \times 10^{-6}$) and dominant ($V_D = 1.27 \times 10^{-6}$) genetic effects. The effect of individual environment ($V_{Ew} = -3.70 \times 10^{-8}$) also was smaller than the effect of common environment ($V_{Ec} = 2.62 \times 10^{-8}$). When age was controlled, there was significant differentiation in growth (Wald $F_{5,747} = 4.24$, $P < 0.001$; Fig. 5.1) of the stickleback between populations. Variation in size at a fixed age was also clearly higher between, than within populations (Wald $F_{5,23} = 4.70$, $P < 0.001$ and Wald $F_{52,179} = 3.45$, $P < 0.001$ respectively; Table 5.1). There was no significant differentiation of mortality rate among populations (Wald $F_{5,52} = 1.74$, $P = 0.12$; Table 5.1). The average of mortality rate from all families was 5.7% (21 out of 366). The earliest dead fish, after standardisation, was observed at 38 days old, and no more dead fish were recorded after an age of 207 days.

Table 5.1. Number of families, and indices of body size and mortality on North Uist freshwater stickleback during (270 days) common garden experiment

Populations	Number of families				Body size (mm) $\pm$ S.E		Mortality rates (%) $\pm$ 95% CI
	Crossed	Not hatched	Dead*	Survived	Wild yearlings†	270 days old	
Chru	12	0	0	12	29.82+1.16	39.9+0.67	2.38+1.61
Stru	16	4	1	11	31.70+1.08	41.1+1.27	3.12+2.13
Torm	16	2	2	12	29.19+0.63	41.7+0.67	3.62+1.91
Daim	12	2	3	7	32.27+0.45	42.2+0.64	1.19+1.19
Reiv	12	0	0	12	34.92+1.06	44.8+1.13	10.42+4.25
Host	4	0	0	4	36.56+1.17	45.4+0.95	10.71+3.57

*at 0-17 days post-hatching. †Wild yearlings are from chapter three of this thesis.

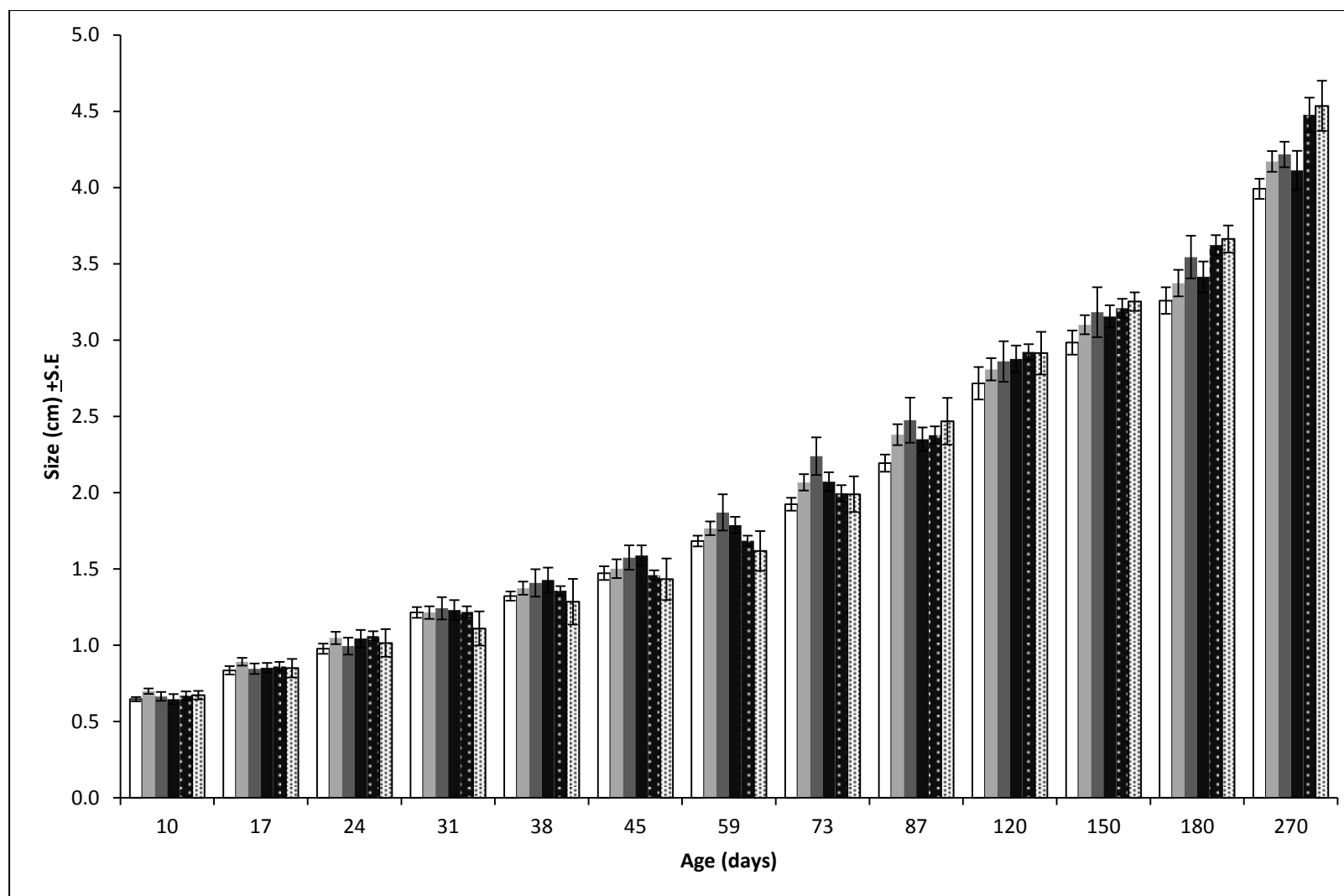


Figure 5.1. The comparison of growth among populations of the North Uist stickleback ($\pm$ S.E) No fill=Chru, light grey=Torm, dark grey=Daim, solid black=Stru, black dotted=Reiv, white dotted=Host.

5.4.2 The relationship of traits in the wild and in the common garden

Mortality rates during the experiment were positively correlated with larger number of eggs (Wald $F_{1,4}=17.35$, $P=0.014$; Fig. 5.2), but were unrelated to egg size (Wald $F_{1,4}=0.33$, $P=0.598$). Higher indexes of mortality were also related to larger size of yearlings in the wild (Wald $F_{1,4}=9.26$, $P=0.038$; Fig. 5.3). The size of stickleback at 270 days (end of the experiment) in the common garden was positively correlated with size in the wild (Wald $F_{1,4}=19.91$, $P=0.011$; Fig.5.4), but was independent from egg size (Wald $F_{1,4}=1.01$, $P=0.37$).

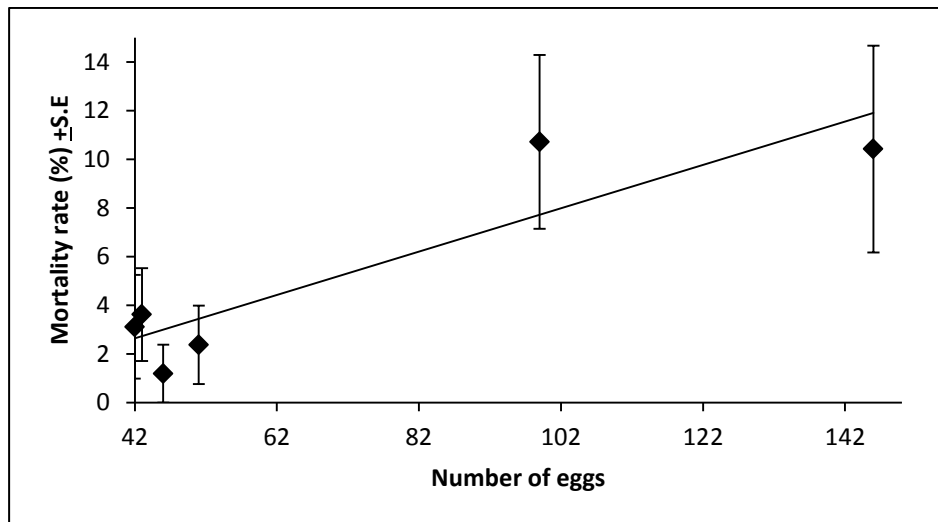


Figure 5.2. Relationship of mortality rate ($\pm$ S.E) in the common garden experiment and number of eggs in the wild among six populations of freshwater stickleback in North Uist.

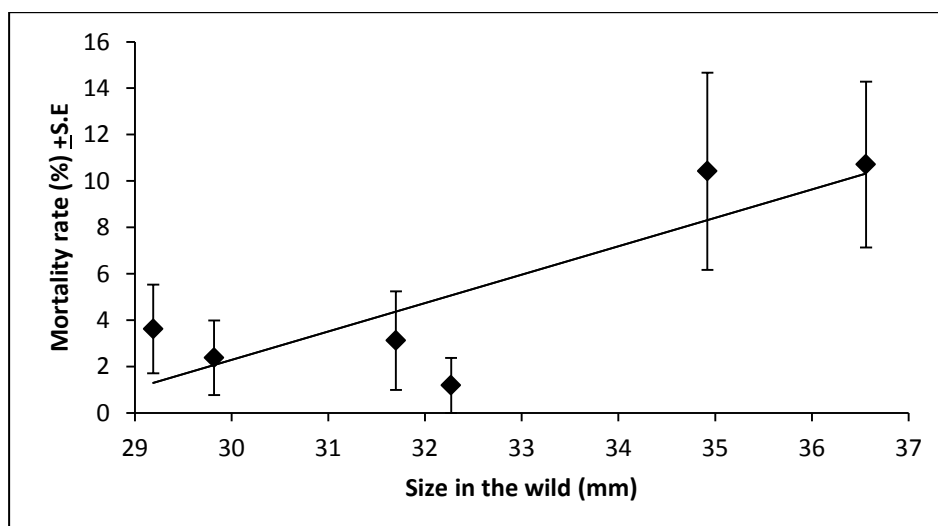


Figure 5.3. Relationship of mortality rate ($\pm$ S.E) in the common garden experiment and yearlings size in the wild among six populations of freshwater stickleback in North Uist.

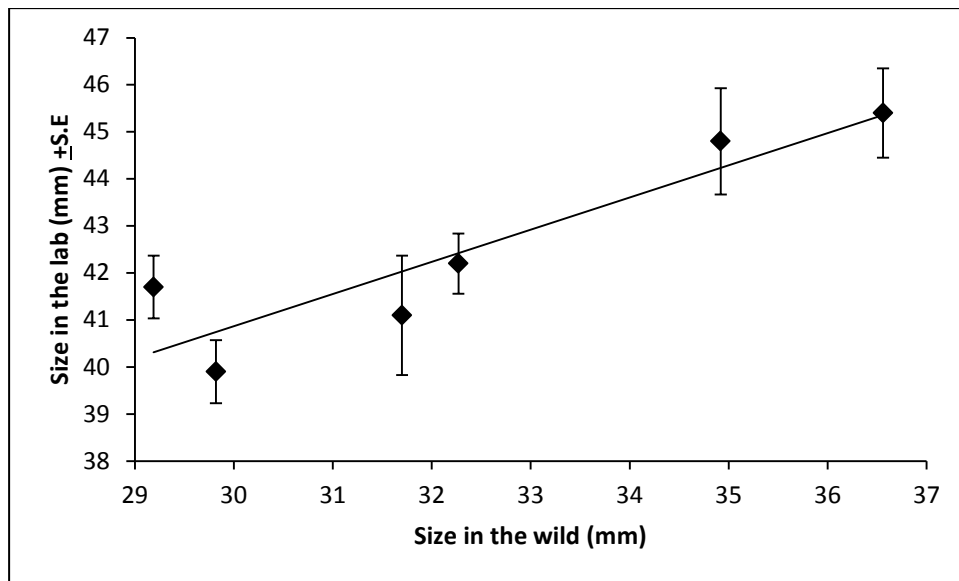


Figure 5.4. Relationship of size at 270 days old in the common garden experiment ($\pm$ S.E) and yearling size in the wild among six populations of freshwater stickleback in North Uist.

5.5 Discussion

My results showed that the growth rate of North Uist stickleback is highly heritable, suggesting that variation between populations is largely the result of genetic differences, and occurred as a part of selected responses to the ancestral environment. The evolution of stickleback in freshwater on North Uist is assumed to have begun around 16,000 years ago as a result of the invasion of freshwater bodies by marine populations shortly after this island was deglaciated (MacColl *et al.*, 2013). This is more than enough time for body size to evolve, since evolution of single traits in stickleback can be substantial in only 100 years (Lucek *et al.*, 2014).

The estimate of heritability in this study is contrary to Mousseau & Roff's (1987) finding that the heritability of life history traits is generally lower than 40% in most ectotherm animals. Maternal effects, a common confounding factor in estimates of heritability (Hutchings, 2011; Oomen & Hutchings, 2016), are unlikely to explain this result. The North Carolina II design allowed me to make an estimate of the dam effect, which was negative and a magnitude lower than the estimation of additive or dominance, suggesting that the maternal effect is likely to be small.

The assumption of a strong effect of selection on the divergence of body size on North Uist stickleback is coincident with a study by Magalhaes *et al.*, (2016). By conducting molecular analysis through RAD sequencing, and analysing divergence in morphological traits i.e. body size, body shape and body armour in an overlapping set of populations, they showed that populations of freshwater stickleback in North Uist have high genetic divergence (the average of $F_{ST}=0.2$). This value is still higher even in comparison to studies of geographically more widely separated stickleback populations.

The extreme divergence of ecological factors between North Uist lochs probably contributed to this high degree of heritability, through a rapid selection process. Lochs in North Uist are characterised by a contrast in ecological harshness between the east and west sides of the island (Chapter 3; Giles, 1981; MacColl *et al.*, 2013; MacColl & Aucott, 2014). Rich resources and a higher density of the main competitor, *Pungitius pungitius*, in most of the lochs in the west side favours a larger size of three-spined stickleback. While, in contrast, poor resource and a higher density of small brown trout, *Salmo trutta*, a potential predator of stickleback, on the east side probably select for dwarf stickleback (MacColl *et al.* 2013). The contrasting level of harshness on the two sides of the island probably drove rapid selection on most of the phenotypic traits of North Uist stickleback. Each side of ecological harshness will select for specific traits, including the phenotypic ones, that are distinct to another, and it may have resulted in a higher heritability of body size than was expected. Estimates of the heritability of other phenotypic traits are required to test this idea.

High heritability of phenotypic variation in stickleback has been ubiquitous among studies in the last decade (e.g. Hermida *et al.*, 2002; Leinonen *et al.*, 2011; Leaver & Reimchen, 2012; Loehr *et al.*, 2012; Mccairns & Bernatchez, 2012; Voje *et al.*, 2013). Leinonen's *et al.*, (2011) study on Baltic stickleback found heritabilities of 0.40 (± 0.18) for standard length and 0.75 (± 0.08) for body depth. A similar value (0.40+0.36/-0.26) for standard length was found by Mccairns & Bernatchez (2012) for Canadian stickleback. A more complex exploration by Leaver & Reimchen (2012) on 20 related defence and trophic traits in Canadian stickleback revealed high heritabilities of these traits with plasticity making a significant contribution to only two traits.

In addition to the high heritability, my results were consistent with the prediction of a positive relationship between mortality rate and egg number, but contrary to the prediction of a negative relationship with egg size (Smith & Fretwell, 1974; Stearns, 1992). As all of the populations were raised in similar conditions with the absence of environmental factors that

potentially drove these relationships in the wild, the positive relationship of mortality rate with egg number needed more explanation. I assumed higher mortality in larger-fecundity populations were related to faster growth in these populations. Faster growth in higher-fecundity populations (Chapter 3 & 4) probably required more resources for growth and standardising resources in the lab may have resulted in higher mortality for these populations compared to their counterparts with slower growth. Indeed, it is also possible that my findings are the result of differentiation in the intrinsic mortality rates of individuals between populations.

I also found that the size of stickleback grown in common garden conditions in the lab was significantly correlated with size of the size of stickleback in the wild. This result should be expected given the high heritability of growth, but provides further confirmation that much of the variation in size is likely to have a genetic basis. A positive relationship of size in common garden conditions and size in the wild in stickleback has been widely reported by previous studies (e.g. Leinonen *et al.*, 2011; Voje *et al.*, 2013; MacColl *et al.*, 2013).

To summarise, my results showed high heritability of growth rate of North Uist stickleback. This result was consistent with previous research on overlapping populations (MacColl *et al* 2013, Magalhaes *et al* 2016). The mortality rates of these populations in the common garden were positively correlated with number of eggs in the wild, but were unrelated with egg size. Furthermore, size of the stickleback between populations during the experiment was highly interrelated with their size in the wild.

Chapter 6. Resources are more important than predation in driving the size of maturation on freshwater three-spined stickleback

6.1 Abstract

Resource availability and predation are supposed to be the most important ecological factors shaping time of maturation. They are expected to have a similar effect in causing populations to mature earlier, but a contrasting effect on size at maturation. Higher resources have a positive effect on size at maturation by increasing growth rate, while heavier predation has a negative effect since it induces populations to mature earlier (and thus at smaller size). By using probabilistic maturation reaction norm (PMRN) and size at maturation data of freshwater three-spined stickleback (*Gasterosteus aculeatus*) living in naturally variable environments on the Scottish island of North Uist, I address the question of which one is more important in shaping the size at maturation when resources and predation covary in the environment. My results show that size at maturation is strongly influenced by resources, but not by predation, and this is consistent in both the PMRN and size at maturation approaches. Populations in higher resource environments, even with higher predation, tend to mature at larger size and older age. The large increase in fecundity achieved by delaying maturation could provide an explanation for this. Populations living in resource-rich environments also clearly have a faster growth rate as indicated by a larger average size of individuals at one year old. On the other hand, populations with higher predation have a significantly lower life span as indicated by a lower proportion of fish older than one year old.

Keywords: resources, predation, PMRN, size at maturation, three-spined stickleback

6.2 Introduction

Time of maturation is a crucial event in the life history of an organism, since it will affect other life history traits such as future growth, fecundity, adult survival rate and the survival rate of the next cohort. The most important thing to be considered in choosing the time of maturation, in terms of age and size at maturation, is fitness: the balance of costs and benefits of maturing earlier or later (Stearns, 1992). Maturing earlier reduces generation time and the probability of dying before maturation (Stearns, 1992). However, delaying maturation, if growth is still possible, has a direct positive consequence for fecundity because larger body size results in a greater number or larger size of propagules (Stearns & Koella, 1986; Roff 1992). As larger propagules tend to increase fitness in the next generation, delaying maturation is likely to have an indirect positive effect on the survival rate of the next cohort. Delaying maturation can also extend growth and increase adult survival if there is a trade-off between growth and reproduction (Reznick, 1983), or a negative relationship between adult survival and age at maturation (Stearns, 1992).

From a life history perspective, time of maturation is strongly influenced by growth rate, and juvenile and adult mortality rate. Most studies (Stearns & Koella, 1986; Reznick *et al.*, 1990; Roff, 1992; Heino *et al.*, 2002) agree that individuals or populations with a faster growth rate are expected to mature earlier and at a bigger size. However, if the faster growth leads to increasing adult or adult and juvenile mortality, this general pattern can reverse to produce later maturation at a bigger size (paraboloid and keel shaped reaction norms) (Stearns & Koella, 1986). Higher juvenile mortality should force individuals to mature later in order to compensate for reduced fecundity since higher fecundity correlates positively with juvenile survival. In contrast, higher adult mortality should force individuals to mature earlier to avoid being killed before maturation (Roff, 2001).

From an ecological perspective, resource availability and predation are supposed to be the most important factors shaping age and size maturation of organisms (Beckerman *et al.*, 2010; Abrams, 2011). This is because resource availability is the most important factor in shaping growth, while predation strongly affects the survival rate of organisms. Better resources will lead to faster growth rates while higher predation will increase the probability of dying before reproduction. Due to these reasons, resources and predation have a similar impact in causing populations to mature earlier, but have a contrasting effect on size at maturation. Higher resources have a positive effect on size at maturation by increasing growth rate, while heavier

predation has a negative effect since it induces populations to mature earlier (and thus at smaller size).

In theoretical studies of life history evolution, agents of selection, such as resource availability and predation, are generally assumed to be independent of each other, but this may often not be true. There are good reasons to believe that resources and predation may covary in natural environments (MacColl 2011; MacColl et al. 2013), since naturally richer environments may support more complex food webs (Grenouillet *et al.*, 2002), or because predation reduces prey population size (Abrams & Rowe, 1996; Reznick *et al.*, 2001; Abrams, 2011). A study by Arendt & Reznick (2005) on Trinidadian guppies (*Poecilia reticulata*) found that higher resources decrease the negative effect of predation on growth rate. On the other hand, Martin et al. (2011) located that high predation risk can force broods of Passerine birds to grow faster even when they have lower resources. A lab experiment by Gale *et al.* (2013) also indicated that predation is more important in shaping the growth rate of Costa-Rican livebearing fish (*Rachyrhaphis rhabdophora*). Their results showed that the impact of fluctuating resources on growth rate was only significant for those fish from high-predation environments. Starting from these unpredicted results, I address the question of which one is more important in shaping the size at maturation if resources and predation covary.

Maturation has traditionally been quantified as a deterministic reaction norm linking age and size for individuals or populations (Stearns & Koella, 1986; Stearns, 1992). Individuals are assumed to mature as they pass a critical point along the maturation curve at a fixed age and size. More recently it has been suggested that it is more accurate to view maturation as a probabilistic phenomenon, in which the likelihood of maturing is a function of size and age called the probabilistic maturation reaction norm (PMRN) (Heino *et al.*, 2002; Dieckmann & Heino, 2007). Heino and co-workers argue that not all individuals will mature at the same critical point of maturation line because they have different growth curves. When growth rate varies among the individuals, the maturation reaction norms will occur as a gradual (not a simultaneous) process following each individual's size and condition (Heino *et al.*, 2002; Dieckmann & Heino, 2007). Therefore, maturation reaction norms should be more accurate to view as the probability of maturing at specific age and size.

Quantifying maturation in PMRNs also was thought to provide a better disentangling of phenotypic plasticity and selection on maturation time. As PMRNs remove the effect of variation in mortality and juvenile growth among populations, it will reduce the plasticity

effects on the maturation function (Dieckmann & Heino, 2007). For this reason, PMRNs are used widely to analyse selection, especially in harvested populations (e.g. Law, 2000; Conover & Munch, 2002; Ernande et al., 2004). Even though PMRNs cannot be ascribed purely to genetic variation (Kraak, 2007), it can assume they are mostly shaped by the selection they have experienced.

To date the use of PMRNs has been mostly applied to marine populations and especially the impacts of commercial fishing (Pérez-Rodríguez *et al.*, 2013; Marty *et al.*, 2014; Hunter *et al.*, 2015). It is well established that intensive fishing will decrease the age and size at maturation (Heino & Godo 2002; Jørgensen et al. 2007; Kokkonen et al 2015). There are two possible reasons for this: plastic or genetic responses to harvesting. The PMRN has been widely applied to harvested marine fisheries to try to resolve this debate. However the PMRN approach has seldom been applied to the study of life history responses to natural variation in ecological circumstances in freshwater fishes species (e.g. Kokkonen et al. 2015). Here, I investigate the role of natural variability in resources and predation on inter-population variation in the PMRNs of freshwater three-spined stickleback (*Gasterosteus aculeatus*) from populations on the Scottish island of North Uist. There is a strong covariance between resource availability and density of predators in these populations (MacColl et al. 2013; MacColl & Aucott 2014). My main question is which factor is more important in shaping size at maturation: resources or predation?

6.3 Methods

6.3.1 Populations and stickleback sampling

Females from six different populations of North Uist stickleback were sampled in 2014-2015 (Table 6.1). These six populations were chosen because of their apparent diversity in yearling (1+) size (Chapter 3; MacColl et al 2013), resource availability and predation intensity (Chapter 3). I chose three pairs of populations across the resource gradient. In each pair of populations, trout were present in one and absent from the other, to ensure the greatest contrast in predation potential. In a fastStructure analysis for 20 North Uist populations, based on genome wide data (~9,000 SNPs) from RADseq, all six analysed here form their own clearly distinct genetic unit (Magalhaes *et al.*, 2016).

Fish sampling was carried out using unbaited minnow traps which were set overnight from the shore in water 0.3 - 3 m deep. The trapped stickleback were emptied into buckets and were sorted on the loch shore to exclude males as far as possible, since the PMRN analysis was restricted to females. The remaining samples were selected haphazardly and brought to a field laboratory for dissection and measurement. I aimed to get at least 50 immature and 50 mature females from each population. Male fish were excluded from data collection once their sex had been established by visual inspection of dissected gonads.

For each individual stickleback I recorded standard length in millimetres, weight in grams, reproductive status (maturing/mature or immature) (Chapter 2) and weight of gonads. Fish were preserved in ethanol for later determination of the age of each fish by otolith analysis (Chapter 2). Using the otolith data, I estimated average size (length) at one and two years old, and the proportion of fish older than one year (a majority of stickleback in these populations mature at about one year of age). I used the average size in each year class as an estimate of the growth rate in each population, while the proportion of older fish was used as an estimate of life expectancy.

6.3.2 Data analysis

Principal components analysis was used to summarise the resource factors (concentration of calcium and sodium ions, pH, conductivity and dissolved organic carbon), and the first principal component (PC1) used as an overall summary of 'resource availability'. A generalized linear model (GLM) with normal errors and identity link function was used to test for differences in size at one year old. In comparison of age distributions between loch, fish were coded as one year old or older, and the data were analysed by GLM with binomial error

distribution and a logit link function. Regression analysis was then used to examine the relationship between both mean size at one year and the ‘proportion of older’ fish in each population, and measures of resource availability and predation (brown trout CPUE).

PMRN analysis was conducted using a GLM with binomial errors and a logit function, following Heino et al (2002). Maturation of each fish (0 or 1) was used as the response variable. Size, age (in years from otoliths) and loch of origin were included in the fixed model. Size at 50 per cent probability of maturation (lp50) was calculated for each loch following Heino et al (2002). Lp50 was then regressed on environmental (resource and predation) variables.

A GLM with normal errors and identity link function was used to test for differences in the size and age (square root transformed) of the matured fish. The average length and age of these mature fish in each population were then used to estimate traditional maturation reaction norms (Stearns & Koella 1986). Estimated means were regressed on resource availability and brown trout CPUE. I also estimated the difference (‘bias’, Heino et al, 2002) between mean size at maturation and the lp50, and examined how this was related to mean body size in each population using simple regression analysis. All statistical analyses were conducted in Genstat 15th edition (VSN International, 2011).

6.4 Results

I found there was a significant difference in size at one year old (Wald $F_{5,540} = 68.71$, $P < 0.001$) and age distributions (Wald $F_{5,667} = 10.56$, $P < 0.001$) between populations (Table 6.1). Larger size at one year old was clearly associated with measures of resource availability (Wald $F_{1,4} = 37.08$, $P = 0.004$), but not with trout catch rate (Wald $F_{1,4} = 1.14$, $P = 0.34$). Proportion of older fish was significantly lower in populations with higher trout catch rate (Wald $F_{1,4} = 11.42$, $P = 0.028$), but there was no correlation with resources (Wald $F_{1,4} = 0.07$, $P = 0.810$) (Fig. 6.1:a, b, c and d for more detail).

The PMRN analysis showed that there was significant variation in size, but not in age at maturation, among lochs (Table 6.1 and 6.2). The 50% probability of maturing occurred at larger sizes in lochs with richer resources (Wald $F_{1,4} = 62.00$, $P = 0.001$) but differed little with the presence of trout (Wald $F_{1,4} = 0.82$, $P = 0.415$) (Fig. 6.1e and f). There was no significant difference of size at lp50 between one and two year old fish (Fig. 6.2).

Mean age and size at maturation (Stearns's approach) differed significantly between populations (Wald $F_{5,396} = 10.62$, $P < 0.001$ for age at maturation and Wald $F_{5,396} = 138.14$, $P < 0.001$ for size at maturation). Again, mean size at maturation was more strongly associated with resource variation (Wald $F_{1,4} = 31.07$, $P = 0.005$), but unrelated to variation in predation (Wald $F_{1,4} = 1.41$, $P = 0.301$) (Fig. 6.1g and h). Mean age at maturation decreased with increasing trout catch rate (Wald $F_{1,5} = 19.54$, $P = 0.012$), but was unrelated with resources (Wald $F_{1,4} = 0.04$, $P = 0.851$) (Fig. 6.1i and j).

Table 6.1. Population mean ($\pm$ S.E.) values of size and age at maturation statistics of three-spined stickleback in six North Uist lochs. See text for details.

Loch	Number of sample		Average size at:		Proportion of older fish (%)	Mean values at maturation		Lp 50 of PMRN		Deviation (bias)	
	F0 [†]	F1 [‡]	1 year	2 years		Size	Age	Size	Age	Size	Age
Bhar	51	70	30.28 $\pm$ 0.51	33.80 $\pm$ 0.99	15.00 (4.74,6.23)	32.82 $\pm$ 0.45	1.21 $\pm$ 0.04	28.1 $\pm$ 1.96	0.91 $\pm$ 0.68	4.72	0.30
Iala	24	81	33.86 $\pm$ 0.48	37.16 $\pm$ 0.72	35.24 (6.60,7.13)	37.22 $\pm$ 0.41	1.55 $\pm$ 0.07	30.1 $\pm$ 2.06	N/A	7.12	N/A
Chru	44	58	32.44 $\pm$ 0.60	43.81 $\pm$ 1.27	17.65 (4.84,6.01)	38.74 $\pm$ 0.59	1.34 $\pm$ 0.05	32.1 $\pm$ 2.16	0.97 $\pm$ 0.77	4.38	0.37
Stru	50	69	31.01 $\pm$ 0.40	34.13 $\pm$ 4.06	2.52 (1.59,3.65)	33.78 $\pm$ 0.31	1.03 $\pm$ 0.02	29.4 $\pm$ 2.03	0.83 $\pm$ 0.71	6.64	0.20
Host	51	54	40.52 $\pm$ 0.79	51.98 $\pm$ 2.67	17.14 (4.90,6.10)	48.87 $\pm$ 0.68	1.22 $\pm$ 0.04	41.5 $\pm$ 2.58	1.11 $\pm$ 0.82	7.37	0.11
Reiv	51	70	42.34 $\pm$ 0.63	57.80 $\pm$ 0.93	27.27 (6.29,7.30)	51.99 $\pm$ 0.67	1.45 $\pm$ 0.05	43.6 $\pm$ 2.67	1.05 $\pm$ 0.78	8.39	0.40

*[†]immature, [‡]mature, Size in mm and age in year, n/a= not available, S.E for proportion of older fish and PMRN as 95% confidence interval

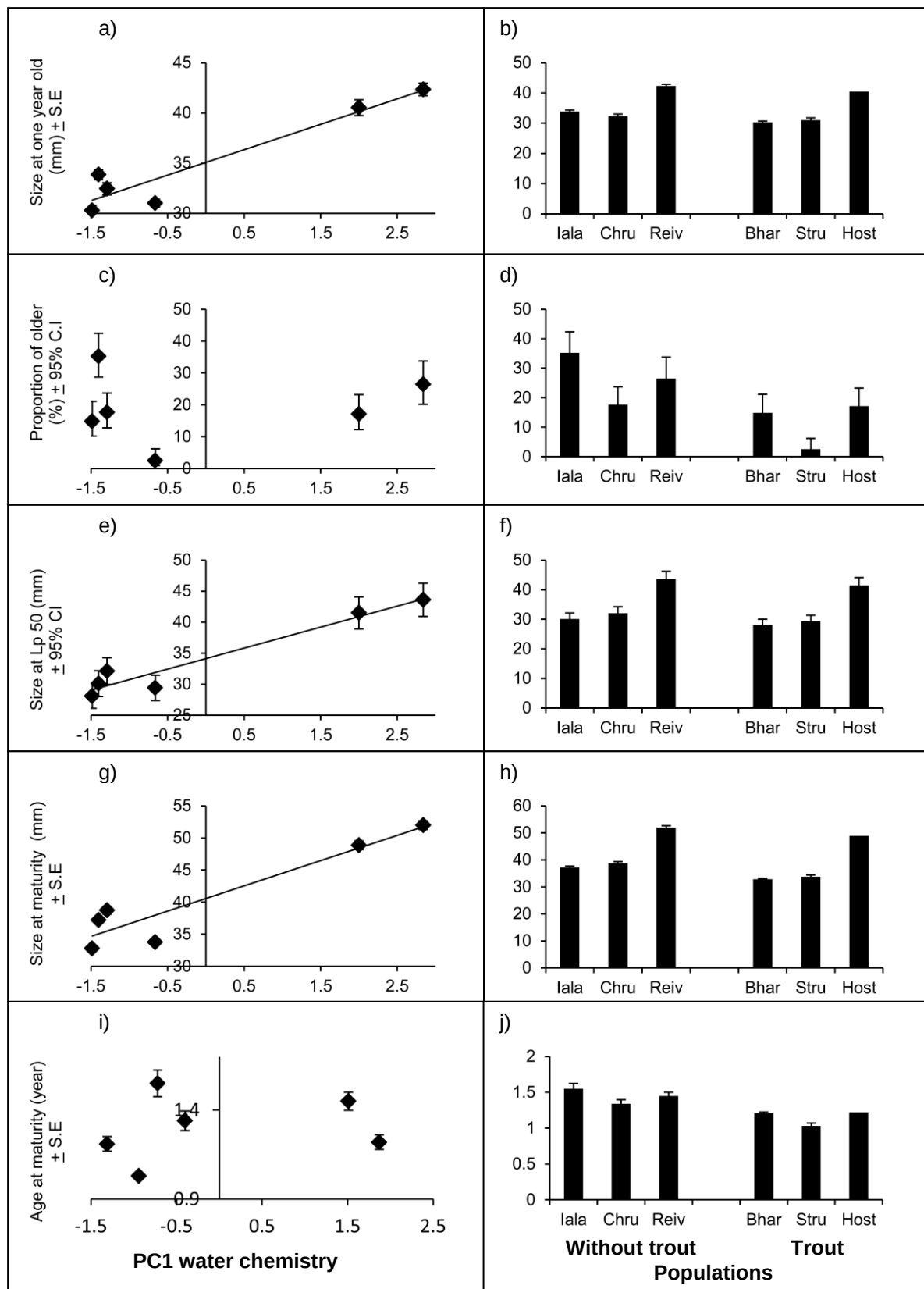


Figure 6.1. Relationships between life history variation and resource availability and predator density among North Uist three-spined stickleback populations, CI=confidence interval.

Table 6.2. Results of a GLM to estimate PMRNs in six populations of North Uist three-spined stickleback. The model fitted ‘reproductive status’ (0 or 1) as a binary response variable, and used binomial errors and a logit link function.

Explanatory variable	χ^2	df	p
Loch	42.47	5, 670	<0.001
Length	67.0	1, 666	<0.001
Age	0.07	1, 666	0.798
Loch.Length	39.87	11, 661	<0.001
Loch.Age	8.63	11, 661	<0.001
Length.Age	64.30	3, 669	<0.001

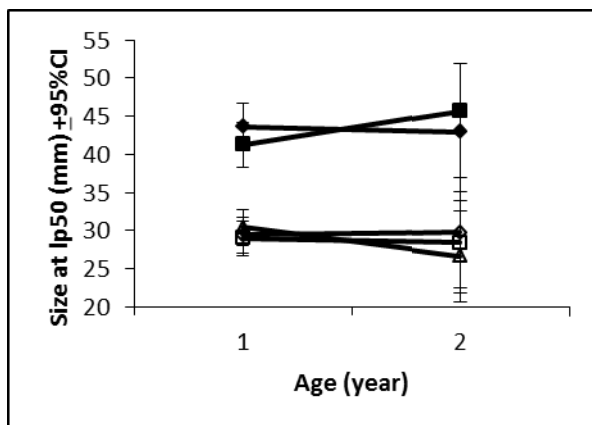


Figure 6.2. Size at lp50 (+95%CI) among different age classes in populations of North Uist three-spined stickleback. Bhar=open square, Iala=triangle, Stru=open diamond, Host=solid square, Reiv=solid diamond.

I also found that populations with higher growth rates, as indicate by a larger mean size at one year old, mature at a bigger size, and this is consistent when using the PMRN (Wald $F_{1,4}=102.86$, $P<0.001$) or mean size at maturation approaches (Wald $F_{1,5}=164.05$, $P<0.001$). Populations with faster growth rate also tended to mature at older age (Wald $F_{1,4}=9.66$, $P=0.053$ for PMRNs; Wald $F_{1,4}=0.64$, $P=0.467$ for Stearns'). There was a significant positive correlation between the proportion of older fish in populations, and mean age at maturation (Wald $F_{1,4}= 84.14$, $P<0.001$; Wald $F_{1,4}=4.52$, $P=0.123$ for PMRNs), suggesting a negative relationship between earlier maturation and life span.

The bias between size at maturation calculated from the PMRNs and Stearns's mean size at maturation was positively related to body size (size at one year old) (Wald $F_{1,4}=11.26$, $P=0.028$). The bias ranged from 14.12 to 21.03 % of the mean size at one year old.

Finally, fecundity (measured as gonad weight of mature females), was strongly associated with length (Wald $F_{1,400}=419.40$, $P<0.001$) and weight (Wald $F_{1,400}=723.39$, $P<0.001$) of the fish. Populations with higher resources experienced a larger increase in investment between their first and second year compared with resource-poor populations (Fig. 6.3). In Host and Reiv there were 28.3 % and 36.5% increases in length respectively between one year old and two year old fish, while in Bhar and Iala the equivalent increases were only 11.62% and 9.7%. As a result, the fecundity of Host and Reiv increased by 55.61% and 87.52%, while the equivalent increases in Bhar and Iala were only 10.80% and 5.37% (Fig. 6.3).

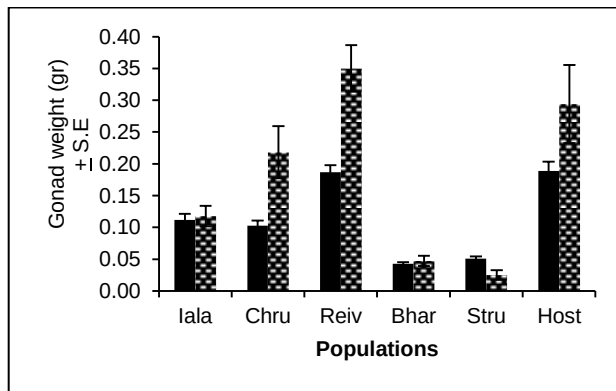


Figure 6.3. Changes in mean ovary weights ($\pm$ S.E) between one and two year old mature female three-spined stickleback in six populations on North Uist. Solid bars = gonad weight at one year old, striped bars = gonad weight at two years old.

6.5 Discussion

The results clearly show that when resources and predation covary, resources are more important in driving evolution of size at maturation in stickleback populations. My analyses show that the effect of resources overrides the effect of predation on size at maturation. This result is similar to the one obtained by Arendt & Reznick (2005) when examining the effect of resources and predation on the growth of Trinidadian guppies. They declared, when resources and predation covary, resources are more important in shaping growth rate. Since choice of maturation time is highly dependent on cost and benefits (Stearns 1992), it is interesting to consider how populations overcome the effect of predation.

The most likely reason is that the advantage of delaying maturation more than offsets the risk of being killed before maturation. The main advantage of delaying maturation is the increase in fecundity, since larger females produce more or bigger offspring (Stearns 1992; Roff 2001). However, if continuing growth is not possible, delaying maturation will become disadvantageous since it will increase the generation time. In my study, I found that the populations which live in higher resource lochs continue growing after they reach one year old while those from resource-poor populations exhibited little change in size. Delaying maturation in resource-poor populations seems unlikely to outweigh the cost of increasing the generation time.

The relationship between age at maturity and predation suggests that trout mainly eat adult stickleback, thereby selecting for earlier maturation (Stearns, 1992; Roff, 2001). This is consistent with the observed reduction in the proportion of older stickleback in lochs with trout present, and with Scharf et al.'s (2000) observation that fish predators will take prey up to 20% of their size: even adult stickleback are generally less than this proportion of the size of trout.

Overall it appears that maturation will occur at smaller size with increasing predation (Fig 1f and 1h), and this is consistent with many results that have examined the effect of commercial fishing (Pérez-Rodríguez *et al.*, 2013; Marty *et al.*, 2014; Hunter *et al.*, 2015; Kokkonen *et al.*, 2015). However, my study suggests that if predation in North Uist system is sufficient to drive such changes they can be offset by increases in resources. This may be relevant to fisheries or wildlife management.

An unexplained result from this study is that the Reiv population, with high resources, appears to have a lower life span than resource-poor Iala (Fig 1d). Fish predators are absent

from both of these populations and Reiv has better resources. Since better resources should favour longer life span and there is a trade-off between maturation time and future growth (Reznick 1983; Stearns 1992), this result requires explanation. The most obvious one is that stickleback densities are very high in Reiv, where the competitor species *P. pungitius* is also present (MacColl unpublished data). This population also has a higher prevalence of most parasites than does Iala (de Roij 2010; AR, personal observations), and a higher density of avian predators (ADCM, personal observations). These facts combined may explain the lower life expectancy in Reiv.

As I expected, the bias between the PMRN lp50 estimate and mean size at maturation is positively related to body size. This is a useful result, since it is much easier to estimate mean size at maturation, and means that in larger samples of populations it should be acceptable to estimate the lp50 from mean size at maturation.

Finally, it is interesting to note that, at least in some populations, it appears that not all stickleback die after breeding. In Iala 76% of individuals were maturing at one year old, when I sampled in May, which is relatively early in the breeding season. In my sample 35% of Iala had survived to two years old. If these proportions remain constant across years, then this suggests that some individuals survive until the next spring even after breeding.

Overall, I can conclude that resources are more important than predation in shaping the evolution of size at maturation. On North Uist the combination of the two factors leads to substantial variation in age at maturation. Modelling could be useful to further understand the complex relationship of these two factors to maturation. Another interesting outcome in this research is the presence of a unique population i.e. Iala. This population is characterised as having poor resources, and showed signs of maturation in their yearlings, but it has the highest percentage of old fish among all of the populations analysed. This indicates that stickleback in this population can either postpone their breeding time until the following year or reproduce multiple times across different years.

Chapter 7. General Discussion

My study on the evolution of life history traits in North Uist stickleback has produced four main results that are novel with regard to the findings of previous research on stickleback life history. Firstly, I provided a new and improved method for the ageing of stickleback via otolith examination (Chapter 2) that clearly differs from the established one, Jones & Hynes (1950). Secondly, I found that established models which predict a relationship of larger body size with earlier maturation (Stearns & Koella, 1986; Roff, 1992; Stearns, 1992) do not apply for North Uist stickleback (Chapter 3). Instead, on North Uist, populations of stickleback with a larger size at one year old matured later, while populations with smaller size at one year old were the opposite. This pattern was found to be consistent across multiple analyses, whether looking at age at maturation (Stearns & Koella, 1986; Chapter 3) or probabilistic maturation reaction norms (PMRNs) (Heino *et al.*, 2002; Chapter 6). Thirdly, my finding on the relationship of maternal size with egg size (Chapter 4), as far I know is the first report of a negative correlation in stickleback. Other previous studies (e.g. Snyder & Dingle, 1989; Baker, 1994; Ali & Wootton, 1998; Wootton & Fletcher, 2009) found either a positive or no relationship. Fourthly, I revealed that the positive effect of resources on size and time of maturation, and life expectancy can overcome the negative effect of predation (Chapter 6). Despite the widely reported contrasting effect of these two factors on size of maturation (Stearns, 1992; Heino & Dieckmann, 2008), how they interact in shaping the size of maturation of stickleback in a real environment is now unravelled. Another interesting result in this thesis (Chapter 5) is the high heritability (0.66 ± 0.16) of growth rate in North Uist stickleback. Below, I will discuss the contribution of each finding to the understanding of life history of fish, and particularly the three-spined stickleback.

7.1 New method for ageing three-spined stickleback

Although the Jones & Hynes method has been well established and cited for more than a half a century, my results show this method does not apply for most stickleback populations. A big bias with Jones & Hynes's (1950) method is that stickleback ages are often underestimated by one year. This is because they state that the first transparent ring (S type otolith) will be prominent in the stickleback's otolith by June and/or early July of the year the stickleback was born, and therefore this type of otolith belongs to young of the year. In fact, my results demonstrate that, as in other fishes (Williams & Bedford, 1974; Borland, 1986;

Jones, 1992; Uriarte *et al.*, 2016), stickleback with S type otoliths during these months are the yearlings that were born in the previous year. Moreover, by following Jones & Hynes (1950), fish with S+ otolith that are found after July until the end of the year would be counted as young of the year, because the second opaque ring is starting to grow by July. Again, my result showed a different pattern that S+ otoliths only appear by the following spring and persist until the end of August in more than a half of the yearling samples. Therefore, those S+ fish should be counted as yearlings, not young of the year. Since many stickleback populations are essentially annual, Jones & Hynes method would result in very significant error.

A second issue with Jones & Hynes's (1950) method was that in their method the growth pattern of the seasonal rings on the otoliths of stickleback was contradicted by the findings of later researchers (Panella *et al.*, 1971; Borland, 1986; Uriarte *et al.*, 2016; Chapter 2). As the opaque ring was expected to grow from October to May (Jones & Hynes, 1950), sampling in late autumn i.e. October to end of winter i.e. February could lead to confusion because the majority of otolith edges during these months were transparent. In contrast, the expectation of transparent growth during June to September (Jones & Hynes, 1950) will seldom be found during this period because most young of the year still have opaque edges until late summer i.e. end of August. Therefore, the transparent edges will be expected merely from stickleback in breeding/post breeding condition or those with slow growth that had still not started their new opaque growth.

Previous researchers on stickleback (Borland, 1986; Defaveri & Merila, 2013) have displayed their awareness of the inaccuracy of Jones & Hynes. Borland (1986) for example found in his analysis that the ageing of stickleback is more consistent with those of other fishes (e.g. Panella *et al.*, 1971; Williams & Bedford, 1974). However, Borland's idea has not become widely known because it was only an appendix to his PhD thesis. Defaveri & Merila, (2013) also seem to have differed in their approach from Jones & Hynes. They did not clearly describe their method for ageing, but they stated that fish with a couple of seasonal rings were counted as one year old. If they followed Jones & Hynes (1950), a couple of seasonal ring should be counted as young of the year, since (according to Jones & Hynes) the first transparent ring was completed by September of the year the fish were born.

Apart from a result contradicting Jones & Hynes (1950), I noticed that the otolith growth in my samples was highly dependent on the somatic growth of the fish. Therefore, researchers applying ageing methods which rely on counting seasonal rings of the otolith should be aware

of the effects of growth patterns in their samples. In keeping with Panella *et al.*, (1971; Williams & Bedford, 1974), I found that the formation of opaque rings was highly correlated with faster growth, that usually occurs under conditions with ample resources i.e. spring and summer. The transparent rings were mostly laid down during periods of slower/no growth, which usually correlate with low resource conditions i.e. autumn and winter. Therefore, specific habitat conditions and the duration of season in each sampling site could result differences in otolith growth.

The specific otolith growth in each sampling site was also probably the main reason for the differentiation of my results from Jones & Hynes (1950). Based on Hynes (1950), the Birket river, the site where Jones & Hynes's (1950) study was conducted, was choked by vegetation and had reduced in flow in summer. In addition, there was a conspicuous shortage of large prey items in stickleback stomachs during June to September (Hynes, 1950, Table 8). It therefore seems likely that for stickleback, growth conditions in the Birket were at their poorest in the summer, and improved in the winter. The pattern of resource conditions in the Birket also probably explains why the otolith growth pattern of stickleback in this river was reversed in comparison to other locations in most of the northern hemisphere.

Indeed, part of the ageing method in my results could lead to a lack of certainty for those otoliths which were sampled in late summer. The presence of a clear transparent edge, which mostly belonged to older fish, could lead to doubt over whether these rings were grown during the previous winter or from the current year. I assumed these rings were either from the previous winter or part of maturation rings as the formation of the first transparent ring was more likely just completed after winter, while the similar ring should be formed later in older individuals (Williams & Bedford 1974). The fact that appearance of this ring was significantly commoner in older age classes was consistent with the findings of Williams & Bedford, (1974). As other researchers (e.g. Panella *et al.*, 1971; Williams & Bedford, 1974; Campana, 2001) that have claimed the presence of transparent rings in older classes is often related to the maturation or spawning of the fish, I also found a significant correlation between reproductive status and the presence of this transparent ring in my data.

7.2 Ecological constraint on body size, maturation time, reproductive strategies and life expectancy of three-spined stickleback

My results came with a clear pattern that body size and size at maturation of North Uist stickleback were mostly driven by the combination of the availability of resources and the

presence of their main competitor, nine-spined stickleback (*Pungitius pungitius*) (Chapter 3). Both of these factors were strongly associated with larger body size, and this probably acted as the primary factor contributing to larger size at maturation. The positive relationship between larger body size and rich resources was robust even in the lochs where *Pungitius* are absent, but this relationship became stronger with the presence of this main competitor. Thus larger body size in stickleback may be selected for when in competition with *Pungitius*, and when supported by better resources.

A positive impact of the relative density of *Pungitius* on body size of North Uist stickleback suggests that the effect of inter-specific competition in this study was consistent with the findings of Arendt, (1997), but not with Mittelbach, (1988). Arendt & Reznick (1997) argued that higher inter-specific competition can select for larger size in order to outcompete a sympatric competitor by resulting in a larger hunting area and/or by shifting niche. Meanwhile, the effect of inter-specific competition on resource limitation (Mittelbach 1988) may not apply in North Uist lochs, especially in lochs with rich resources, although it may still be important in resource-poor conditions.

Stickleback populations with better resources and a higher relative density of *Pungitius* also tended to mature later and have a longer life expectancy than their counterparts with poorer resources and lower relative density of *Pungitius*. While a longer life expectancy in resource-rich populations is reasonable, maturing later in these populations was unusual. As rich resources correlated with larger body size (Chapter 3) and the established models (Stearns & Koella, 1986; Roff, 1992; Heino *et al.*, 2002) predicted that larger body size should favour early maturation, these populations were expected to mature earlier than their counterparts in resource-poor lochs.

From an ecological perspective, later maturation in resource-rich populations was thought to be correlated with a higher density of juvenile predation in these lochs. This assumption was attributed to the presence of large insect larvae i.e. Odonata and Coleoptera larvae, that are a well known predator of juvenile stickleback in rich-resource lochs, but absent from lochs with poor resources. Moreover, this assumption was supported by a larger fecundity (Chapter 4) in rich-resource populations that is consistent with the higher mortality rate of juveniles in these populations.

Resources and the relative density of *Pungitius* also acted as the main factor in driving the pattern of reproductive strategies in North Uist stickleback. Better resources in the lochs

selected for larger ovaries and larger fecundity of the stickleback, while populations with higher relative density of *Pungitius* had significantly lower GSI. Of these correlations, a negative relationship of relative density of *Pungitius* and GSI tended to require more explanation. Since the presence of *Pungitius* is coincident with better resources (Chapter 3) and there is a positive correlation of GSI with resources (Morris, 1987; Erikstad *et al.*, 1998), the GSI in resource-rich populations are expected to be higher.

A possible explanation for the negative correlation of relative density of *Pungitius* with GSI is the conflicting effect of evolutionary and plastic effects. Populations of stickleback in better resources probably tend to increase their GSI, but as their density increases, it also will lead to increasing *Pungitius* density. Increasing the density of these two species leads to resources becoming the limiting factor. Therefore, rather than maximising the GSI, populations in better resource conditions may be selected to optimise their GSI. Meanwhile the negative correlation of GSI and the relative density of *Pungitius* probably was related to the trade-off of growth and reproduction (below). As populations with higher relative density of *Pungitius* tended to have a larger body size, they reduced their reproductive investment.

In contrast to the life history traits above, egg size of North Uist stickleback was mostly shaped by the density of predators, not by resources or relative density of *Pungitius*. A higher density of trout (*Salmo trutta*), a potential predator of North Uist stickleback, was positively related with larger egg size. A larger egg size in populations with higher density of trout was consistent with the prediction as these populations were also characterised by a lower availability of resources (Chapter 4). Both of these factors, higher density of predation and poor resources are expected to correlate with larger egg size (Hendry *et al.*, 2001; Jørgensen *et al.*, 2011; Rollinson & Hutchings, 2013a).

The positive correlation of egg size and predator density in this study suggested the effect of trout on North Uist stickleback is mostly felt by younger age classes. The established theories (Roff, 1992; Stearns, 1992) predict that the relationship of egg size and predator density will be positive when predators favour juvenile age class or the predator is gape-size limited. As the proportion of older stickleback in these populations was unrelated to trout density (Chapter 3), it can be assumed the impact of predation was less intense in adult age classes. A study by MacColl *et al.*, (2013) on these populations also suggested that trout in poor-resource lochs were more likely to act as a larger competitor for adult stickleback rather than a predator.

The strong impact of resources and interspecific competition on body size, maturation time and life expectancy on North Uist stickleback may have meant that the effect of predation became unclear. Indeed, the impact of predation on body size and age at maturation was detected by comparing populations with similar resources (Chapter 6). However, this impact tended to disappear when it covaried with the effect of resources, especially when resources are rich (Chapter 3). Furthermore, increasing resources also tend to correlate positively with the density of stickleback, suggesting that resources may also have acted as a limiting factor for population size. On the other hand, it can be stated that life history traits of North Uist stickleback are shaped mostly by resources and inter-specific competition, while the effect of predation was mostly applicable to egg size. This result had a similar pattern to the findings of Arendt & Reznick (2005) in Trinidadian guppy populations. By comparing the effect of predator regime and resource level on juvenile growth rate, they found that growth rate was primarily determined by resource level. Therefore, the effect of resources in reducing the age and size at maturation may be relevant to fisheries or wildlife management.

7.3 Life history trade-offs in North Uist three-spined stickleback

My results concerning the relationship of body size with maturation time in North Uist stickleback tended to oppose the established models (Stearns & Koella, 1986; Reznick, 1990; Roff, 1992; Heino *et al.*, 2002), which predicted individuals/populations with larger body size would mature at a younger age, but larger size. Instead of maturing earlier, stickleback in populations with a larger size at one year old matured later than their counterparts in populations with smaller body size. Although not consistent with the established models, my result was compatible with Baltz & Moyle, (1982; Fox, 1994) and those acknowledged by Stearns and Koella (1986).

Higher juvenile mortality rate seems the most likely reason for the delay in maturation in populations with larger body size. Stearns & Koella's, (1986) model predicts that the curve of maturation time will shift to the right when the juvenile mortality is high. By delaying maturation, organisms expect to make a larger reproductive investment, and therefore, a better quality of offspring (Roff, 1992; Stearns, 1992). Sticking to the assumption of higher juvenile mortality rates in larger body size populations, Odonata larvae, which are known to prey upon juvenile stickleback are common in the littoral invertebrate communities of lochs with larger stickleback (Reiv and Maga) but absent in those of lochs with smaller body size (Bhar, Fada and Torm) (Chapter 4). Populations of stickleback with larger body size also

produce larger numbers of eggs than those with smaller body size (Chapter 4), which is consistent with a presumably higher juvenile mortality rate in these populations.

Another reason for these populations to extend their maturation time, even populations under high predation, is because the fitness gained by increasing size and therefore fecundity, may outweigh the negative effect of longer generation time and the increased chance of mortality before reproduction. Following Stearns (1992; Roff 2001), delaying maturation could lead to increased fitness as it increases fecundity, since larger females produce more or bigger offspring. However, if continuing growth is not possible, delaying maturation will become disadvantageous since it will increase the generation time. In my study (Chapter 6), I found that the populations which live in higher resource lochs continue growing after they reach one year old while those from resource-poor populations exhibited little change in size.

On the other hand, early maturation in populations with slower growth is probably due to limitation of resources, and therefore a lower adult survival rate. Consistent with Fox's (1994) result for pumpkinseed sunfish (*Lepomis gibbosus*), I also found a strong relationship between resources and life expectancy as judged by the proportion of older fish across populations (Chapter 3). Populations in resource-poor environments thus have a lower survival rate. Therefore earlier maturation is likely to increase fitness in these populations, even though it occurs at smaller size. This assumption is supported by the fact that delaying maturation in poor-resource populations only has a small advantage in terms of increasing reproductive investment (Chapter 6). It emphasizes Fox's (1994) statement that mortality rate, not growth, is more important in shaping the maturation time.

A negative relationship between interspecific-competition and GSI of North Uist stickleback is probably related to the established theory on the trade-off between growth and reproduction. It is widely believed (Reznick, 1983; Jennings & Philipp, 1992; Stearns, 1992) that an increase in reproductive effort will be costly in terms of somatic growth or vice versa. Since populations with higher interspecific competition in North Uist stickleback also had faster growth rate i.e. larger size at one year old (Chapter 3), it can be assumed they reduced their reproductive investment in order to grow faster. Indeed, part of this larger body size was correlated with better resources, but the effect of higher inter-specific competition was clearly stronger (Chapter 3).

My result also concurs with Smith & Fretwell (1974)'s model that expected a negative correlation between number of eggs and eggs size. Consistent with their model, I found there

was a trade-off of number of eggs with mean egg diameter on a fixed investment of North Uist stickleback. However, I did not find any correlation of this trade-off with the maternal size or with the quality of environment as found by Räsänen *et al.*, (2008; Ford & Seigel, 2015). A study by Ford & Seigel, (2015) on the viviparous garter snake (*Thamnophis proximus*) found the egg number-egg size trade-off was more prominent in smaller females, while study of Räsänen *et al.*, (2008) on moor frogs (*Rana arvalis*) found this trade-off was stronger in lower acidity.

7.4 The role of maternal effects in fecundity aspects of North Uist stickleback

My results concerning the pattern of maternal investment in North Uist stickleback showed that larger mothers had larger ovaries and greater fecundity, but smaller eggs. These results were exactly compatible with Hendry *et al.* 's, (2001) predictions. If the positive relationship of maternal size with ovary size and fecundity is easily understood, a negative correlation of maternal size and egg size probably requires more explanation. Hendry *et al.*, (2001) argued that in most fishes, if maternal size does not bring advantages in terms of better parental care of the offspring, larger mothers tend to prioritise increasing fecundity rather than maximising egg size. This is because maximising egg size without providing a better environment can make offspring vulnerable, as larger egg will correlate with higher oxygen demand during incubation process (Hendry *et al.*, 2001).

However, although my result fit with the prediction of (Hendry *et al.*, 2001), a negative correlation of maternal size and egg size is contrary to previous studies on similar species (Snyder & Dingle, 1989; Baker, 1994; Ali & Wootton, 1999; Wootton & Fletcher, 2009; Oravec & Reimchen, 2013). As the previous studies found either positive or no correlations, my results showing a negative correlation of maternal size and egg size on stickleback stand alone. This negative correlation is only found in a minority of studies, even when considering only other fishes for which females are not involved in parental care (Hendry *et al.*, 2001; Braun *et al.*, 2013; Kasimatis & Riginos, 2015). A literature study by Kasimatis & Riginos, (2015) found that a negative correlation of maternal size-egg size among fishes was most prominent in egg scatterers rather than demersal and pelagic spawners.

A negative correlation of maternal size and egg size in my results also brings into question the Rollinson & Rowe, (2015) statement that claimed maternal size per se is not a good predictor for maternal-offspring size relationship. They argued that rather than maternal size, maternal condition or maternal nutritional should be more appropriate for analysing maternal-

offspring size relationships. However, a different test which included maternal condition and/or maternal nutrition is required to confirm if this is the case in North Uist stickleback.

7.5 Plasticity versus evolutionary aspect in the life history traits of North Uist stickleback

Both plasticity and evolutionary factors seemed to contribute to the relationship of ecological factors and life history traits in North Uist stickleback. Data from the common garden experiment (Chapter 5) showed a really high contribution of heritable genetic variation in the differentiation of body size among the six populations analysed. A parallel result was also found in the probabilistic maturation reactions norm (PMRNs) analysis that revealed size at Lp 50 was highly related with size of maturation (Chapter 6). Although the value of Lp 50 of PMRNs does not purely reflect evolutionary variation (Kraak, 2007), this method could estimate the degree of selection (Heino *et al.*, 2002; Heino & Dieckmann, 2008). Involving immature samples in PMRNs analysis was thought to reduce the effect of plasticity because the immature individuals only have a short period of exposure to the different ecological conditions.

The large effect of selection and evolution prominent throughout my results was consistent with Magalhaes *et al.*, (2016). By conducting molecular analysis using RAD sequencing, and analysing divergence in morphological traits i.e. body size, body shape and body armour in an overlapping set of populations, Magalhaes *et al.*, (2016) showed that populations of freshwater stickleback in North Uist have high genetic divergence (average $F_{ST}=0.2$). These F_{ST} values were high even in comparison to studies of geographically more widely separated stickleback populations (Magalhaes *et al.*, 2016).

Therefore, based on the data above, it can be assumed the differentiation in phenotype of North Uist stickleback is largely the result of evolutionary responses to selection brought about by different environments. As the evolution of stickleback in freshwater on North Uist was assumed to have begun around 16,000 years ago (MacColl *et al.*, 2013), there has been more than enough time for phenotypic traits in stickleback to evolve. Following Lucek *et al.*, (2014), single traits can evolve substantially in stickleback in only 100 years. Moreover, the extreme contrast in environmental harshness on the two sides of the island (Chapter 3; Giles, 1981; MacColl *et al.*, 2013; MacColl & Aucott, 2014) probably drove a more rapid selection on most of the phenotypic traits of North Uist stickleback. Further heritability analyses in different traits e.g. fecundity and life span are required to test this assumption.

7.6 Conclusions and future research

Overall, my thesis identifies four novel findings that contribute significant new information to the study of life history variation in fish, especially three-spined stickleback. Those four main points with other results in this study are summarised in Table 1. As this study has refuted the theoretical prediction of larger body size being accompanied by early maturation, a new maturation curve that accommodates this pattern needs to be considered. Furthermore, more research which examines life history in fishes without maternal care is required to confirm the minority finding on the negative correlation of maternal size-egg size. Another vibrant remaining question specifically related to North Uist stickleback is to disentangle the plasticity and evolutionary effects upon other life history traits i.e. fecundity and life span of these populations.

Table 7.1. Summary of hypotheses/questions and results related in this thesis.

Chapter	Main question/hypotheses	Results
II	a. What is the pattern of otolith growth in the stickleback?	a. Transparent rings of the stickleback otolith are mostly laid down in autumn and winter, and opaque rings in the other months. There is neither transparent growth during the first summer and/or second opaque growth before the first winter.
III	a. How do ecological factors interact in shaping the body size of stickleback?	a. The combination of resources and inter-specific competition selects for larger body size and outweigh the opposite effect of predation.
	b. Stickleback with larger body size will mature earlier and at larger size.	b. Rejected. Stickleback with larger body size tend to mature later and at larger size, while smaller body size are the opposite.
IV	a. Larger mothers have larger ovaries and fecundity, but smaller egg size.	a. Accepted.
	b. How do the confounding effect of ecological factors affect the reproductive traits of stickleback?	b. There is a positive correlation of resources and inter-specific competition with fecundity, and between predation and egg size. In contrast, a negative relationship was found between inter-specific competition and reproductive investment.
	c. There is a trade-off between fecundity and egg size.	c. Accepted.
V	a. Differentiation in growth rate has a heritable basis.	a. Accepted. Of $66 \pm 16\%$ of this differentiation is heritable.
VI	a. Which factor is more important in shaping size at maturation: resources or predation?	a. Resources.

Literature Cited

- Abrams, P.A. 2011. Simple life-history omnivory: responses to enrichment and harvesting in systems with intraguild predation. *Am. Nat.* **178**: 305–319.
- Abrams, P.A. & Rowe, L. 1996. The effects of predation on the age and size at maturity of prey. *Evolution (N. Y.)* **50**: 1052–1061.
- Ali, M. & Wootton, R.J. 1998. Do random fluctuations in the intervals between feeding affect growth rate in juvenile three-spined sticklebacks? *J. Fish Biol.* **53**: 1006–1014.
- Ali, M. & Wootton, R.J. 1999. Effect of variable food levels on reproductive performance of breeding female three-spined sticklebacks. *J. Fish Biol.* **55**: 1040–1053.
- Allen, J.R.M. & Wootton, R.J. 1982. Age, growth and rate of food consumption in an upland population of the three-spined stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol.* **21**: 95–105.
- Altermatt, F. 2010. Climatic warming increases voltinism in European butterflies and moths. *Proc. Biol. Sci.* **277**: 1281–1287.
- Arendt, J.D. 1997. Adaptive intrinsic growth rates: An integration across taxa. *Q. Rev. Biol.* **72**: 149–177.
- Arendt, J.D. & Reznick, D.N. 2005. Evolution of juvenile growth rates in female guppies (*Poecilia reticulata*): predator regime or resource level? *Proc. R. Soc. B* **272**: 333–337.
- Ariyomo, T.O., Carter, M. & Watt, P.J. 2013. Heritability of boldness and aggressiveness in the zebrafish. *Behav. Genet.* **43**: 161–167.
- Baker, J.A. 1994. Life history variation in female threespine stickleback. In: *The evolutionary biology of the threespine stickleback* (M. . Bell & S. . Foster, eds), pp. 144–187. Oxford University Press, New York.
- Baker, J.A., Foster, S.A., Heins, D. c, Bell, M.A. & King, R.W. 1998. Variation in female life-history traits among Alaskan populations of the threespine stickleback, *Gasterosteus aculeatus* L. (Pisces: Gasterosteidae). *Biol. J. Linn. Soc. Lond.* **63**: 141–59.
- Baker, J.A., Heins, D.C., King, R.W. & Foster, S.A. 2011. Rapid shifts in multiple life history traits in a population of threespine stickleback. *J. Evol. Biol.* **24**: 863–870.
- Ballantyne, C.K. 2010. Extent and deglacial chronology of the last British-Irish Ice Sheet: Implications of exposure dating using cosmogenic isotopes. *J. Quat. Sci.* **25**: 515–534.
- Baltz, D.M. & Moyle, P.B. 1982. Life history characteristics of tule perch (*Hysterocarpus traski*) populations in contrasting environments. *Environ. Biol. Fishes* **7**: 229–242.
- Bårdsen, B.J., Henden, J.A., Fauchald, P., Tveraa, T. & Stien, A. 2011. Plastic reproductive allocation as a buffer against environmental stochasticity - linking life history and population dynamics to climate. *Oikos* **120**: 245–257.
- Baumann, H., Peck, M.A. & Herrmann, J.P. 2005. Short-term decoupling of otolith and

- somatic growth induced by food level changes in postlarval Baltic sprat, *Sprattus sprattus*. *Mar. Freshw. Res. Freshw. Res* **56**: 539–547.
- Beckerman, A.P., Rodgers, G.M. & Dennis, S.R. 2010. The reaction norm of size and age at maturity under multiple predator risk. *J. Anim. Ecol.* **79**: 1069–1076.
- Bell, A.M., Dingemanse, N.J., Hankison, S.J., Langenhof, M.B.W. & Rollins, K. 2011. Early exposure to nonlethal predation risk by size-selective predators increases somatic growth and decreases size at adulthood in threespined sticklebacks. *J. Evol. Biol.* **24**: 943–953.
- Bell, M. & Foster, S. 1994. *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford.
- Berner, D. & Blanckenhorn, W.U. 2007. An ontogenetic perspective on the relationship between age and size at maturity. *Funct. Ecol.* **21**: 505–512.
- Biggs, J., Fox, G., Nicolet, P., Walker, D., Whitfield, M. & Williams, P. 1998. *A guide to the methods of the National Pond Survey*. Oxford.
- Biro, P.A., Abrahams, M. V., Post, J.R. & Parkinson, E.A. 2006. Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. *J. Anim. Ecol.* **75**: 1165–1171.
- Blanc, J.M. 2003. Comparison of experimental designs for estimating quantitative genetic parameters in sh. *Aquaculture* **34**: 1099–1105.
- Blouw, D.M. & Hagen, D.W. 1981. Ecology of the fourspine stickleback, *Apeltes quadracus*, with respect to a polymorphism for dorsal spine number. *Can. J. Zool.* **59**: 1677–1692.
- Borland, M. 1986. Size-assortative mating in threespine sticklebacks from two sites on the Salmon river, British Columbia. The University of British Columbia.
- Braun, D.C., Patterson, D.A. & Reynolds, J.D. 2013. Maternal and environmental influences on egg size and juvenile life-history traits in Pacific salmon. *Ecol. Evol.* **3**: 1727–1740.
- Buckmeier, D.L., Irwin, E.R., Betsill, R.K. & Prentice, J.A. 2002. Validity of otoliths and pectoral spines for estimating ages of channel catfish. *North Am. J. Fish. Manag.* **22**: 934–942.
- Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* **59**: 197–242.
- Campana, S.E. 2005. Otolith science entering the 21st century. *Mar. Freshw. Res.* **56**: 485–495.
- Campana, S.E. & Neilson, J.D. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci* **42**: 1014–1032.
- Campana, S.E. & Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci* **58**: 30–38.
- Chan, Y.F., Marks, M.E., Jones, F.C., Villarreal, G., Shapiro, M.D., Brady, S.D., *et al.* 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. *Science (80-.)*. **327**: 302–5.

- Charnov, E.L. 1997. Trade-off-invariant rules for evolutionary stable life histories. *Nature* **387**: 393–394.
- Comstock, R.. & Robinson, H.. 1948. The Components of Genetic Variance in Populations of Biparental Progenies and Their Use in Estimating the Average Degree of Dominance. *Biometrics* **4**: 254–266.
- Conover, D. & Munch, S.B. 2002. Sustaining fisheries yields over evolutionary time scales. *Science* (80-.). **297**: 94–96.
- Crozier, L.G. & Hutchings, J.A. 2014. Plastic and evolutionary responses to climate change in fish. *Evol. Appl.* **7**: 68–87.
- Dahlke, F.T., Politis, S.N., Butts, I.A.E., Trippel, E.A. & Peck, M.A. 2016. Fathers modify thermal reaction norms for hatching success in Atlantic cod, *Gadus morhua*. *J. Exp. Mar. Bio. Ecol.* **474**: 148–155.
- Day, T., Pritchard, J. & Schluter, D. 1994. A Comparison of Two Sticklebacks. *Evolution* (N. Y). **48**: 1723–1734.
- Day, T. & Rowe, L. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *Am. Nat.* **159**: 338–350.
- de Lira, J.J.P.R., Calado, T.C. dos S., Rezende, C.F. & Silva, J.R.F. 2015. Comparative biology of the crab *Goniopsis cruentata*: geographic variation of body size, sexual maturity, and allometric growth. *Helgol. Mar. Res.* **69**: 335–342.
- De Roij, J. 2010. Spatial variation in host-parasite interactions in the three-spined stickleback. The University of Nottingham.
- De Roij, J., Harris, P.D. & MacColl, A.D.C. 2011. Divergent resistance to a monogenean flatworm among three-spined stickleback populations. *Funct. Ecol.* **25**: 217–226.
- Defaveri, J. & Merila, J. 2013. Variation in age and size in Fennoscandian three-spined sticklebacks (*Gasterosteus aculeatus*). *PLoS One* **8**: 8–13.
- Dieckmann, U. & Heino, M. 2007. Probabilistic maturation reaction norms: Their history, strengths, and limitations. *Mar. Ecol. Prog. Ser.* **335**: 253–269.
- Dobson, M., Pawley, S., Fletcher, M. & Powell, A. 2012. *Guide to Freshwater Invertebrates*. Freshwater Biological Association, Cumbria.
- Einum, S. & Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc. Biol. Sci.* **266**: 2095–2100.
- El Nagar, A. & MacColl, A.D.C. 2016. Parasites contribute to ecologically dependent postmating isolation in the adaptive radiation of three-spined stickleback. *Proc. R. Soc. B - Biol. Sci.* **283**: 201606.
- Erickson, C.M. 1983. Age determination of Manitoban walleyes using otoliths, dorsal spines, and scales. *North Am. J. Fish. Manag.* **3**: 176–181.
- Erikstad, K.E., Fauchald, P., Tveraa, T. & Steen, H. 1998. On the Cost of Reproduction in Long-Lived Birds : The Influence of Environmental Variability. *Ecology* **79**: 1781–

1788.

- Ernande, B., Dieckmann, U. & Heino, M. 2004. Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. *Proc. R. Soc. Lond. B* **271**: 415–423.
- Ford, N.B. & Seigel, R.A. 2015. The influence of female body size and shape on the trade-off between offspring number and offspring size in two viviparous snakes. *J. Zool.* **295**: 154–158.
- Fox, C.. & Rauter, C.. 2003. Bet-hedging and the evolution of multiple mating. *Evol. Ecol. Res.* **5**: 273–286.
- Fox, M.G. 1994. Growth , density , and interspecific influences on pumpkinseed sunfish life histories. *Ecology* **75**: 1157–1171.
- Fox, M.G. & Keast, A. 1991. Effect of overwinter mortality on reproductive life history characteristics of pumpkinseed (*Lepomis gibbosus*) populations. *Can. J. Fish. Aquat. Sci.* **48**: 1792–1799.
- Gale, B.H., Johnson, J.B., Schaalje, G.B. & Belk, M.C. 2013. Effects of predation environment and food availability on somatic growth in the Livebearing Fish *Brachyrhaphis rhabdophora* (Pisces : Poeciliidae). *Ecol. Evol.* **3**: 326–333.
- Gambling, S.J. & Reimchen, T.E. 2012. Prolonged life span among endemic *Gasterosteus* populations. *Can. J. Zool. Can. Zool.* **90**: 284–290.
- Giles, N. 1981. Predation effects upon the behaviour and ecology of Scottish *Gasterosteus aculeatus* L. populations. University of Glasgow.
- Green, B.S., Gardner, C., Hochmuth, J.D. & Linnane, A. 2014. Environmental effects on fished lobsters and crabs. *Rev. Fish Biol. Fish.* **24**: 613–638.
- Greenbank, J. & Nelson, P. 1959. Life history of the threespine stickleback *Gasterosteus aculeatus* Linnaeus in Karluk lake and Bare lake, Kodiak island, Alaska. *Fish B-NOAA* **59**: 537–559.
- Grenouillet, G., Pont, D. & Seip, K.L. 2002. Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. *Ecography (Cop.)*. **25**: 641–650.
- Hart, P.J.B. 2003. Habitat use and feeding behaviour in two closely related fish species, the three-spined and and nine-spined stickleback: an experimental analysis. *J. Anim. Ecol.* **72**: 777–783.
- Harvey, J.T., Loughlin, T.R., Perez, M.C. & Oxman, D.S. 2000. Relationship between fish size and otolith length for 63 species of fishes from the Eastern North Pacific Ocean. *NOAA Tech. Rep. NMFS* **150**.
- Heino, M. & Dieckmann, U. 2008. Detecting fisheries-induced life-history evolution : An overview of the reaction-norm approach. *Bull. Mar. Sci.* **83**: 69–93.
- Heino, M., Dieckmann, U. & Godø, O.R. 2002. Measuring probabilistic reaction norms for age and size at maturation. *Evolution (N. Y.)*. **56**: 669–678.

- Heino, M. & Godo, O.R. 2002. Fisheries-induced selection pressures in the context of sustainable fisheries. *Bull. Mar. Sci.* **70**: 639–656.
- Heins, D. & Baker, J.A. 1988. Egg sizes in fishes: do mature oocytes accurately demonstrate size statistics of ripe ova? *Copeia* **1988**: 238–240.
- Hendry, A.P., Day, T. & Cooper, A.B. 2001. Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *Am. Nat.* **157**: 387–407.
- Hippel, F.A. Von, Smayda, L.C., Zimmerman, C.E. & Bell, M.A. 2013. Validation of daily growth increments in otoliths to age threespine stickleback (*Gasterosteus aculeatus*). *Evol. Ecol. Res.* **15**: 947–957.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.
- Howells, G.D., Brown, D.J.A. & Sadler, K. 1983. Effects of Acidity, Calcium, and Aluminium on Fish Survival and Productivity-A Review. *J. Sci. Food Agric.* **34**: 559–570.
- Hunter, A., Speirs, D.C. & Heath, M.R. 2015. Fishery-induced changes to age and length dependent maturation schedules of three demersal fish species in the Firth of Clyde. *Fish. Res.* **170**: 14–23.
- Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. *Heredity (Edinb.)* **106**: 421–437.
- Hynes, H.B.N. 1950. The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *J. Anim. Ecol.* **19**: 36–58.
- Jackson, J.R. 2007. Earliest references to age determination of fishes and their early application to the study of fisheries. *Fisheries* **32**: 321–328.
- Jennings, M.J. & Philipp, D.P. 1992. Reproductive investment and somatic growth rates in longear sunfish. *Environ. Biol. Fishes* **35**: 257–271.
- Jones, C.M. 1992. Development and application of the otolith increment technique. In: *Otolith Microstructure Examination and Analysis* (D. K. Stevenson & S. E. Campana, eds), pp. 1–11. Can. Spec. Publ. Fish. Aquat. Sci.
- Jones, C.M. & Chen, Z. 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with plasma mass spectrometry (SF-ICP-MS). *Proc. 26th Annu. Larval Fish Conf.* 431–443.
- Jones, J.W. & Hynes, H.B.N. 1950. The age and growth of *Gasterosteus aculeatus*, *Pygosteus pungitius* and *Spinachia vulgaris*, as shown by their otoliths. *J. Anim. Ecol.* **19**: 59–73.
- Jonsson, B., Finstad, A.G. & Jonsson, N. 2012. Johnson 2012, Effect of temperature and food on age at maturity of salmon.pdf. *Can. J. Fish. Aquat. Sci.* **69**: 1817–1826.
- Jørgensen, C., Auer, S.K. & Reznick, D.N. 2011. A model for optimal offspring size in fish, including live-bearing and parental effects. *Am. Nat.* **177**: E119–E135.

- Jørgensen, C., Enberg, K., Dunlop, E.S., Arlinghaus, R., Boukal, D.S., Brander, K., *et al.* 2007. Ecology: managing evolving fish stocks. *Science* (80-.). **318**: 1247–1248.
- Kalbe, M., Eizaguirre, C., Dankert, I., Reusch, T.B.H., Sommerfeld, R.D., Wegner, K.M., *et al.* 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proc. Biol. Sci.* **276**: 925–34.
- Kasimatis, K. & Riginos, C. 2015. A phylogenetic analysis of egg size, clutch size, spawning mode, adult body size, and latitude in reef fishes. *Coral Reefs* **35**: 1–11.
- Katsiadaki, I., Sanders, M., Sebire, M., Nagae, M., Soyano, K. & Scott, A.P. 2007. Three-spined stickleback: an emerging model in environmental endocrine disruption. *Environ.Sci.* **14**: 263–283.
- Kneale, D. & Viles, H. 2000. Beach cement: Incipient CaCO₃-cemented beachrock development in the upper intertidal zone, North Uist, Scotland. *Sediment. Geol.* **132**: 165–170.
- Koeda, K., Ishihara, T., Fukagawa, T. & Tachihara, K. 2016. Life cycle differences between two species of genus *Pempheris* based on age determination around Okinawa-jima Island. *Ichthyol. Res.* **63**: 519–528.
- Kokkonen, E., Vainikka, A. & Heikinheimo, O. 2015. Probabilistic maturation reaction norm trends reveal decreased size and age at maturation in an intensively harvested stock of pikeperch *Sander lucioperca*. *Fish. Res.* **167**: 1–12.
- Kraak, S.B.M. 2007. Does the probabilistic maturation reaction norm approach disentangle phenotypic plasticity from genetic change? *Mar. Ecol. Prog. Ser.* **335**: 295–300.
- Kuparinen, A., Cano, J.M., Loehr, J., Herczeg, G., Gonda, A. & Merilä, J. 2011. Fish age at maturation is influenced by temperature independently of growth. *Oecologia* **167**: 435–443.
- Law, R. 2000. Fishing, selection, and phenotypic evolution. *ICES J. Mar. Sci.* **57**: 659–668.
- Leaver, S.D. & Reimchen, T.E. 2012. Abrupt changes in defence and trophic morphology of the giant threespine stickleback (*Gasterosteus* sp.) following colonization of a vacant habitat. *Biol. J. Linn. Soc.* **107**: 494–509.
- Leinonen, T., Cano, J.M. & Merilä, J. 2011. Genetics of body shape and armour variation in threespine sticklebacks. *J. Evol. Biol.* **24**: 206–218.
- Leips, J., Richardson, J.M.L., Rodd, F.H. & Travis, J. 2009. Adaptive maternal adjustments of offspring size in response to conspecific density in two populations of the least killifish, *heterandria formosa*. *Evolution (N. Y.)* **63**: 1341–1347.
- Litvak, M.K. & Leggett, W.C. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. *Mar. Ecol. Prog. Ser.* **81**: 13–24.
- Loehr, J., Leinonen, T., Herczeg, G., O'Hara, R.B. & Merilä, J. 2012. Heritability of asymmetry and lateral plate number in the threespine stickleback. *PLoS One* **7**.
- Lucek, K., Sivasundar, A., Kristjansson, B.K., Skulason, S. & Seehausen, O. 2014. Quick divergence but slow convergence during ecotype formation in lake and stream

- stickleback pairs of variable age. *J. Evol. Biol.* **27**: 1878–1892.
- MacColl, A.D.C. 2011. The ecological causes of evolution. *Trends Ecol Evol* **26**: 514–522.
- MacColl, A.D.C. & Aucott, B. 2014. Inappropriate analysis does not reveal the ecological causes of evolution of stickleback armour: a critique of Spence et al. 2013. *Ecol. Evol.* 3509–3513.
- MacColl, A.D.C., El Nagar, A. & De Roij, J. 2013. The evolutionary ecology of dwarfism in three-spined sticklebacks. *J. Anim. Ecol.* **82**: 642–652.
- Magalhaes, I.S., D’Agostino, D., Hohenlohe, P.A. & MacColl, A.D.C. 2016. The ecology of an adaptive radiation of three-spined stickleback from North Uist, Scotland. *Mol. Ecol.* **25**: 4319–4336.
- Magurran, A.. 2004. *Measuring biological diversity*. Blackwell Science Ltd, Oxford.
- Mapp, J.J.I., Fisher, M.H., Atwood, R.C., Bell, G.D., Greco, M.K., Songer, S., *et al.* 2016. Three-dimensional rendering of otolith growth using phase contrast synchrotron tomography. *J. Fish Biol.* **44**: 1–6.
- Marshall, D.J. & Uller, T. 2007. When is a maternal effect adaptive? *Oikos* **116**: 1957–1963.
- Martin, T.E., Lloyd, P., Bosque, C., Barton, D.C., Biancucci, A.L., Cheng, Y.-R., *et al.* 2011. Growth rate variation among Passerine species in tropical and temperate Sites: an antagonistic interaction between parental food provisioning and nest predation risk. *Evolution (N. Y.)*. **65**: 1607–1622.
- Marty, L., Rochet, M.J. & Ernande, B. 2014. Temporal trends in age and size at maturation of four North Sea gadid species: Cod, haddock, whiting and Norway pout. *Mar. Ecol. Prog. Ser.* **497**: 179–197.
- Matta, M.E., Helser, T.E. & Black, B.A. 2016. Otolith biochronologies reveal latitudinal differences in growth of Bering Sea yellowfin sole *Limanda aspera*. *Polar Biol.* 1–13.
- McBride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A., Wuenschel, M.J., *et al.* 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. *Fish Fish.* **16**: 23–57.
- Mccairns, R.J.S. & Bernatchez, L. 2012. Plasticity and heritability of morphological variation within and between parapatric stickleback demes. *J. Evol. Biol.* **25**: 1097–1112.
- McGinley, M., Temme, D. & Geber, M. 1987. Parental investment in offspring in variable environments: Theoretical and empirical considerations. *Am. Nat.* **130**: 370–398.
- Merilä, J. & Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evol. Appl.* **7**: 1–14.
- Mittelbach, G.G. 1988. Competition among refuging sunfishes and effects of fish density on littoral zone invertebrates. *Ecology* **69**: 614–623.
- Morris, D.W. 1987. Optimal allocation of parental investment. *Oikos* **49**: 332–339.
- Morrongiello, J.R., Bond, N.R., Crook, D. a. & Wong, B.B.M. 2012. Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. *J. Anim.*

- Ecol.* **81**: 806–817.
- Moser, D., Roesti, M. & Berner, D. 2012. Repeated Lake-Stream Divergence in Stickleback Life History within a Central European Lake Basin. *PLoS One* **7**.
- Mousseau, T.A. & Roff, D.A. 1987. Natural selection and the heritability of fitness components. *Heredity (Edinb)*. **59**: 181–197.
- Murphy, A.D., Goedert, D. & Morris, M.R. 2014. Maternal effects are long-lasting and influence female offspring's reproductive strategy in the swordtail fish *Xiphophorus multilineatus*. *J. Evol. Biol.* **27**: 1613–1622.
- Nilsson, J., Backström, T., Stien, L.H., Carlberg, H., Jeuthe, H., Magnhagen, C., *et al.* 2016. Effects of age and rearing environment on genetic parameters of growth and body weight and heritability of skin pigmentation in Arctic charr (*Salvelinus alpinus* L.). *Aquaculture* **453**: 67–72.
- Oomen, R.A. & Hutchings, J.A. 2016. Genetic variation in life-history trait plasticity between Atlantic cod (*Gadus morhua*) populations exposed to contrasting thermal regimes. *Can. J. Zool.* **94**: 257–264.
- Oravec, T.J. & Reimchen, T.E. 2013. Divergent reproductive life histories in Haida Gwaii stickleback (*Gasterosteus* spp.). *Can. J. Zool.* **91**: 17–24.
- Panella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science (80-.)*. **173**: 1124–1127.
- Parker, G.A. & Begon, M. 1986. Optimal Egg Size and Clutch Size: Effects of Environment and Maternal Phenotype. *Am. Nat.* **128**: 573–592.
- Peichel, C.L., Nereng, K.S., Ohgi, K. a, Cole, B.L., Colosimo, P.F., Buerkle, C. a, *et al.* 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* **414**: 901–905.
- Pérez-Rodríguez, A., Morgan, J., Koen-Alonso, M. & Saborido-Rey, F. 2013. Disentangling genetic change from phenotypic response in reproductive parameters of Flemish Cap cod *Gadus morhua*. *Fish. Res.* **138**: 62–70.
- Plaistow, S.J., Lapsley, C.T., Beckerman, A.P. & Benton, T.G. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. *Proc. Biol. Sci.* **271**: 919–924.
- Price, T.D., Qvarnström, A. & Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. - Biol. Sci.* **270**: 1433–1440.
- Räsänen, K., Söderman, F., Laurila, A. & Merilä, J. 2008. Geographic variation in maternal investment: Acidity affects egg size and fecundity in *Ran arvalis*. *Ecology* **89**: 2553–2562.
- Reimchen, T.E. 1992. Extended longevity in a large-bodied stickleback, *Gasterosteus*, population. *Can. F. Nat.* **106**: 122–125.
- Reznick, D. 1983. The Structure of guppy life histories: The tradeoff between growth and reproduction. *Ecology* **64**: 862–873.

- Reznick, D.A., Bryga, H. & Endler, J.A. 1990. Experimentally induced life-history evolution. *Nature* **346**: 183–187.
- Reznick, D., Butler IV, M.J. & Rodd, H. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat.* **157**: 126–140.
- Reznick, D.N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): An experimental evaluation of alternative models of development. *J. Evol. Biol.* **3**: 185–203.
- Robertson, S., Bradley, J.E. & Maccoll, A.D.C. 2016. Measuring the immune system of the three-spined stickleback - investigating natural variation by quantifying immune expression in the laboratory and the wild. *Mol. Ecol. Resour.* **16**: 701–713.
- Roesti, M., Kueng, B., Moser, D. & Berner, D. 2015. The genomics of ecological vicariance in threespine stickleback fish. *Nat. Commun.* **6**: 8767.
- Roff, D.A. 2001. Age and Size at Maturity. In: *Evolutionary Ecology; Concepts and Case Studies* (C. W. Fox et al., eds), pp. 99–112. Oxford University Press, Oxford.
- Roff, D.A. 1992. *The Evolution of Life Histories Theory and Analysis*. Chapman & Hall, Inc, New York.
- Rollinson, N. & Hutchings, J.A. 2013a. Environmental quality predicts optimal egg size in the wild. *Am. Nat.* **182**: 76–90.
- Rollinson, N. & Hutchings, J.A. 2013b. The relationship between offspring size and fitness: Integrating theory and empiricism. *Ecology* **94**: 315–324.
- Rollinson, N. & Rowe, L. 2015. The positive correlation between maternal size and offspring size: Fitting pieces of a life-history puzzle. *Biol. Rev.* **91**: 1134–1148.
- Scharf, F.S., Juanes, F. & Rountree, R. a. 2000. Predator size - Prey size relationships of marine fish predators: Interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Mar. Ecol. Prog. Ser.* **208**: 229–248.
- Scheiner, S.M.. 1993. Genetics and Evolution of Phenotypic Plasticity. *Annu. Rev. Ecol. Syst.* **24**: 35–68.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**: 676–682.
- Schrader, M. & Travis, J. 2012. Assessing the roles of population density and predation risk in the evolution of offspring size in populations of a placental fish. *Ecol. Evol.* **2**: 1480–1490.
- Sih, A. & Moore, R.D. 1993. Delayed Hatching of Salamander Eggs in Response to Enhanced Larval Predation Risk. *Am. Nat.* **142**: 947–960.
- Skogland, T. 1985. The effects of density-dependent resource limitations on the demography of wild reindeer. *J. Anim. Ecol.* **54**: 359–374.
- Smith, B. & Wilson, J.B. 1996. A Consumer &sup>TM&sup> s Guide to Evenness Indices. *Oikos* **76**:

- Smith, C.C. & Fretwell, S.D. 1974. The Optimal Balance between Size and Number of Offspring. *Am. Nat.* **108**: 499–506.
- Snyder, R.J. 1991. Quantitative genetic analysis of life histories in two freshwater populations of the threespine stickleback. *Copeia* **1991**: 526–529.
- Snyder, R.J. & Dingle, H. 1989. Adaptive, genetically based differences in life history between estuary and freshwater threespine sticklebacks (*Gasterosteus aculeatus* L.). *Can. J. Zool. J. Can. Zool.* **67**: 2448–2454.
- Spence, R., Wootton, R.J., Barber, I., Przybylski, M. & Smith, C. 2013. Ecological causes of morphological evolution in the three-spined stickleback. *Ecol. Evol.* **3**: 1717–1726.
- Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Stearns, S.C. & Koella, J.C. 1986. The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. *Evolution (N. Y.)* **40**: 893–913.
- Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J. & Baguette, M. 2012. How is dispersal integrated in life histories: A quantitative analysis using butterflies. *Ecol. Lett.* **15**: 74–86.
- Swan, S.C. & Gordon, J.D.M. 2001. A review of age estimation in macrourid fishes with new data on age validation of juveniles. *Fish. Res.* **51**: 177–195.
- Thorrold, S.R. & Shuttleworth, S. 2000. In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled plasma mass spectrometry. *Can. J. Fish. Aquat. Sci.* **57**: 1232–1242.
- Trip, E.D.L., Clements, K.D., Raubenheimer, D. & Choat, J.H. 2014. Temperature-related variation in growth rate, size, maturation and life span in a marine herbivorous fish over a latitudinal gradient. *J. Anim. Ecol.* **83**: 866–875.
- Turbill, C., Bieber, C. & Ruf, T. 2011. Hibernation is associated with increased survival and the evolution of slow life histories among mammals. *Proc. R. Soc. B Biol. Sci.* **278**: 3355–3363.
- Uriarte, A., Rico, I., Villamor, B., Duhamel, E., Duenas, C., Aldanondo, N., *et al.* 2016. Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L) in the Bay of Biscay. *Mar. Freshw. Res.* **67**: 951–966.
- Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D. & Van Tienderen, P.H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* **10**: 212–217.
- Voje, K.L., Mazzearella, A.B., Hansen, T.F., Østbye, K., Klepaker, T., Bass, A., *et al.* 2013. Adaptation and constraint in a stickleback radiation. *J. Evol. Biol.* **26**: 2396–2414.
- Vøllestad, L.A., Peterson, J. & Quinn, T.P. 2004. Effects of Freshwater and Marine Growth Rates on Early Maturity in Male Coho and Chinook Salmon. *Trans. Am. Fish. Soc.* **133**: 495–503.

- VSN International. 2011. Genstat for Windows 14th Edition. VSN International, Hemel Hempstead.
- Waldron, M.E. & Kerstan, M. 2001. Age validation in horse mackerel (*Trachurus trachurus*) otoliths. *ICES J. Mar. Sci. – ICES J. Mar. Sci.* **58**: 806–813.
- Waterston, A.R., Holden, A. V., Campbell, R.N. & Maitland, P.S. 1979. The Inland waters of the Outer Hebrides. *Proc. R. Soc. Edinburgh* **77**: 329–351.
- Wilbur, H.M. & Collins, J.P. 1973. Ecological Aspects of Amphibian Metamorphosis: Nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science* **182**: 1305–1314.
- Williams, N.M., Crone, E.E., Roulston, T.H., Minckley, R.L., Packer, L. & Potts, S.G. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biol. Conserv.* **143**: 2280–2291. Elsevier Ltd.
- Williams, T. & Bedford, B. 1974. The use of otoliths for age determination. In: *Ageing of Fish* (T. Bagenal, ed), pp. 114–123. Unwin Brothers Limited, Surrey.
- Wootton, R.J. & Fletcher, D.A. 2009. Effect of spawning number and ration on reproductive performance of the batch-spawning three-spined stickleback *Gasterosteus aculeatus*. *J. Fish Biol.* **75**: 618–629.
- Wund, M.A., Valena, S., Wood, S. & Baker, J. a. 2012. Ancestral plasticity and allometry in threespine stickleback fish reveal phenotypes associated with derived, freshwater ecotypes. *Biol. J. Linn. Soc.* **105**: 573–583.
- Zera, A.J. & Harshman, L.G. 2001. The Physiology of Life History Trade-Offs in Animals. *Annu. Rev. Ecol. Syst.* **32**: 95–126.