The impacts of river impoundments on the biology of the three-spined stickleback, *Gasterosteus aculeatus*

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Statement of contributors

I hereby declare that all of the work in the following thesis is my own research and was carried out by myself, unless otherwise stated. The following exceptions apply:

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Abstract

THE IMPACTS OF RIVER IMPOUNDMENTS ON THE BIOLOGY OF THE THREE-SPINED STICKLEBACK, *GASTEROSTEUS ACULEATUS*

Swati Nettleship

River impoundments cause a dramatic shift in the environment from a fluvial habitat to a static one. As such, they are likely to cause considerable changes to the biology of species living in those rivers, including the three-spined stickleback, *Gasterosteus aculeatus*.

Sticklebacks from streams were wider and deeper bodied than those from reservoirs, consistent with differences observed between lake-stream pairs (e.g. Hendry *et al*., 2002; Berner *et al*., 2010). Variation in armour morphology was not as associated with habitat type and was more likely affected by variation in the predation regime. Although stream fish had wider mouths and shorter gill rakers than those in reservoirs, differences expected based on the likely changes in prey type availability as a result of impoundments (Berner *et al*., 2009), there were no associated changes in prey preferences or feeding efficiency. Overall, feeding efficiency was best predicted by an individual's standard length.

The degree of morphological divergence was not correlated with levels of neutral genetic differentiation, suggesting that divergence has been the result of natural selection acting on traits differently in diverse habitats. However, the extent to which traits were heritable was population specific and highlights the importance of assessing and estimating the strength of selection for populations separately.

There were also differences in the timing of the onset of the breeding season. Although reservoir sticklebacks began breeding up to 2 months before stream sticklebacks, stream fish showed compensatory growth so that there were no differences in size at the onset of winter and subsequent end of the growing season.

This thesis demonstrates how reservoir-stream systems can be used as a proxy for studying divergence in lake-stream systems but with additional information about the age of the system. A recurrent theme is that of variation between systems and highlights the importance of studying multiple systems simultaneously.

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Chapter 1

General impacts of river impoundments on fish biology

1.1. INTRODUCTION

River impoundments convert previously lentic (flowing) ecosystems into lotic (static) lakes and cause significant changes to both physical and ecological aspects of aquatic environments. Aquatic organisms specialised to fluvial ecosystems may therefore be lost following impoundments. Those species able to survive the conversion of fluvial habitats to still waters face a range of physical challenges, including altered flow regime, water chemistry, oxygen levels and water temperature (Gilvear *et al.* 2002). Lakes and rivers also vary considerably in food web structure, so that survival in the new environment is expected to require a modified diet (de Mérona & Vigouroux 2006; Roberts *et al.* 2007). Furthermore, changes to an environment can bring new and different species into the area that may compete for resources. Some of these may predate on native species, forcing them to adapt behaviourally and morphologically if they are to avoid becoming prey (Bertrand *et al.* 2008; Sharma & Borgstrøm 2008).

The main focus of this thesis has been on understanding the impacts of river impoundments on populations of three-spined sticklebacks, *Gasterosteus aculeatus* – small, ubiquitous fish found across the temperate regions of the northern hemisphere and often used as a model in evolutionary ecological research (Wootton 1976). The formation of man-made reservoirs over short evolutionary time scales provides the opportunity to study how reservoirs can be used as a tool for studying how species adapt to rapid and substantial environmental changes. By studying the changes in biology of a species following its impoundment in man-made lakes, it is possible to gain an insight into the mechanisms by which organisms adapt to novel environments.

1.2. PHYSICAL CHANGES TO THE AQUATIC ECOSYSTEM ASSOCIATED WITH RIVER IMPOUNDMENTS

1.2.1. Flow regimes

The flow regime of streams and rivers is typically highly variable and is affected as much by land use as it is by precipitation (Hynes 1979). Differences in flow are the result of evaporation from the environment, which is greater in forested areas than it is in areas of heather or grassland, the level of precipitation and the physical attributes of the river or stream (i.e. width, depth and land gradient) (Johnson 1995). In the UK, the monthly flow of rivers, measured as cubic metres per second (m^3s^{-1}), is generally lowest in the south of England and greatest in Scotland, and can range from less than $1m^3s^{-1}$ to over $800m^3s^{-1}$ (Marsh & Hannaford 2008).

One of the most obvious and immediate effects of damming rivers is the dramatic cessation of flow in the newly created reservoir and the associated artificially enhanced increase in flow downstream of the reservoir. Although water currents may still be evident in the newly-created reservoir, these are typically multidirectional and the result of water management practises and prevailing wind direction rather than a continuous uni-directional flow generated by gradient. This means that areas that act as a refuge from currents on one day may become areas highly exposed to prevailing winds and currents the next.

Common uses for reservoirs include water supply, hydroelectric power, flood control, irrigation, navigation and the downstream regulation of river flow, which are likely to determine the site that a reservoir is built (British Dam Society, 2010). For example, the Galloway Hydro-Electric Scheme uses a network of eight dams to produce 109MW of power, enough energy to power in the region of 20,000 homes (Scottish Power, 2010). Water is stored in the various reservoirs and the force with which it is

released to downstream power stations is harnessed to produce energy. Hence, reservoirs used for hydroelectric purposes undergo rapid filling and emptying and benefit from a fast inflowing river. This is in stark contrast to reservoirs that are primarily used for recreation or flood control, where filling and emptying is less extreme and are usually in lowland areas.

1.2.2. Silt and sediment deposits affecting macrophyte growth

Flowing rivers invariably carry particles of silt and sediment. As water enters a reservoir and becomes stagnant, particles carried in the river are deposited and begin to accumulate (Moss 1988). As they do, they release metallic elements such as manganese and iron in addition to phosphorus compounds which, under normal lentic conditions, would be washed away and diluted. However, due to the lack of water current, they are deposited in the reservoir and this encourages the growth of aquatic macrophytes. Aquatic plants prevent sediment re-suspension and thus lower water turbidity (Horppila & Nurminen 2003). Macrophytes provide food and shelter for zooplankton and invertebrates, contributing to the food web structure (Mustapha 2008). However, extremely high densities of aquatic plants can also lower oxygen concentrations and have a detrimental impact on fish (Petr 2000). Furthermore, as they decay, aquatic plants decrease dissolved oxygen concentrations and lower the quality of the collecting water further (Ogbeibu & Oribhabor 2002).

1.2.3. Temperature and thermal stratification

The turbulent flow of water in rivers and streams means that they do not form clearly stratified thermal layers and as such, temperatures vary little with depth (Hynes 1979) and tend to remain cooler than still waters (Null *et al*. 2010). Instead, they show

diurnal cycles of warming and cooling that are controlled by the immediate surroundings and upstream conditions (Macan 1958; Johnson 2003).

Impounded bodies of water, on the other hand, are much more prone to thermal stratification, as both daily and seasonal occurrences (Zoran *et al.* 1994). Stratification leads to warm, well oxygenated surface waters (epilimnion) appropriate for fish life but cooler anoxic bottom waters (hypolimnion) where toxic metabolites quickly accumulate (Gilvear *et al.* 2002). Separating the epilimnion and hypolimnion is the metalimnion, a zone of steep decline in temperature with depth.

Some countries (e.g. Israel and India) have adapted reservoirs to use for fish culture (Milstein & Zoran 2001; Jayasinghe *et al.* 2006), which affects where water is drawn from and thus further impacts temperature. If the reservoir is to be used as a source of drinking water, water is primarily drawn from the epilimnion, leaving the remaining water more anoxic. Conversely, if used for fish culture, then water is drawn from the hypolimnion, removing anoxic waters that can be dangerous to fish (Milstein & Zoran 2001).

1.2.4. Ionic concentrations

Although most rivers are considered to be fresh water, all natural waters, including a virgin stream emerging from a mountain watershed, contain salts, predominantly carbonates, chlorides and sulphates of calcium, magnesium and sodium (Pillsbury 1981). Salts in freshwater are the result of rain and weathering processes, by which mineral crystals that constitute rock are oxidised and fractured, leading to the production of salts. Thus, as water percolates through permeable rock, salts are dissolved. This underlying geology dictates ionic concentration; for example, in limestone regions, water is often very high in calcium bicarbonate (Hynes 1979).

The fluvial nature of rivers and streams means that salts are eventually transferred out to seas and oceans and continually replenished, but following impoundment, evaporation of water from the reservoir means that the concentration of salts increases in the water, only falling when the reservoir is emptied and re-filled. As such, salinity in reservoirs is a direct function of water residency time i.e. the average age of water in any given location (An & Jones 2000).

It is clear that lakes and rivers vary dramatically in a number of physical properties, and by impounding rivers and creating man-made lakes, many of the riverine features will be lost and replaced by those similar to natural lakes. As a result of the physical properties of flowing and standing waters, there are also differences in sources of food and energy flow, which in turn has ecological consequences on the aquatic organisms that are able to survive and flourish in them (Moss 1988).

1.3. BIOLOGICAL AND ECOLOGICAL CONSEQUENCES OF RIVER IMPOUNDMENTS

1.3.1. Changes in food web structure

Litterfall accounts for much of the organic matter in streams and rivers and provides food for benthic macroinvertebrates (Moss 1988). Studies looking at the composition of stream communities have found that there is a relationship between composition and the physico-chemical environment, which is primarily related to pH (Townsend *et al.* 1983; Rundle & Ramsay 1997). Because invertebrates form a major component of the food web in rivers and streams, pH also indirectly affects the composition of the fish community.

In contrast to streams, the major source of carbon in still waters is due to photosynthetic carbon uptake by phytoplankton. Phytoplankton growth flourishes in

lakes and reservoirs and densities are associated with water residency time (Søballe & Kimmel 1987). However, phytoplankton abundance is also affected by the intensity of light exposed to suspended algae and so is lower in reservoirs with greater water turbidity (Brylinsky & Mann 1973). Phytoplankton biomass is positively correlated with zooplankton abundances (Smyly 1979; Talling 2003), which forms the major component of aquatic food webs in reservoirs.

1.3.2. Changes in the composition of aquatic communities

The morphology of rivers and streams creates a mosaic of microhabitats, whereas a large proportion of reservoir habitats are pelagic in nature. That is not to say that reservoirs are completely homogeneous, as they too have bottoms and edges that create a littoral zone, which in general, support a wider range of communities than the pelagic zone. However, reservoirs created by river impoundments are often accompanied by species loss and colonisation by invasive alien species (Taylor *et al.* 2001; Ogbeibu & Oribhabor 2002; Locke *et al.* 2003). As such, the ecological impact of river impoundments can be assessed by surveying the species present, and using them as environment indicators (Fenoglio *et al.* 2002; Moreno & Callisto 2006). For example, in a study of benthic invertebrates above, within and below a reservoir, oligochaetes were found at higher densities within the reservoir than above or below it, and were attributed to organic enrichment and macrophyte growth (Learner *et al.* 1978; Ogbeibu & Oribhabor 2002). In contrast, decapod crustaceans were completely absent in the reservoir and the number of taxa and density of dipteran larvae significantly reduced (Ogbeibu & Oribhabor 2002).

1.3.3. Impoundment consequences for fish

1.3.3.1. Barriers to fish migration

Many temperate freshwater species migrate between freshwater and marine environments at particular life-cycle stages including several commercially important salmonids (Banks 1969). Dams therefore pose a significant problem as they create barriers to migration, preventing fish from moving to breeding grounds and this can lead to a reduction in species richness in impounded waters (Lundqvist *et al*. 2008; Rolls 2011). Although many dams have been modified to include fish ladders (artificial structures enabling fish to pass around dams using several small 'ladder' steps), these are not always 100% successful (Agostinho *et al.* 2002) and can also cause artificial selection for certain morphological-physiological traits (Volpato *et al.* 2009).

1.3.3.2. Changes in community structure

Impoundments can also cause significant changes to fish species composition (Banks 1969; Porto *et al.* 1999; Gehrke *et al.* 2002; Anderson *et al.* 2006; Guenther & Spacie 2006). In a large scale survey of fish fauna of the Suriname River before and after impoundment, Mol *et al*. (2007) documented a marked drop in species evenness and diversity. Just four years after impoundment, the number of species collected from the newly formed reservoir had dropped dramatically when compared to the number of species that had been identified in the same area prior to the impoundment. The decline continued for a further 11 years before stabilising. Many of the original species with strict habitat requirements did not survive the closure; in one tributary, just 3 of 57 species survived. The loss of species has been uneven, with significant differences in fish community structure evident from the dominance of only a few species.

1.4. SUCCESS IN A NEWLY CREATED ENVIRONMENTS: THE ROLES OF PLASTICITY AND ADAPTIVE DIVERGENCE

1.4.1. Plasticity

A common finding in studies of impoundments have shown that species initially surviving the change from a fluvial to static environment are habitat generalist (Quinn & Kwak 2003; Matthews *et al.* 2004; Guenther & Spacie 2006). Species that survive the transition often exhibit changes in behaviour, morphology and/or physiology, in order to exploit the new environmental conditions. For example, freshwater Mary River turtles *Elusor macrurus* reduce their diving time as a consequence of low oxygen conditions following river impoundments (Clark *et al.* 2009).

Phenotypic plasticity is the ability of an organism to express different phenotypes depending on the biotic or abiotic environment (Agrawal 2001). During periods of environmental change, success is reliant on the ability to exploit resources in novel environments (Schluter 2000). Plasticity of prey choice therefore, is a fundamentally important and favoured trait (Fernando & Holčík 1982; Araya *et al.* 2005; de Mérona & Vigouroux 2006; Pereira *et al.* 2007; Barquete *et al.* 2008).

South American boga, *Lepoinus obtusidens* are commercially important omnivorous fish, naturally inhabiting both lotic and lentic environments, which feed on aquatic macrophytes, snail fragments and Asian clams *Corbicula fluminea* (Mastrarrigo, 1950; cited in García & Protogino 2005). After construction of the Yacyretá dam, the diet of boga adapted to include an Asian golden mollusc *Limnoperna fortune* that had invaded the area in large numbers as a consequence of the impoundment (Araya *et al.* 2005). As a result, there was a dominance of younger boga and an increase in the maximum lifespan from 8 to 13 years. The success of boga was

attributed to its plasticity in prey choice, allowing it to exploit the newly invaded and highly abundant golden mollusc.

In addition to plasticity in prey selection, changes in feeding behaviours may also act to benefit individuals in new or altered habitats. Golden galaxias *Galaxias auratus* usually feed during the day in areas of heavy vegetation and throughout the night in open water. However, following the introduction of brown trout *Salmo trutta*, golden galaxias reduce the amount of time spent foraging in open water, moving to heavily vegetated areas (Stuart-Smith *et al.* 2008). These behavioural changes in foraging habitat use are considered a plastic response as a result of increased predation that minimise the risk of mortality and ensures survival.

1.4.2. Adaptive divergence

Although the ability to undertake plastic responses to changing environmental regimes appears critical for colonising new habitats and surviving ecological change, longer term success is often dependent on subsequent specialisation, particularly in resource-limited environments (Schluter 2000). Prior to impoundment of the Suriname River for example, 172 species of fish fauna were recorded but 14 years after the dam closure, only 41 species were recorded in the recently created reservoir (Mol *et al.* 2007). In particular, species with strict habitat requirements did not survive. Studies of stomach content analyses of fish before and after impoundment have shown that there were significant changes to trophic organisations of fish communities so that post-impoundment, the majority of community biomass was from specialist feeders (de Mérona *et al.* 2001).

Whereas in rivers and streams the main source of food is benthic macroinvertebrates, in lakes and reservoirs, the greatest food source comes from

zooplankton and other small fish (Hynes 1979). Hence successful colonisation and long-term survival in newly impounded waters is often associated with adaptations that maximise feeding efficiency on zooplankton prey (Holčík 1998). Fish yields from natural and ancient lakes are three times those of recently created man-made lakes (Holčík 1998) and it has been suggested that this is due to the absence of true lacustrine fish, i.e. those that are endemic to the lake and specialised for a lake-type environment (Fernando & Holčík 1982). Specialisation is frequently the result of adaptive genetic divergence, which is the divergence of phenotypic traits that influence survival and reproduction between populations, as a consequence of being exposed to different ecological environments (Hendry 2001). Specialisations arise as a result of natural selection on phenotypes, which, in addition to trait variation and the fitness consequences of those variations, require traits to be heritable so that the benefits may be passed on to successive generations (Endler 1986).

Darwin's finches *Geospiza* sp. provide a classic example of adaptive morphological divergence (Lack 1947). Diversity in beak size and shape has been attributed to specialised adaptations for exploiting particular types of foods (Schluter 2000). However, the limited distribution of Darwin's finches makes it difficult to use the species for understanding the processes involved in ecological divergence and speciation. Postglacial fishes, on the other hand, are widespread across the northern hemisphere and show rapid evolution of reproductive isolation, making them ideal for studying the impact of ecological changes.

There are several examples where morphologically distinct populations of a single species coexist in lakes less than 15,000 years but show little cross-breeding (see Schluter 1996, for a summary). For example, based on trophic polymorphisms, distinct morphs of Arctic charr *Salvelinus alpinus* have been identified in a Scottish loch, for

which there is a genetic basis (Hartley *et al.* 1992; Adams *et al.* 1998; Verspoor *et al.* 2010). The pelagic morph is streamlined and feeds primarily on pelagic zooplankton whereas the benthic morph is deep bodied and less streamlined and feeds on benthic macroinvertebrates. Similar patterns of differences have also been observed in other lakes (Skúlason *et al.* 1993; Garduno-Paz & Adams 2010) in addition to other species (McPhail 1992; Taylor & Bentzen 1993; Bernatchez *et al.* 1999; Dynes *et al.* 1999).

1.5. THREE-SPINED STICKLEBACKS AS A MODEL FOR STUDYING ADAPTIVE DIVERGENCE IN RESPONSE TO RAPID ECOLOGICAL CHANGES

The three-spined stickleback, *Gasterosteus aculeatus*, is an ideal study species for investigating the effect of environmental changes on species ecology and evolution because of its broad geographical and ecological distribution (Bell & Foster 1994). The species is characterised by its ability to inhabit both marine and freshwater habitats (rivers and lakes) across the northern hemisphere and has three major life history forms: marine, anadromous and freshwater (Bell *et al.* 1993; McPhail 1994). It is generally agreed that the ancestral state of the stickleback is the marine form (Bell & Foster 1994). The similarity of both Eastern Pacific and Atlantic marine populations to modern anadromous populations, which are temporally and spatially phenotypically stable, implies that they are the most likely source of the colonising individuals in freshwater habitats (Walker & Bell 2000).

1.5.1. Rapid adaptation to a freshwater environment

The existence of distinct anadromous and stream-resident sticklebacks highlights the ability of anadromous stickleback populations to adapt to freshwater

conditions (Hagen 1967). Colonisation of freshwaters across Europe and North America is believed to have occurred after the last glacial retreat, circa 12,000 years ago. It is thought that as the ice sheets retreated, pockets of rising sea water containing stickleback refugia were trapped (Wootton 1976) and most freshwater population of stickleback in North America are in areas that were submerged by the sea after glaciers retreated (McPhail 1994).

Studies in Norwegian populations have shown that divergence from a marine ancestor to a freshwater-tolerant phenotype can occur in less than 40 years (Klepaker 1993). More recently, Kitano et al. (2008a) have discovered that in a similar timeframe, freshwater forms can also revert back to their marine phenotypes. Other studies have demonstrated that sticklebacks show distinct morphological adaptations to changing environments in as little as 12 generations (Kristjánsson *et al.* 2002; Bell *et al.* 2004). Moreover, common-garden experiments have shown that fish from marine tidal pools transferred to freshwater ponds show morphological changes in just one generation (Kristjánsson 2005). The authors have suggested that this extremely rapid divergence is due to high phenotypic plasticity and/or very strong natural selection acting on the first freshwater generation. This raises the possibility that adaptations to newly created reservoirs are likely following the impoundment of rivers.

1.5.2. Variation in body shape and swimming performance

Aquatic environments provide a mosaic of microhabitats and consistent variation in the body shape of fish is often associated with the physical and biological characteristics of habitats most often encountered (Spoljaric & Reimchen 2007; Langerhans & Reznick 2010). This is because of the close link between form and function in teleost fish, with numerous features of body shape being associated with

swimming performance and foraging behaviour (Webb 1984; Walker 1997). In general, streamlined bodies with a narrow caudal peduncle are best suited to open water, sustained swimming whereas deep bodies with a deep caudal peduncle improve burst swimming performance (Webb 1982). Hydrodynamic drag can be reduced by minimising surface area hence fish that can collapse their fins or have slimmer bodies are also able to move through the water with less resistance (Webb 1984; Blake 2004). The morphology of any given fish is therefore a balance of swim requirements as maximising performance in one will often preclude maximisation of the other (Langerhans 2009).

Among freshwater sticklebacks, individuals with a smaller overall surface area and streamlined body show less hydrodynamic drag and improved endurance during prolonged swimming than do large, heavy and deep-bodied individuals with a greater body surface area (Blake *et al.* 2005). Fast-start burst swimming is usually in response to predation and individuals with different body shape morphology often differ little in escape fast-start velocities (Law & Blake 1996; Hendry *et al.* 2011), although deeper-bodied fish show improved manoeuvrability (Hendry *et al.* 2011). In general, freshwater sticklebacks show significantly higher fast-start velocities than ancestral-type anadromous individuals (Law & Blake 1996; Blake *et al.* 2005) and has been associated with a reduction in plate number (Bergstrom 2002; Hendry *et al.* 2011) which may improve flexibility or decrease drag.

1.5.3. Variation in trophic morphology and foraging behaviour

The major components of the trophic structure of fish include the snout (Adams *et al.* 2003; Gillespie & Fox 2003; Whiteley 2007) and gill rakers (Malmquist 1992; Gillespie & Fox 2003), and these differ both between and within species according to

the food types consumed. Gill rakers are small, bony protrusions that project from the inner edge of the branchial gill arch forming a sieve that prevent prey from being lost from the bucco-pharyngeal chamber during feeding (Bell & Foster 1994).

During feeding, the manner of attack largely depends on the location of the prey. Sticklebacks are visual predators and a detailed account of stickleback feeding can be found in Tugendhat (1960). Sticklebacks feeding on substrate-dwelling prey (i.e. benthic invertebrates) tilt their bodies from their usual horizontal swimming position to fixate on the prey item before seizing the prey with a snapping motion. The snapping motion is exaggerated by the protrusion of the jaw and further so by the action of sucking water (and the prey) into the mouth (Wootton 1976). Sticklebacks feeding on free-swimming prey in the main water column (e.g. zooplankton) show greater variation depending on the location of the prey item relative to the individual. If prey are taken from the same position in the water column as the individual, then no body tilt is required, merely a swift strike towards the prey. However, in addition to tilting their bodies downwards to consume prey swimming below them, sticklebacks can also tilt upwards and even break the surface of the water to take prey from the very top.

Zooplankton feeding involves charging and striking at prey in open water and so long and numerous gill rakers are necessary for preventing the loss of small prey; feeding on benthos on the other hand, often involves engulfing large amounts of sediment with the benthic prey, which is then expelled before reingesting the individual prey item (Schluter 1993). Closely spaced gill rakers may become clogged up and hinder breathing in the latter situation, so fewer and shorter gill rakers are better suited. Feeding on benthic prey is also associated with a short snout and relatively wide gape, whilst open water feeding is associated with a longer snout and relatively small gape (Walker & Bell 2000).

Numerous studies have shown that in polymorphic populations of fish, intra-specific variation in trophic morphology is associated with differences in prey type availability and abundance in different habitats (Gross & Anderson 1984; Lavin & McPhail 1986; Adams *et al.* 2003; Hellig *et al.* 2010). In sticklebacks, stomach content analyses have shown that individuals feeding on primarily benthic prey items, such as *Chironomus* sp. larvae and *Gammarus* sp. tend to have shorter and fewer gill rakers than those feeding on zooplankton prey (Gross & Anderson 1984; Lavin & McPhail 1986; Berner *et al.* 2009). In a test of foraging efficiency, sticklebacks with a relatively small gape also do significantly better when feeding on zooplankton than do their larger gaped conspecifics (Ibrahim & Huntingford 1988).

1.5.4. Stickleback armour morphology: spines and plates

Unlike most teleost fish, sticklebacks lack scales but instead are protected by a row of bony lateral plates (Wootton 1976). Sticklebacks vary considerably in the number of these lateral plates (Fig. 1.1): fully plated morphs have been 30-35 plates whereas low plated morphs have between two and five plates located in the central trunk region and are thought to function as a buttress to the pelvic and dorsal spines (Reimchen 1983). Partial or intermediate morphs show lateral plates in the central trunk and additional plates at the tail.

Figure 1.1 Variation in the number of plates seen in the three-spined stickleback. (a) A fully plated morph with around 30-35 plates. (b) A partial plated morph with lateral plates in the central trunk region and additional plates at the tail. (c) A low plated morph with just 5 plates in the central trunk region.

The relatively large dorsal spines, from which the three-spined stickleback derives its name, pelvic spines and lateral plates form armour against predation. Armour defences however, provide the final defence mechanism, and behavioural adaptations may negate the need for potentially costly armour. The spines, which vary between population in length, placement and serration, function to deter predators by increasing the size-appearance of the individual (Hoogland *et al.* 1957; Reimchen 1992), but are also sharp and can injure the oral cavity of potential predators. The pelvic structure consists of a pelvic girdle which, together with the spines and plates, forms an armoured ring around the trunk of the fish (Bell & Orti 1994). Loss or reduction of the pelvic structure has been reported independently in several freshwater stickleback populations in Scotland and North America (Moodie & Reimchen 1976; Reimchen 1980; Bell *et al.* 1993; Bell & Orti 1994; Kristjánsson *et al.* 2002; Shapiro *et al.* 2004).

1.5.4.1. Two hypotheses for the reduction of armour

There are two main hypotheses that have been proposed for the reduction in armour morphology observed in freshwater populations of three-spined stickleback. The predation hypothesis states that the extent of pelvic reduction in lakes and streams is dependent on the abundance and composition of predators (Reimchen 1980). Sticklebacks are predated upon by a wide range of piscivorous fish, birds and invertebrates (Reimchen 1994) hence the extent of armour is likely to be a complex interaction between numbers and types of predators. In habitats where predator communities are dominated by fast-swimming predatory fishes where the likelihood of capture is high, lateral plates, which minimise damage, may improve post-capture survival (Reimchen 1992). Protection from bird predation, on the other hand, may be

better achieved by longer spines so that the predatory bird is more likely to be pieced as it attempts to manipulate the fish (Reimchen 1980). Conversely, it has also been suggested that predation by grappling predators such as dragonfly nymps, *Aeshna palmate* and *A. eremite* which use the spines to grasp young stickleback, may result in the loss or reduction of spines (Reimchen 1980).

Alternatively, the calcium limitation hypothesis (Giles 1983) is based on observations that pelvic reductions also occur in lakes with predatory fishes but where calcium concentrations are low. Sticklebacks absorb calcium from water for skeletal development so in waters of low calcium concentration, there are likely to be additional energetic costs to producing bony armour.

It is likely that neither of the proposed hypotheses above forms a complete answer. In a study of two populations from Cook Inlet, Alaska, Bell *et al*. (1993) demonstrated that the two hypotheses are not necessarily mutually exclusive. Neither low calcium concentrations nor the presence or absence of predatory fishes alone could accurately predict the occurrence or extent of pelvic reduction. Regression analysis of mean pelvic scores suggests that both calcium concentrations and predation influence pelvic reduction, but that the latter limits the effect of the former. Thus, in low calcium concentration waters, whether or not to expend the extra energy required to extract calcium appears to be dependent on the level of predation by piscivorous fish.

1.5.5. A genetic basis for morphological divergence

Sticklebacks provide an excellent system for studying the genetics of single and complex trait adaptations because of the dramatic variation in skeletal structures across different populations, which can be readily crossed in the laboratory for genetic mapping experiments. Crosses between ancient marine and more recent freshwater

sticklebacks yield phenotypically intermediate F_1 generations (Ziuganov 1983). Similarly, crosses between reproductively isolated populations produce highly polymorphic F_1 hybrids, suggesting greater genetic diversity between populations than within (Schluter 1993; Ahn & Gibson 1999; Peichel *et al.* 2001).

Recent studies of morphology have tended to focus on the molecular basis of armour morphology. Polymorphisms in the *Eda* gene are closely related to plate number variation and can accurately group individuals into complete and low plated morphs (Colosimo *et al.* 2005). Genetic differentiation between complete and low plated morphs in the *Eda* gene exceed that between neutral markers implying that divergence is due to natural selection rather than genetic drift and that these differences are adaptive (Cano *et al.* 2006).

Pelvic reduction too is thought to have evolved repeatedly and independently from marine ancestors (Cresko *et al.* 2004; Shapiro *et al.* 2004; Shapiro *et al.* 2006; Coyle *et al.* 2007). Differences in pelvic structure have been found to be associated with differentiation in the expression of the *Pitx1* gene during normal development (Shapiro *et al.* 2004). Although larvae from pelvic-reduced populations show expression of *Pitx1*, it is weaker when compared to fish with a full pelvic structure.

In addition to the genetic control of armour morphology, several studies have found that differences in gill raker morphology and body depth are maintained in the laboratory, suggesting that that there is a genetic component these traits (Gross & Anderson 1984; Lavin & McPhail 1993; Hendry *et al.* 2002; Leinonen *et al.* 2006; Sharpe *et al.* 2008).

1.5.6. Stickleback 'pairs' showing morphological variation

There are several model systems of stickleback pairs that have been used to study and understand speciation in nature, each one involving a pair of phenotypically divergent forms that coexist in nature and often with some degree of reproductive isolation (see McKinnon & Rundle 2002 for a review). Some of these are discussed below.

1.5.6.1. Anadromous and freshwater-resident stickleback

In rivers where both anadromous (migratory) and freshwater resident stickleback co-exist, anadromous sticklebacks are larger than those that reside solely in freshwater streams and lakes, but also tend to be narrower i.e. less deep bodied, relative to their length (Hagen 1967). These differences are thought to be adaptations in the freshwater form to a life-history that no longer requires extensive migrations (Hagen 1967; Klepaker 1993; Kristjánsson 2005). Anadromous sticklebacks are also usually fully plated and have numerous, long gill rakers whereas freshwater-resident fish are often of the low plate morph and have a few, short gill rakers (Ziuganov 1983; McPhail 1994; Marchinko & Schluter 2007).

Three-spined sticklebacks in Loberg Lake, Alaska were founded by anadromous individuals between 1983 and 1989 (Aguirre *et al.* 2004). Although initially showing complete platedness, over the years since colonisation, the population has shown a significant reduction in plate number and numerical dominance is now primarily of the low plated morph. A notable absence of large numbers of partially plated morphs implies a genetic bias against them, and is consistent with work by Cresko et al. (2004) who found few of the partially plated morphs during experimental laboratory crosses between full and low plated individuals. Loberg Lake sticklebacks also show a

significant positive correlation between plate number and gill raker number, implying genetic linkage or a correlated response (Aguirre *et al*. 2004). Evidence cited by the authors is in favour of the latter: plankton feeding anadromous stickleback showing higher gill raker number and feeding in open waters, are vulnerable to predation, therefore plates afford some level of protection. In contrast, benthic feeding freshwater sticklebacks with fewer gill rakers, forage on larger prey in sheltered areas away from fish predation (McPhail 1994).

1.5.6.2. Differentiation along the benthic-limnetic axis

In some cases, extreme morphological differences between populations living in sympatry have lead to reproductive isolation. One case in which this has occurred is in a small number of lakes in British Colombia in which sticklebacks have been described as 'benthic' and 'limnetic', so named due to their feeding habits. Limnetic individuals forage mainly in the pelagic regions of the lake, have a streamlined body, extensive body armour and are usually small bodied fish. Benthic individuals, which are conspicuously less armoured, have a deeper bodied with fewer, short gill rakers and a wide mouth – making them particularly well adapted to feeding on invertebrates near the shore (McPhail 1994).

Crosses between benthic and limnetic individuals generate F_1 progeny that show an intermediate phenotype and although lower levels of fitness are not detectable in the lab, there is evidence for selection against hybrids in the wild (Schluter 1993; Hatfield 1995; Schluter 1995). In sticklebacks living in sympatry, mating behaviour is thought to play an important role for maintaining reproductive isolation. Benthic females show a preference for laying eggs in the nest of a benthic rather than a limnetic male (Rundle & Schluter 1998) and there is evidence to suggest preferences are learnt through

experience during development (Kozak & Boughman 2009). Limnetic males show a decrease in courting effort with increasing female size and may explain why benthic females are less inclined to lay eggs in a limnetic nest (Rundle & Schluter 1998).

However, the fragile nature of this biological segregation means that it is highly susceptible to erosion and recent research has suggested that the species pair in at least one system may be collapsing (Kraak *et al.* 2001). The benthic-limnetic dichotomy is primarily based on feeding adaptations with fish separating into two distinct clusters based on the number of gill raker (Bentzen & McPhail 1984). However, in a recent survey, although fish continue to cluster into two groups, the distinction between them has lessened (Kraak *et al.* 2001). Further research has shown that the genetic structure of the populations has also changed so that it now appears as though there is only a single population where previously there had been two (Taylor *et al.* 2006) and that this is the result of increased hybrid viability (Behm *et al.* 2010).

1.6. STICKLEBACKS IN PARAPATRIC LAKES AND STREAMS

Differentiation in the shape of three-spined sticklebacks has been noted in several parapatric lake and stream drainage systems in North America (Moodie 1972a, 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993; Thompson *et al.* 1997; Hendry *et al.* 2002) and Europe (Gross & Anderson 1984; Berner *et al.* 2010). Sticklebacks from lakes are more slender bodied than those from streams, which tend to be deeper bodied. Similar patterns of variation across continents suggest that differences have arisen independently and are adaptation to ecological differences between habitats.

Walker and Bell (2000) noted that in stickleback, the ectocoracoid, a paired bone immediately anterior to the pelvic structure and part of the pectoral girdle (Bowne 1994) gives rise to the deep adductor muscle (the major muscle active during the power

stroke). They have shown that freshwater sticklebacks, which do not need to make extensive migrations like their anadromous ancestors, show a reduced ectocoracoid, possibly due to reduced power requirements. Here, deeper bodied stream stickleback are like anadromous fish in that they are likely to have greater power requirements. Thus, if body depth is correlated with weight and musculature, then deeper bodied stream sticklebacks are likely to have greater inertia in flowing water conditions.

Although both lake- and stream-dwelling stickleback consume zooplankton and benthic invertebrates, lake fish consume more zooplankton and less benthic prey than do stream fish (Gross & Anderson 1984; Berner *et al.* 2009). As expected from their diet, sticklebacks from lakes consistently show fine, closely spaced and long gill rakers whereas stream sticklebacks have relatively few, short gill rakers (Moodie 1972a, 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993; Hendry *et al.* 2002).

Although research has shown differences in the armour morphology of fish from lakes and streams, studies have not been consistent in the direction of differences. Whereas some research has demonstrated a greater number of lateral plates in stream fish (e.g. Baumgartner 1990; Lavin & McPhail 1993; Hendry *et al.* 2002) other work has shown the opposite (e.g. Moodie 1972a; Reimchen *et al.* 1985). Similarly, some studies have found that spine length is longer in streams than it is in lakes (e.g. Hendry *et al.* 2002) whereas others have shown the opposite (e.g. Lavin & McPhail 1993).

1.6.1. Reservoirs as newly created, man-made lakes

Reservoirs share many of the same characteristics as natural lakes (Moss 1988) hence patterns of differences observed in lake-stream pairs, may also be evident in reservoir-stream pairs. Consistent differences in the morphology of lake-stream pairs across continents (Gross & Anderson 1984; e.g. Lavin & McPhail 1993; Berner *et al.*

2010) suggests that the differences are due to independent, parallel evolution. However, because most lakes are ancient, it is difficult to ascertain over what timeframe adaptations have occurred and what impact human interference may have had. UK reservoirs, on the other hand, have been built over several hundred years with the date of filling and various other human-related factors often precisely known.

It is likely that sticklebacks resident in present day reservoirs are the descendants of fish previously resident in the streams that were dammed, and this assumption is made throughout this research. The availability of definitive data on the dates of impoundments of reservoirs in the UK means that it is possible to investigate divergence between reservoir-stream pairs where the maximum time available for adaptation to the lake-type habitat is known as that of the reservoir age.

1.7. PROJECT AIMS

The main focus of this thesis was to investigate whether reservoir-stream stickleback pairs show differences in morphology that are akin to those in lake-stream pairs. Information on the age of UK reservoirs is known hence reservoirs were selected to cover a broad age range.

In addition to using traditional linear measures of morphology (e.g. length and depth etc.), this thesis utilises geometric morphometrics (GM) to analyse overall shape. GM methods use information about the relative spatial configuration of landmarks and hence preserve information about overall shape. Landmarks are discrete anatomical loci that are homologous in all specimens and information about the relative position of each landmark is recorded as coordinates.

Chapter 2 studied morphological variation in seven reservoir-stream systems with an age range between 16-154 years. The age of the reservoir indicates the

maximum time available for adaptation to the newly created lake-type habitat. I set out to test whether divergence between habitats types was in the same direction as those previously observed in lake-stream pairs and also to see if divergence between habitats types was associated with time since impoundment.

In addition to assessing the morphological divergence between reservoir-stream pairs, Chapter 3 tested whether reservoir and stream populations could be distinguished based on differentiation at neutral genetic loci. It also assessed whether there was genetic divergence between systems. If so, it would suggest that morphological differences may have arisen independently in each system.

Chapter 4 compared the morphology of fish from streams, reservoirs and natural lakes. If the morphology of reservoir fish is tending towards that of fish from natural lakes, then sticklebacks from reservoirs were expected to show a phenotype that was intermediate between those from streams and natural lakes.

The aim of Chapter 5 was to assess whether the morphological differences observed in reservoir-stream pairs was the result of adaptive divergence or plasticity. Using fish from a single reservoir-system system, sticklebacks were bred and reared under common-garden conditions, removing the effect of habitat type on the growth and development of traits. The persistence of differences in the F_1 progeny would suggest that traits were underpinned by a genetic mechanism and were heritable. However, a lack of morphological differences in the F_1 progeny would suggest that traits were phenotypically plastic and the result of the rearing environment.

A single reservoir-stream system was also selected to examine whether other aspects of stickleback biology might also show variation between habitats. Chapter 6 focussed on whether foraging behaviours had altered as a consequence of changes in

locally available food sources from primarily benthos in streams to plankton in reservoirs (Berner *et al.* 2008).

Sticklebacks show indeterminate growth and so their growth is a function of their environment (Wootton 1976). It is unclear however, whether there are differences in growth between fish resident in different habitats and the purpose of Chapter 7 was to assess whether sticklebacks in reservoirs and stream have different growth rates.

Chapter 2

Morphological variation of three-spined sticklebacks from UK reservoir-stream systems

2.1. INTRODUCTION

Intraspecific variation in morphology is widely attributed to differences in the habitat experienced by a population (Goodman *et al.* 2008; Bhagat *et al.* 2011; Spoljaric & Reimchen 2011). Adaptation to different environments have been demonstrated by mountain whitefish, *Prosopium williamsoni* (Whiteley 2007), arctic charr, *Salvelinus alpinus* (Adams *et al.* 2003), gilthead seabream, *Sparus aurata* (Russo *et al.* 2007), rainbow fish, *Melanotaenia eachamensis* (McGuigan *et al.* 2003), rock bass, *Ambloplites rupestris* (Brinsmead & Fox 2002) and three-spined stickleback, *Gasterosteus aculeatus* (Moodie 1972b).

In fish, occupying either flowing or still water can have a major effect in intra-specific variation in morphology. For example, in 34 populations of rainbow trout, *Oncorhynchus mykiss* sampled across British Colombia, Canada, the most dramatic differences in morphology occurred between stream- and lake-dwelling populations (Keeley *et al.* 2005). Further work has suggested that these differences are strongly influenced by inherited differences and can thus be considered as different ecotypes (Keeley *et al.* 2007). .

In recent years there has been an expansion of interest in the morphological variation of the three-spined stickleback, with studies of phenotypic variation now into their hundreds. In many instances, the emerging morphological differences are considered to be ecotypes, showing adaptations to a particular habitat (McKinnon & Rundle 2002). In the case of lake-stream pairs, evidence for adaptive morphological divergence arises from the existence of similar patterns of variation in several inherited traits on different continents (Hagen & Gilbertson 1972; Gross & Anderson 1984). Given that reservoirs are effectively man-made lakes, looking at morphological

differences in lake-stream pairs may give an indication of the changes that might be expected in sticklebacks following the impoundment of rivers.

2.1.1. Shape and swimming performance

A number of studies have shown that sticklebacks from natural lakes typically have a slender, more streamlined body whereas stream forms tend to be deeper-bodied (Moodie 1972a, 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993; McPhail 1994; Walker & Bell 2000; Hendry *et al.* 2002). Walker and Bell (2000) noted that the ectocoracoid gives rise to the deep adductor muscle, which is the major muscle active during the power stroke of pectoral swimming. They have shown that freshwater sticklebacks, which unlike their anadromous ancestors do not need to make extensive migrations, show a reduced ectocoracoid, possibly due to reduced power requirements (Cowen, 1991; cited in Walker & Bell 2000). Thus, if body depth is correlated with weight and musculature, then deeper bodied individuals are likely to be better able to hold their position against a flowing current.

Similarly, stream sticklebacks also show a shorter and deeper caudal peduncle, which is associated with increased manoeuvrability and greater inertia (Walker 1997), than lake-resident sticklebacks (Sharpe *et al.* 2008). When foraging on typically benthic prey such as gammarids or chironomid larvae lying on the substratum, sticklebacks approach the ground and visually fixate the prey before striking (Tugendhat 1960). Thus, in complex stream environments, increased manoeuvrability and inertia are likely to be important for gaining access to bottom-dwelling invertebrates and for foraging under flowing water conditions, respectively (Berner *et al.* 2008). Lake sticklebacks, on the other hand, forage in the open water of lakes by striking at more motile zooplankton prey (Gross & Anderson 1984), likely cruise for

longer periods of time at steady velocities and so the observed longer and narrower caudal peduncle may be an aid to efficient sustained swimming required for foraging in open waters (Webb 1984).

2.1.2. Trophic morphology and feeding

In fish, gill rakers serve as a sieve to prevent the loss of prey from the bucco-pharyngeal chamber during feeding. Whereas a few, short gill rakers are suitable for foraging on large macroinvertebrates, numerous, long gill rakers are required for preventing the loss of smaller zooplankton prey (Gross & Anderson 1984). Pumpkinseed, *Lepomis gibbosus* captured from the littoral zone in lakes show wider spacing between gill rakers than pelagic individuals and this is associated with the different types of prey consumed in these two habitat-types (Robinson *et al.* 1993; Gillespie & Fox 2003) . The high degree of morphological plasticity and ability to adapt to novel environments has been attributed to the pumpkinseed's ability to exploit prey in different types of habitat, and this, in turn, may explain its success at establishing new populations (Vila-Gispert *et al.* 2007). Similarly, sticklebacks from natural lakes consistently show fine, closely spaced and long gill rakers whereas stream sticklebacks have relatively few, short gill rakers (Moodie 1972a, 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993; Hendry *et al.* 2002). Once again, this variation is associated with differences in prey availability and feeding regime (Bentzen & McPhail 1984; Lavin & McPhail 1993; Schluter 1993).

2.1.3. Armour morphology and predator avoidance

Caudal peduncle morphology also affects other aspects of swimming performance, including the fast start C-type bends used for predator evasion (Law &

Blake 1996; Walker *et al.* 2005; Blake & Chan 2006). However, in addition to fast-start predator evasion tactics, sticklebacks also posses morphological defences against predators, in the form of lateral plates and spines. The relative importance of armour and escape behaviour has been linked to the pursuit efficiency of the most commonly encountered predator (Reimchen 1992). In situations where an individual is subject to predation by predators with high pursuit efficiency, selection should strongly favour armour to minimise damage and increase the chance of post-capture survival as capture likelihood is high (Reimchen 1992); the most likely response against predatory fish. However, Bergstrom (2002) has suggested that lateral plates may also increase drag, thus lowering swimming performance. Hence, the best defence against avian predators, which have a low pursuit efficiency, is improved hydrodynamic efficiency, associated with a loss of lateral plates (Reimchen 1988, 1992). Indeed, where predatory fish are common, stickleback populations show a modal plate frequency greater than in populations where predation pressures are low or primarily from avian piscivores (Hagen & Gilbertson 1972; Moodie & Reimchen 1976). Presumably ancestral, fully-plated marine forms were subject to high levels of predation pressure from several sources, hence complete armour may have served as the optimal defence strategy. However, whereas some authors have shown that plate numbers are greater in stream dwelling sticklebacks than they are in lake dwelling stickleback (Baumgartner 1990; Lavin & McPhail 1993; Hendry *et al.* 2002), others have demonstrated the opposite (Moodie 1972a; Reimchen *et al.* 1985).

The dorsal and ventral spines of the three-spined stickleback also function as a defence mechanism against predators. Orientation of the spines means that when erect and locked into place (Hoogland *et al.* 1957), they effectively increase the cross-sectional size of the individual (Gross 1978; Reimchen 1983). The spines can be

locked without muscular contractions and so they can remain erect even if the individual fatigues. This means that if caught in the jaws of a predator, the spines are difficult to press down and can pierce the sensitive mouthparts of a predator during manipulation . Hoogland *et al*. (1957) conducted feeding experiments using pike, *Esox lucius* and perch, *Perca fluviatilis* as predators to assess the protection afforded to stickleback as a result of their spines. In several cases, three-spined sticklebacks were rejected after being seized and both predators began to actively avoid predating on them, instead showing a preference for the lesser spined species also present. When presented with three- and nine-spined sticklebacks, *Pungitius pungitius*, which posses only very shott spines, it was the nine-spined sticklebacks that were taken the most, suggesting a specialisation of the fewer and larger spines seen in the three-spined species. Furthermore, sticklebacks with their spines removed were taken at the same rate as some naturally spineless brook sticklebacks, *Culea inconstans*. In the absence of predatory fish however, grappling predators such as dragonfly larvae have been documented to prey on stickleback (Reimchen 1980; Ziuganov & Zotin 1995). In these situations, spines may be disadvantageous as these predators may be capable of using the rigid structures to grasp their prey.

2.1.4. Morphological divergence in reservoirs

There are clear and consistent patterns of divergence between sticklebacks inhabiting lakes and streams in several morphological traits (depth, caudal size and trophic structure) which are suggested as being adaptations to the environment. Most natural lakes are ancient, having been formed several thousands of years ago and their age dictates the maximum time available for adaption to a lake habitat (Moss 1988). In most cases, the age and history of a lake is difficult to determine and so it is unclear

over what timeframe adaptations have occurred. Reservoirs, on the other hand, are man-made lakes that can be precisely aged and used as a proxy for studying changes in stickleback morphology over time.

2.1.5. Aims

In the current chapter, I will test the hypothesis that morphological differences previous observed in lake-stream systems of sticklebacks are adaptive by exploring whether similar differences are also apparent in samples of fish from streams and man-made lakes (reservoirs). By comparing the morphology of sticklebacks from a number of reservoirs of differing ages with the streams that flow into those reservoirs, I aim to gain an insight into the timescales over which these adaptations arise. If the morphological features discussed are the result of evolutionary changes in response to environmental differences, I would expect to find a greater degree of divergence from the putative ancestral stream form among sticklebacks inhabiting older reservoirs than in more recently created ones.

2.2. METHODS

2.2.1. Site descriptions

Three-spined sticklebacks were sampled from seven geographically distinct reservoir-stream systems around the UK (Fig. 2.1): Alaw (ALA), Blackbrook (BBK), Cefni (CEF), Carsington (CSG), Kendoon (KND), Stithians (STI) and Thornton (THN). Age of the reservoirs ranged from 17-154 years and sample sizes ranged from 16 to 45 (Table 2.1).

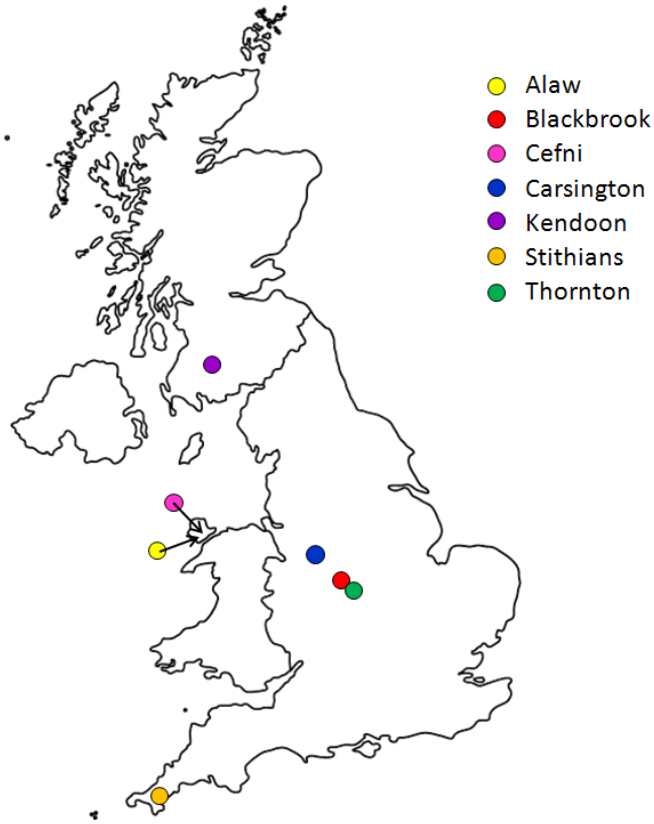


Figure 2.1 Map of the UK showing the location of the seven reservoir-stream systems where sticklebacks were successfully sampled

Table 2.1 Size and age of the reservoirs and streams where sticklebacks were sampled.

System	Code	Reservoir age	Reservoir size (ha)	N_R	Stream width (at sampling point; m)	Stream depth (at sampling point; m)	N_S
Alaw	ALA	42	315	40	0.3-0.5	0.1-0.5	30
Blackbrook	BBK	102	3	26	2	0.5-0.8	40
Cefni	CEF	60	7	45	0.3-0.5	0.1-0.5	44
Carsington	CSG	17	30	21	0.5-1	0.1-1.2	19
Kendoon	KND	73	61	36	2-6	0.1-1.2	16
Stithians	STI	45	110	39	0.3-1	<0.5	30
Thornton	THN	154	30	24	2	0.3-1	21

N_R indicates the number of sticklebacks sampled from the reservoir
N_S indicates the number of sticklebacks samples from the stream

2.2.1.1 Alaw reservoir-stream system

Llyn Alaw was created in 1966 and covers an area of approximately 315ha with a maximum depth of 5.2m. It is a mesotrophic lowland lake located on the island of Anglesey, North Wales (N53°20'25" W4°26'20") and is a stocked rudd *Scardinius erythropthalmus*, and trout (rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*) fishery. Llyn Alaw is a designated Site of Special Scientific Interest (SSSI) on account of its ornithological interest, particular for overwintering waterfowl. Wildfowl species that occur and are possible stickleback predators include the pochard *Aythya ferina*, tufted duck *Aythya fuligula*, common tern *Sterna hirundo*, great-crested grebe *Podiceps cristatus* and common coot *Fulica atra*. During sampling, nine-spined sticklebacks, *Pungitius pungitius* were also caught. Fish were sampled from a small bay in the southwest corner of the reservoir at the dam end of the site (Fig. 2.2).

Figure 2.2 (a) Location map and (b) photograph of the sampling area for Llyn Alaw

Three-spined sticklebacks were successfully captured from an inflowing stream at the North end of the reservoir (N53°22'25" W4°22'33"; Fig. 2.3). The stream was slow flowing with a depth that varied between 0.1-0.5m and a mixed substratum of fine, sandy sediment and small, smooth pebbles. It was narrow stream, around 0.3-0.5m wide located at the bottom of a farming field and although there was overhanging

vegetation at ground level, the stream was otherwise primarily exposed to the sky. Other species of fish caught included the 9-spined stickleback and stone loach, *Barbatula barbatula*.

Figure 2.3 (a) Location map and (b) photograph of the sampling area for the Alaw inflowing stream

2.2.1.2. Blackbrook reservoir-stream system

Blackbrook reservoir was created in 1906 and covers an area of around 3ha. It is a mesotrophic lake, located in Leicestershire, England (N52°44’56” W1°19’05”) and is a stocked brown trout fishery. Blackbrook reservoir is a designated SSSI for its unusual community of marginal and aquatic plants. It also supports flocks of wintering wildfowl and breeding pairs of the great-crested grebe and little grebe, *Tachybaptus ruficollis*. No other species of fish were caught during sampling, which took place in the southeast corner of the reservoir, close to where the inflowing stream joins (Fig. 2.4).

Figure 2.4 (a) Location map and (b) photograph of the sampling area for Blackbrook reservoir

Just one stream feeds the reservoir at the southernmost tip. Three-spined sticklebacks were caught approximately 0.5km away from the point at which the stream meets the reservoir (N52°44'49" W1°18'06"; Fig. 2.5). Depth at the point of capture was between 0.5-0.75m and it had a width of approximately 2m. The water was relatively slow flowing and the stream-bed was soft with overhanging vegetation from the banks. The point of capture was at the bottom of a farming field, near to the main road and under a bridge where it was completely open to the sky.

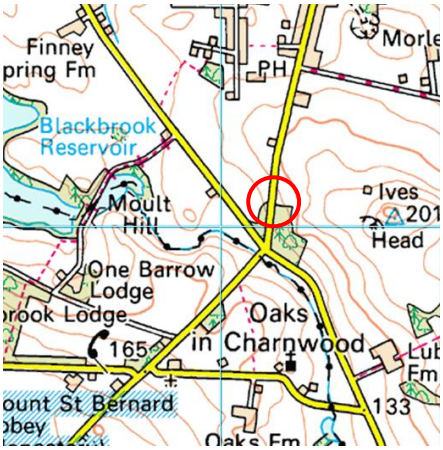


Figure 2.5 (a) Location map and (b) photograph of the sampling area for the Blackbrook inflowing stream

2.2.1.3. Cefni reservoir-stream system

Cefni reservoir was created in 1950 and covers an area of 7ha. It is a mesotrophic lake, located on the island of Anglesey, North Wales (N53°16'32" W4°19'43") and has a population of wild brown trout in addition to stocked rainbow trout. It is fed by several feeder streams and is the source of the Afon Cefni. Potentially piscivorous bird sighted on and around the reservoir included kingfishers *Alcedo atthis* and moorhens *Gallinula chloropus*. Fish were caught in the north of the reservoir along the west bank close to the point where one of the feeder streams joins the reservoir (Fig. 2.6).

Figure 2.6 (a) Location map and (b) photograph of the sampling area for Llyn Cefni

Stream-caught sticklebacks were captured at a site where the stream was at a width of around 0.3-0.5m and depth ranging from 0.1-0.5m. They were caught approximately 0.5km from the mouth of the reservoir (N53°17'13" W4°20'05"; Fig. 2.7) from a section where the water was slow-flowing and the bed of the stream was primarily soft with overhanging and emergent vegetation. However, there were sections of the stream that had a more pebble substratum. The point of capture was at the bottom of a farming field, near to the main road and under a bridge where it was

completely open to the sky. In addition to sticklebacks, brown trout fry and minnows, *Phoxinus phoxinus* were also caught.

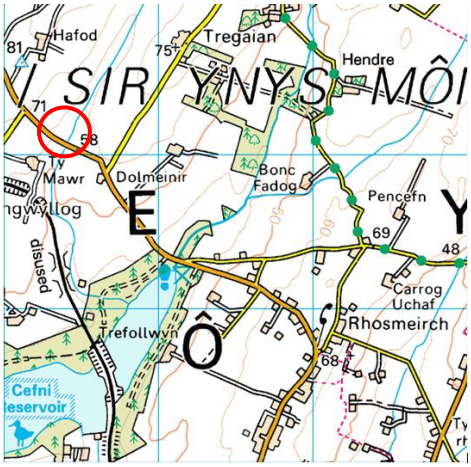


Figure 2.7 (a) Location map and (b) photograph of the sampling area for the Cefni inflowing stream

2.2.1.4. Carsington reservoir-stream system

Carsington reservoir was created in 1992 and covers an area of 30ha. It is a mesotrophic lake located in Derbyshire, England (N53°03'40" W1°38'00") and is a fly fishery stocked with brown and rainbow trout. It is primarily fed by water pumped from the River Derwent but also from local run-off and is based on a site through which a small brook once ran. Several short stretches of inflowing streams still remain but the majority of water is drawn via tunnels and aqueducts direct from River Derwent for storage during periods of high rainfall. Carsington is host to a wide range of wildfowl and waders including the goldeneye *Bucephala clangula*, cormorant *Phalacrocorax carbo*, great created grebe, great northern diver *Gavia immer*, heron *Ardea cinerea*, kingfisher, little grebe, pochard. Fish were caught at the north end of the reservoir, close to and around the nature reserve (Fig. 2.8).

Figure 2.8 (a) Location map and (b) photograph of the sampling area of Carsington Water

Stream-caught sticklebacks were captured at a site where the stream was at a width of around 0.5-1m and the depth ranged from 0.1-1.2m (N53°04'31" W1°36'06"). They were caught approximately 0.5km from the point at which the stream joins the reservoir from a feeder stream at the very North end of the reservoir (Fig. 2.9). Flow was minimal and the stream had a soft, muddy substratum. The site of capture was lined with trees along the bank and so the stream was primarily in the shade and covered from the sky. Kingfishers were spotted at the site in and amongst the trees. No other fish species were caught.

Figure 2.9 (a) Location map and (b) photograph of the sampling area of the Carsington inflowing stream

2.2.1.5. Kendoon reservoir-stream system

Kendoon reservoir was created in 1935 and covers an area of 61ha in Dumfries and Galloway, Scotland (N55°11'54" W4°12'02"). It was built as part of the Galloway Hydro-Electric Scheme and water is used to power the Kendoon Power Station 2km to the south. It is fed by the Water of Ken and Water of Deugh, both of which were dammed during its creation. Kendoon is a coarse fishery although brown trout and salmon, *Salmo salar* are occasionally caught. Sticklebacks were caught on the north side of the reservoir (Fig 2.10), as were minnows.

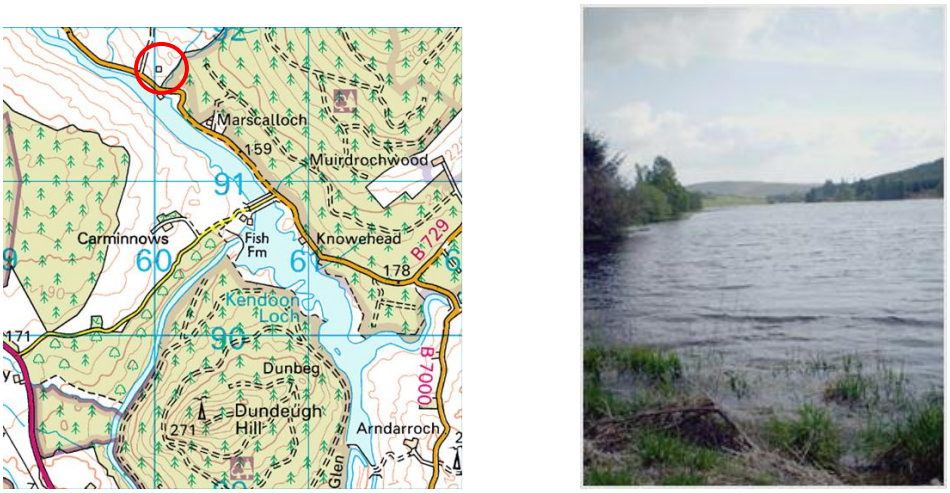


Figure 2.10 (a) Location map and (b) photograph of the sampling area of Loch Kendoon

There are several small tributaries that feed into the two main rivers that run into Loch Kendoon and it was one of these, at the point where it joins the Water of Deugh (N55°12'15" W4°13'01"), where sticklebacks were successfully caught (Fig. 2.11). Water was relatively fast flowing over and around pebbles, rocks and large boulders with pools of slower flowing water. The depth of the water ranged from 0.1-1.2m and the width of the stream varied from 2-6m. At the point where the stream joined the Water of Deugh, there was some submerged vegetation near the bank but on the whole, there was little vegetation. A large number of minnows were also caught, in addition to

some brown trout fry. The stream was located under a bridge, alongside a main road and was completely open to the sky.

Figure 2.11 (a) Location map and (b) photograph of the sampling area of the Kendoon inflowing stream

2.2.1.6. Stithians reservoir-stream system

Stithians reservoir was created in 1965 and covers an area of 110ha. It is a mesotrophic lake located in Cornwall, England (N50°11'22" W5°12'34") and is a stocked rainbow trout fishery with wild brown trout also present. It is owned and operated by South West Water to supply water to West Cornwall. It is host to wide range of wildfowl including the tufted duck, pochard, golden eye, as well as the little grebe and coot. Fish were caught at the north end of the reservoir (Fig 2.12).

Figure 2.12 (a) Location map and (b) photograph of the sampling area of Stithians reservoir

Stream caught sticklebacks were captured at a site north of the reservoir, around 0.5km away (N50°11’33” W5°13’12”; Fig. 2.13). The stream was extremely slow flowing with sections which appeared as though they may dry up during the summer months. The width of the stream varied from 0.3-1m with a depth no more than 0.5m at any point during sampling. The substratum was mixed with some sections have a pebble bed and other with more silt and sediment. The bank along the silty section of stream was lined with overhanging and emergent vegetation but was absent from sections where the bed was primarily stone. Small brown trout fry were also seen.

Figure 2.13 (a) Location map and (b) photograph of the sampling area of the Stithians inflowing stream

2.2.1.7. Thornton reservoir-stream system

Thornton reservoir was created in 1864 and covers an area of 30ha. It is a mesotrophic lake located in Leicestershire, England (N52°40’01” W1°18’34”) and is a stocked rainbow and brown trout fishery. A number of coarse fish including pike *Esox lucius*, perch *Perca fluviatilis* and rudd) are also present. It hosts a number of wildfowl including the tufted duck, as well as the cormorant, heron, great crested grebe, coot and moorhen. Fish were caught in the left arm of the reservoir, just below a weir (Fig.

2.14). In addition to sticklebacks, bullheads *Cottus gobio* were also caught during sampling.

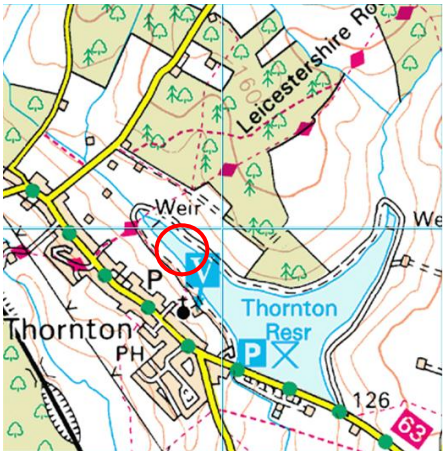


Figure 2.14 (a) Location map and (b) photograph of the sampling area of Thornton reservoir

There are two streams flowing into Thornton reservoir, one at each arm. Sticklebacks were caught in the left arm, approximately 0.5km away from the mouth with the reservoir (N52°40’17” W1°19’01”; Fig. 2.15). The stream had sections of medium flowing water but areas where sticklebacks were caught were primarily slow flowing. The stream bed was a mixture of rocks and muddy sediment, and ranged in depth from 0.3-1m. The width of the stream was approximately 2m. Trees lined both banks of the streams so was not open to the sky and kingfishers were spotted in the area. There was little overhanging vegetation, although fish were primarily caught in and amongst tree roots. A large number of bullheads were also caught.

Figure 2.15 (a) Location map and (b) photograph of the sampling area of the Thornton inflowing stream

2.2.2. Fish sampling procedure

Prior to the commencement of sampling, permission to do so was sought from the owners of each water body visited, angling associations, landowners, Natural England and the Environment Agency. For safety, all sampling was undertaken by at least 2 people. Sticklebacks were sampled using hand nets (approximately 30cm x 30cm with a 1mm mesh) and unbaited galvanised metal minnow traps (3mm or 5mm mesh, 20mm aperture; manufactured by Tackle Factory, USA) deployed from the bankside and left in suitable locations overnight. Following capture, live sticklebacks were externally inspected for infection with the cestode parasite, *Schistocephalus solidus*. The parasite causes an obvious distension of the abdomen and any that were considered infected were returned to the water, or retained separately for other laboratory research. When fewer than 30 non-infected fish were collected, all were processed for morphological analyses. If more than 30 fish were collected, a subset of 30 equally-sized individuals was selected.

On return to the laboratory, fish were photographed individually from above to obtain information on fish body width and then sacrificed with an overdose of 0.1% Benzocaine anaesthetic. Standard length (accurate to 0.1mm) and blotted mass

(accurate to 0.001g) measurements were made and the right pectoral fin removed and preserved in 100% ethanol for DNA analyses. The specimen was subsequently fixed in 10% neutral buffered formalin (NBF).

2.2.3. Morphological analyses

2.2.3.1. Clearing and staining

After a minimum of 14d in NBF, fish were cleared and stained to visualise bone morphology using a modified standard protocol (Dingerkus & Uhler 1977 and described at http://gobiidae.com/methods/method_clear_and_stain.htm) . Briefly, this technique involved dehydrating the specimens before staining the cartilage using Alcian blue. Specimens were then trypsin digested and the bone stained using a potassium hydroxide solution of Alizarin red. Once evenly stained, specimens were bleached before storage in 100% glycerol.

2.2.3.2. Quantifying linear measurements

Digital images of the lateral view of the left hand side of the fish were taken (Fujifilm FinePix s9600) and measurements were made using ImageTool v3.0 following a protocol described in Hendry *et al*. (2002). Linear measurements recorded from lateral and ventral images are given in figure Fig. 2.16. Measures of width were obtained from dorsal images. Gill structures were removed from cleared and stained fish and inspected under a microscope to obtain data on the length of the longest gill raker on the first gill arch and total number of gill rakers on the first gill arch (Gross & Anderson 1984). Finally, the number of lateral plates on each side were determined by visually inspecting specimens.

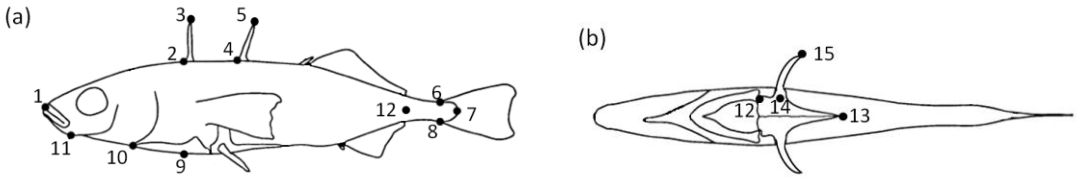


Figure 2.16 Measurements made from the (a) lateral (b) ventral digital photographs of cleared and stained specimens: standard body length (1-7, tip of upper jaw to end of hypural plate), body depth (2-9, anterior insertion of the first pelvic spine to bottom of pelvic girdle, perpendicular to the lateral line), first (2-3) and second (4-5) dorsal spine length, caudal peduncle depth (6-8, depth at the thinnest part of the caudal peduncle) caudal peduncle length (12-7, the horizontal length between vertebrae giving rise to the posterior insertion of the dorsal and ventral fin and the tip of the hypural pate), jaw angle (1-11-10, angle between the anterior tip of the upper jaw, posterior edge of angular and anterior end of ectocoracoid); pelvic spine length (12-13, on the left side) and pelvic girdle length (14-15, tip of posterior process of pelvic girdle to the anterior tip on the left side). Adapted from Walker (1997)

2.2.3.3. Quantifying shape

Fifteen landmarks that describe the overall shape of sticklebacks were digitised using tpsDig v2.16 (Rohlf, 2004; available from http://life.bio.sunysb.edu/morph/). Each one refers to the position of a bone that can be identified on all specimens. The landmarks digitised are given in Fig. 2.17.

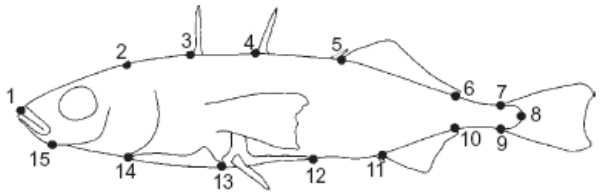


Figure 2.17 Location of the 15 landmarks digitised for shape analysis. (1) anterior tip of the upper lip; (2) supraoccipital notch immediately lateral to the dorsal midline (DML); (3) anterior junction of the first dorsal spine with the DML; (4) anterior junction of the second dorsal spine with the DML; (5) base of the first dorsal fin ray at the DML; (6) insertion of the dorsal fin membrane on the DML; (7) origin of the caudal fin membrane on the DML; (8) caudal border of hypural plate at lateral midline; (9) origin of caudal fin membrane on ventral midline (VML); (10) insertion of anal fin membrane on VML; (11) base of the first anal fin ray on VML; (12) caudal tip of posterior process of pelvic girdle on VML; (13) posterior tip of ectocoracoid; (14) anterior border of ectocoracoid on VML and (15) posterior edge of angular. Adapted from Walker (1997).

2.2.4. Morphological analyses

2.2.4.1. Analyses of linear measurements

A Principal Components Analysis was undertaken to detect which traits best differentiated reservoir and stream fish. All linear traits that correlated with body length (body width, depth, first and second dorsal spine length, pelvic spine length, pelvic girdle length, length and depth of the caudal peduncle and gill raker length) were compared between groups using a multivariate analysis of covariance (MANCOVA). Initially, both standard length and sex were both included as covariates but the results showed no effect of sex so analyses were repeated without sex as a factor. Traits not correlated with length (number of lateral plates, jaw angle and number of gill rakers) were compared between sites using an analysis of variance (ANOVA) for normally distributed data or a Mann Whitney-U for non-normal data.

Data were initially analysed separately for each geographical reservoir-stream system. Data from all samples were then combined to look at overall differences between reservoirs and streams across systems. A paired samples *t*-test was conducted to compare each trait mean between reservoir and stream samples across all systems (n = 7) using marginal means for traits correlated with SL and unadjusted means otherwise.

2.2.4.2. Analysis of shape

Digitised landmark data was used to calculate specimen size, and describe the shape of each fish using the software program tpsRelw (Rohlf, 2004; available from http://life.bio.sunysb.edu/morph/). The program uses the Procrustes superimposition method to calculate a consensus configuration from the coordinates. Procrustes superimposition is performed by scaling, translating and rotating specimens to remove

all information unrelated to shape in a way which minimises differences between configurations (Zelditch et al., 2004). It then compares the set of coordinates for each specimen against the consensus figure using a thin plate spline analysis. This method deforms the specimen landmark towards the consensus configuration and calculates the bending energy required to do so for each individual: the *partial warp score*. The principal components of each partial warp score, called the *relative warp score* (RWS) summarise major trends in shape variation and were tested for significance using a MANOVA. RWS that showed significant differences between populations and the deformation associated with significant RWS, were then plotted to visualise the differentiation. Discriminant function analyses were used to predict habitat membership using the partial warp scores as predictor variables.

To test if variation in shape could separate the seven geographically distinct reservoir-stream systems, RWS were compared between habitats types using a MANOVA. To assess whether landmark data could differentiate fish from different habitats (reservoir or stream), a MANOVA was conducted to investigate if there were significant differences in the RWS for reservoir and stream fish. This was done separately for each system separately and for all systems combined.

2.2.4.3. Correlation between reservoir age and degree of morphological divergence

To test for a relationship between reservoir age and the degree of morphological divergence between stream- and reservoir- resident sticklebacks, a proxy for the extent of morphological divergence in each system was calculated based on the RWS that was best able to discriminate between reservoir and stream fish in that system. The difference in the average RWS between reservoir and stream fish was calculated and

plotted against reservoir age. The correlation between reservoir age and morphological divergence was tested for significance using Spearman's rank correlation coefficient.

2.3. RESULTS

2.3.1. Between-system variation in stickleback morphology

Twenty-six principal components of shape (relative warp scores, RWS) were calculated to describe the variation in shape across all samples. The first five RWS described a total of 78.6% of the variation, with each successive RWS describing less than 5% (Fig. 2.18).

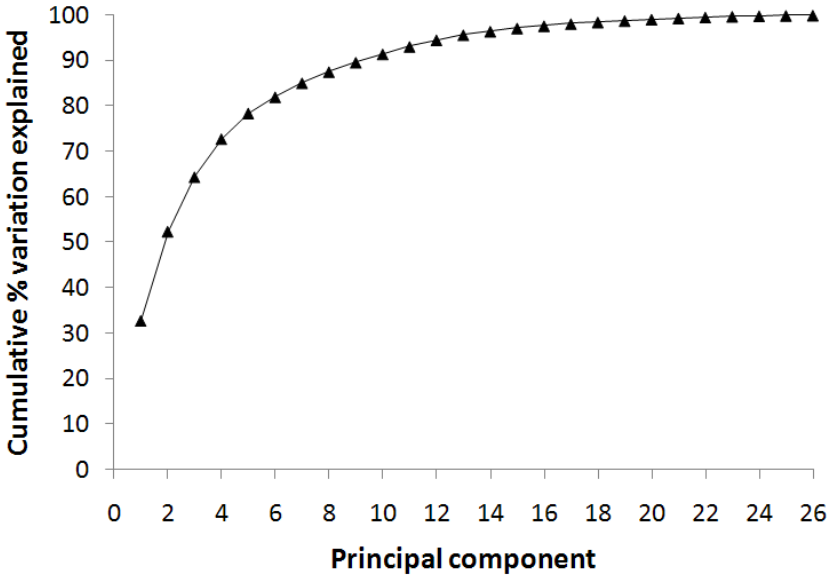


Figure 2.18 Cumulative percentage of variation in shape across all samples described by the 26 principal components of shape (relative warps)

Of those, 24 could significantly differentiate between systems ($p < 0.05$) and explained a total of 99.9% of the variation with RW1-3 explaining 64.5% of variation. RW1 (32.8%, $F_{13,404} = 19.44$, $p < 0.001$) however, was mainly associated with specimen bending. Thus, RW2 (19.7%, $F_{13,404} = 31.41$, $p < 0.001$) and RW3 (12.0%; $F_{13,404} = 15.25$, $p < 0.001$) together described most (31.7%) of the diversity. Using the scores from RW2 and RW3 together, the Alaw, Blackbrook, Kendoon, Stithians and

Thornton and systems showed distinct and tight, although often overlapping, clusters (Fig. 2.19a). The Cefni and Carsington system however, were less tightly clustered (Fig. 2.19b).

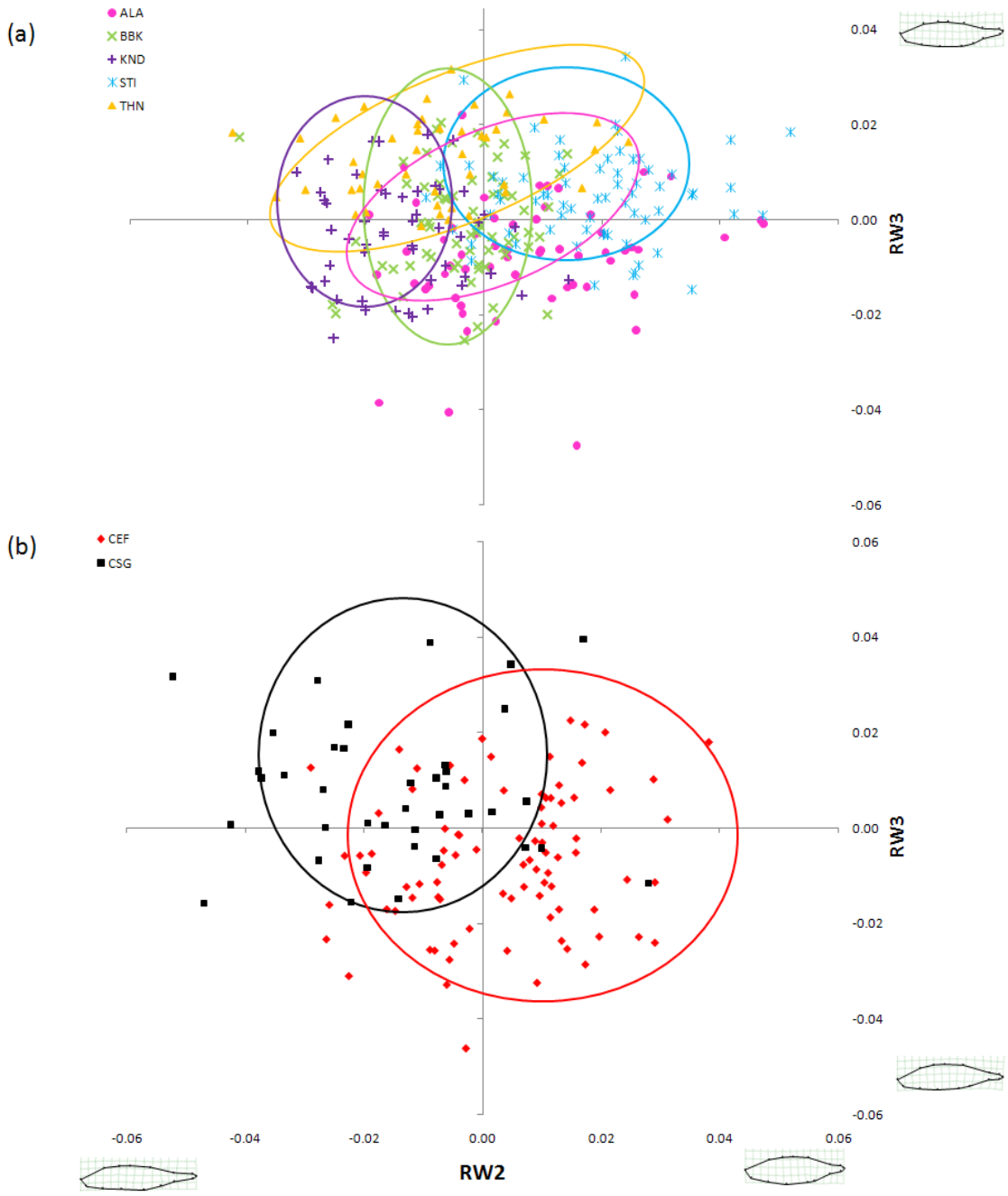


Figure 2.19 Plot of the relative warp scores significantly differentiating sticklebacks from separate reservoir-stream systems. Putative clusters are circled for the (a) Alaw (ALA), Blackbrook (BBK), Kendoon (KND), Stithians (STI) and Thornton (THN) systems, and for the (b) Cefni (CEF) and Carsington (CSG) systems. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

Six canonical discriminant functions were calculated to predict system membership; all were statistically significant and together explained 100% of the variation ($p < 0.05$; Table 2.2). Together the discriminant functions were correctly able to predict group membership for 85.9% of the samples (Table 2.3).

Table 2.2 Tests of canonical discriminant functions to assess suitability of partial warp scores for differentiating between sticklebacks from separate reservoir-stream systems.

Function	Canonical correlation	χ^2	df	*p* value
1	0.862	1622	156	<0.001
2	0.745	1077	125	<0.001
3	0.684	753.4	96	<0.001
4	0.674	500.5	69	<0.001
5	0.529	257.6	44	<0.001
6	0.520	126.0	21	<0.001

Table 2.3 Percentage classification results of sticklebacks from 7 reservoir-stream systems using a discriminant function analysis of shape.

	Predicted group membership						
System	ALA	BBK	CEF	*CSG*	KND	STI	THN
ALA	81.7	0	10	0	6.7	1.7	0
BBK	0	95.5	0	0	0	0	4.5
CEF	9.0	1.1	79.8	2.2	0	6.7	1.1
CSG	2.5	0	2.5	87.5	5.0	0	2.5
KND	1.9	1.9	1.9	0	90.4	3.8	0
STI	7.2	0	2.9	1.4	2.9	82.6	2.9
THN	0	9.5	0	2.4	0	0	88.1

2.3.2. Morphological differences between reservoir and stream caught sticklebacks for the seven systems

2.3.2.1. Alaw system

Principal components analysis revealed the presence of four components with eigenvalues exceeding 1, explaining 78% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 61% of the variance. Component 1 was primarily related to overall size and shape of specimens whereas component 2 appeared to be more closely related to spine length, but also length of the gill rakers and caudal peduncle length (Table 2.4).

Table 2.4 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Alaw reservoir-stream system.

Trait	Component 1	Component 2
Depth	0.962	-0.007
Caudal peduncle depth	0.892	0.122
Width	0.798	-0.420
Mouth width	0.766	0.390
Standard length	0.734	0.551
Pelvic girdle length	0.680	0.325
Number of gill rakers	0.524	0.210
Jaw angle	0.359	-0.157
Second dorsal spine length	-0.128	0.914
First dorsal spine length	-0.033	0.883
Pelvic spine length	0.018	0.872
Gill raker length	0.124	0.679
Caudal peduncle length	0.207	0.656
Average number of lateral plates	0.123	0.245

Sticklebacks from the stream had deeper bodies ($F_{1,56} = 224.0$, $p < 0.001$), deeper ($F_{1,56} = 16.43$, $p < 0.001$) and shorter ($F_{1,56} = 10.99$, $p = 0.002$) caudal peduncles and had shorter pelvic ($F_{1,56} = 18.50$, $p < 0.001$) and first dorsal ($F_{1,56} = 18.03$, $p <$

0.001) spines than those from the reservoir (Figs. 2.20b-g). They also possessed a longer pelvic girdle ($F_{1,56} = 11.01$, $p = 0.002$; Fig. 2.20e).

There was a significant interaction between standard length (SL) and habitat type in the length of the second dorsal spine (DS2) ($F_{1,55} = 10.01$, $p = 0.003$); DS2 increased with SL among both reservoir and stream-caught fish, however, the increase was greater in sticklebacks from the reservoir (Fig. 2.20h). There was also a significant interaction between SL and habitat type in the length of the gill rakers ($F_{1,35} = 11.81$, $p = 0.002$); whereas gill raker length remained relatively constant regardless of SL in stream-caught fish, it increased with length in fish from the reservoir (Fig. 2.20j). A statistically significant interaction was also apparent between SL and habitat type in body width ($F_{1,56} = 13.81$, $p < 0.001$). Inspection of the data (Fig. 2.20a) shows that stream sticklebacks were wider than all but the smallest reservoir fish. Finally, there was also an interaction between SL and mouth width ($F_{1,55} = 7.00$, $p = 0.011$) with smaller stream fish having wider mouths than reservoir fish, at all but the largest sizes (approx. 41mm+) (Fig. 2.20i).

Two of the 26 RW scores showed significant differences between reservoir and stream fish, explaining a total of 60.6% of shape differences (Fig.2.21). Positive values in RW1 (41.7%; $F_{1,58} = 114.2$, $p < 0.001$) related to fish with slimmer bodies, longer heads, longer caudal peduncles, shorter pelvic girdles and a shorter ectocoracoid Positive scores in RW2 (18.8%; $F_{1,58} = 7.53$, $p = 0.008$) were associated with an increase in head and body depth , a thicker caudal peduncle and a longer ventral fin.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.944, χ^2 (26) = 99.9, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 100% of the samples.

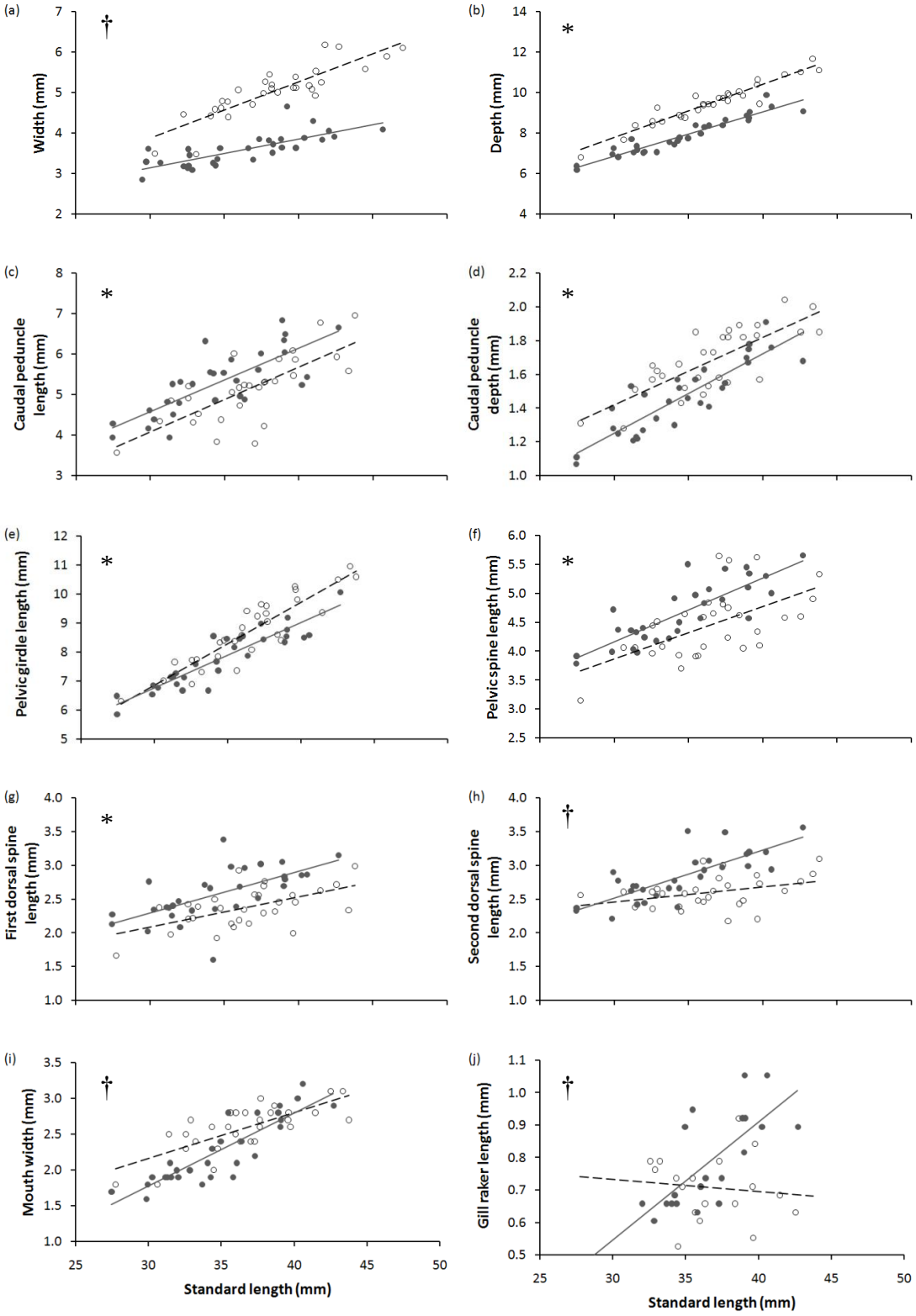


Figure 2.20 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle length (d) caudal peduncle depth and (e) pelvic girdle length in sticklebacks collected from the **Alaw** system (reservoir samples —●—, stream samples -- ○--). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

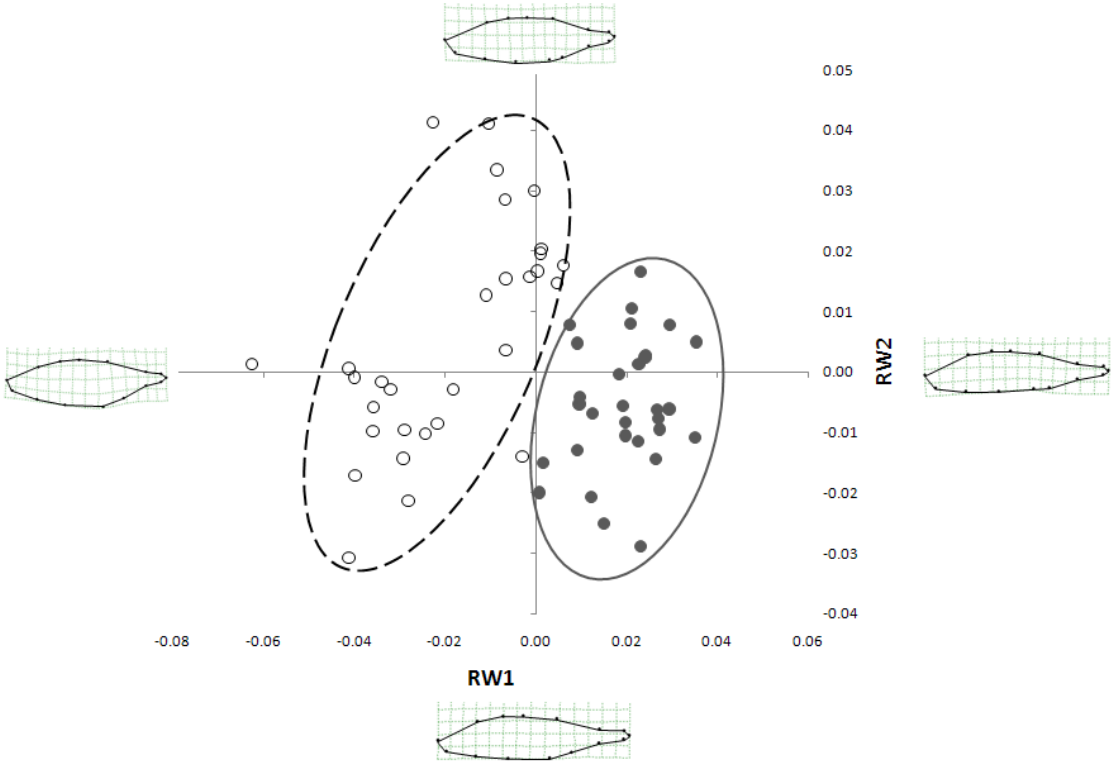


Figure 2.21 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Alaw** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

2.3.2.2. Blackbrook system

Principal components analysis revealed the presence of three components with eigenvalues exceeding 1, explaining 74% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 65% of the variance. Component 1 was primarily related to overall size and shape of specimens including spine lengths, whereas component 2 appeared to be more closely related to feeding morphology and the average number of lateral plates (Table 2.5).

Table 2.5 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Blackbrook reservoir-stream system.

Trait	Component 1	Component 2
Standard length	0.971	-0.008
Depth	0.960	-0.049
Caudal peduncle depth	0.937	-0.045
Pelvic girdle length	0.924	0.172
Second dorsal spine length	0.890	0.147
First dorsal spine length	0.874	0.136
Pelvic spine length	0.851	0.203
Caudal peduncle length	0.813	-0.097
Mouth width	0.794	-0.181
Jaw angle	0.437	-0.642
Gill raker length	0.201	0.591
Average number of lateral plates	0.125	-0.575
Number of gill rakers	0.029	0.495
Width	0.208	0.284

Stream-caught stickleback from the Blackbrook system had wider ($F_{1,63} = 27.98$, $p < 0.001$) and deeper ($F_{1,62} = 16.99$, $p < 0.001$) bodies than reservoir-caught fish (Figs. 2.22a-b). Stream fish also had a longer first dorsal spine ($F_{1,62} = 5.58$, $p = 0.021$), a wider mouth than reservoir fish ($F_{1,62} = 15.47$, $p < 0.001$) and shorter gill rakers ($F_{1,37} = 5.05$, $p = 0.031$) (Figs. 2.22c-e). No other differences in morphology were noted ($p > 0.05$).

There were significant differences between reservoir and stream caught fish in 4 of the RW scores, accounting for a total of 37.9% of the variation observed. RW1 explained 21.5% of the variation; however, although significant, this axis was primarily associated with specimen bending, an artefact of the fixing stage. RW4 and RW6 together explained 12.9% of the variation (Fig. 2.23). Although significant, RW7 only explained 3.5% of the variation. Positive scores in RW4 (7.8%; $F_{1,64} = 12.64$, $p = 0.001$) were associated with an increased separation of the first two dorsal spines, in

addition to a deeper body and caudal peduncle. RW6 (5.1%; $F_{1,64} = 18.21$, $p < 0.001$) was associated with head size, body depth and dorsal fin placement. Fish with high RW6 scores had smaller heads, deeper bodies and longer dorsal fins, which extend anteriorly.

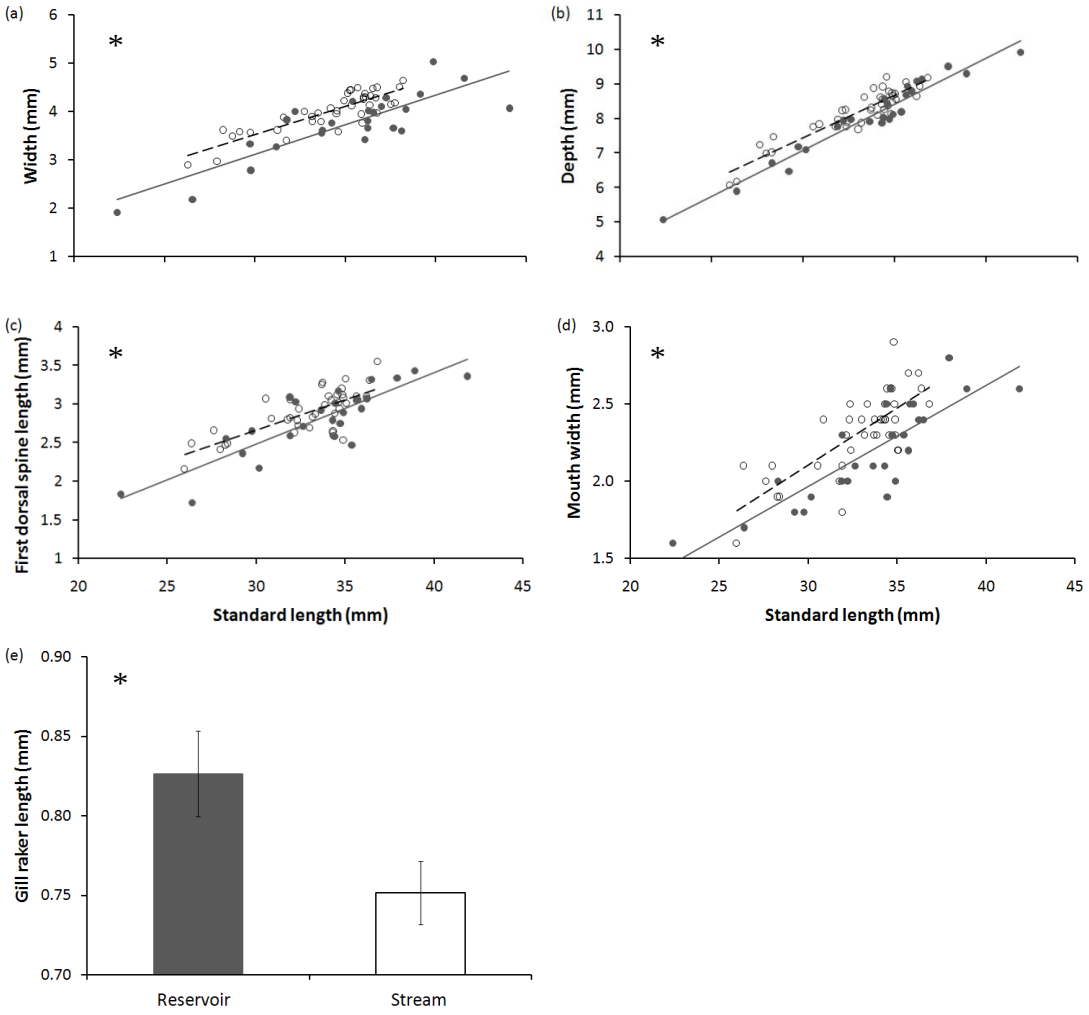


Figure 2.22 The relationship between standard length (SL) and (a) width, (b) depth, (c) first dorsal spine length and (d) mouth width in sticklebacks collected from the **Blackbrook** system (reservoir samples —●—, stream samples -- ○--). (e) Mean (±SE) gill raker length for fish from the reservoir (filled bar) and stream (open bar). *indicates a significant difference at the 5% level.

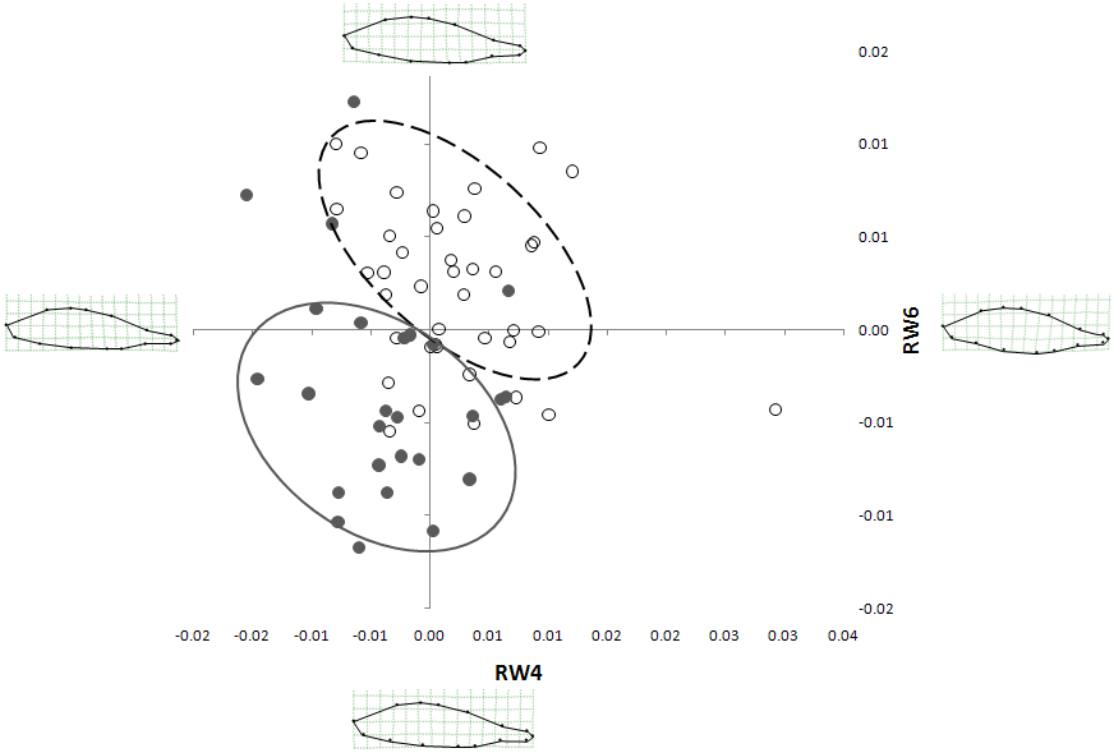


Figure 2.23 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Blackbrook** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.894, χ^2 (26) = 81.8, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 98.5% of the samples (Table 2.6).

Table 2.6 Percentage classification results of sticklebacks from the Blackbrook reservoir-stream system using a discriminant function analysis of shape.

	Predicted group membership	
Habitat	Stream	Reservoir
Stream	97.5	2.5
Reservoir	0	100

2.3.2.3. Cefni system

Principal components analysis revealed the presence of four components with eigenvalues exceeding 1, explaining 78% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 61% of the variance. Component 1 was primarily related to overall size and shape of specimens, whereas component 2 appeared to be more closely related to spine lengths. Jaw angle, gill raker morphology and plate number did not fit well with either component (Table 2.7).

Table 2.7 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Cefni reservoir-stream system.

Trait	Component 1	Component 2
Depth	0.896	0.376
Standard length	0.879	0.378
Pelvic girdle length	0.846	0.417
Caudal peduncle depth	0.839	0.408
Width	0.829	0.264
Caudal peduncle length	0.740	0.327
Mouth width	0.719	0.448
First dorsal spine length	0.043	0.896
Second dorsal spine length	0.323	0.819
Pelvic spine length	0.206	0.786
Jaw angle	0.301	-0.133
Average number of lateral plates	0.268	0.183
Gill raker length	0.448	0.204
Number of gill rakers	-0.311	0.055

A significant effect of habitat type was apparent in measures of body depth (U = 1348, N_S = 44, N_R = 45, p = 0.003), width ($F_{1,84}$ = 11.702, p = 0.001), second dorsal spine length ($F_{1,84}$ = 4.07, p = 0.047), pelvic girdle length ($F_{1,84}$ = 34.22, p < 0.001), and average number of lateral plates (U = 1450, N_S = 44, N_R = 45, p < 0.001). Sticklebacks from streams had deeper and wider bodies, a longer second dorsal spine, a longer pelvic

girdle and fewer lateral plates than their reservoir-dwelling counterparts (Figs. 2.24a-b, d, f, h).

There was a significant interaction between SL and habitat type in depth of the caudal peduncle ($F_{1,83} = 10.66$, $p = 0.002$); although depth increased with SL in fish from both habitats, the increase was greater for stream fish (Fig. 2.24c). There was also a significant interaction between SL and habitat type in pelvic spine length ($F_{1,83} = 6.91$, $p = 0.010$) and also in mouth width ($F_{1,83} = 5.19$, $p = 0.025$). Similar to the pattern observed with caudal peduncle depth, in both of these traits, although the size of the trait increased with SL, the increase was greater in stream fish than it was in reservoir fish (Figs. 2.24e,g).

Geometric morphometric analyses established 3 significant RW axes explaining a total of 31.0% of variation associated with differences in the reservoir-stream pairs of the Cefni system (Fig. 2.25). Positive scores in RW2 (17.4%; $F_{1,85} = 92.36$, $p < 0.001$) were associated with fish with a deeper body, longer dorsal and ventral fins and a wider and shorter caudal peduncle. Positive scores in RW3 (11.2%; $F_{1,85} = 5.06$, $p = 0.027$) were also related to a deeper bodies, a shorter caudal peduncle, longer dorsal and ventral fins and additionally a longer head. RW7 was significant but only accounted for 2.4% of the observed shape variation.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.911, χ^2 (26) = 127.8, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 97.7% of the samples (Table 2.8).

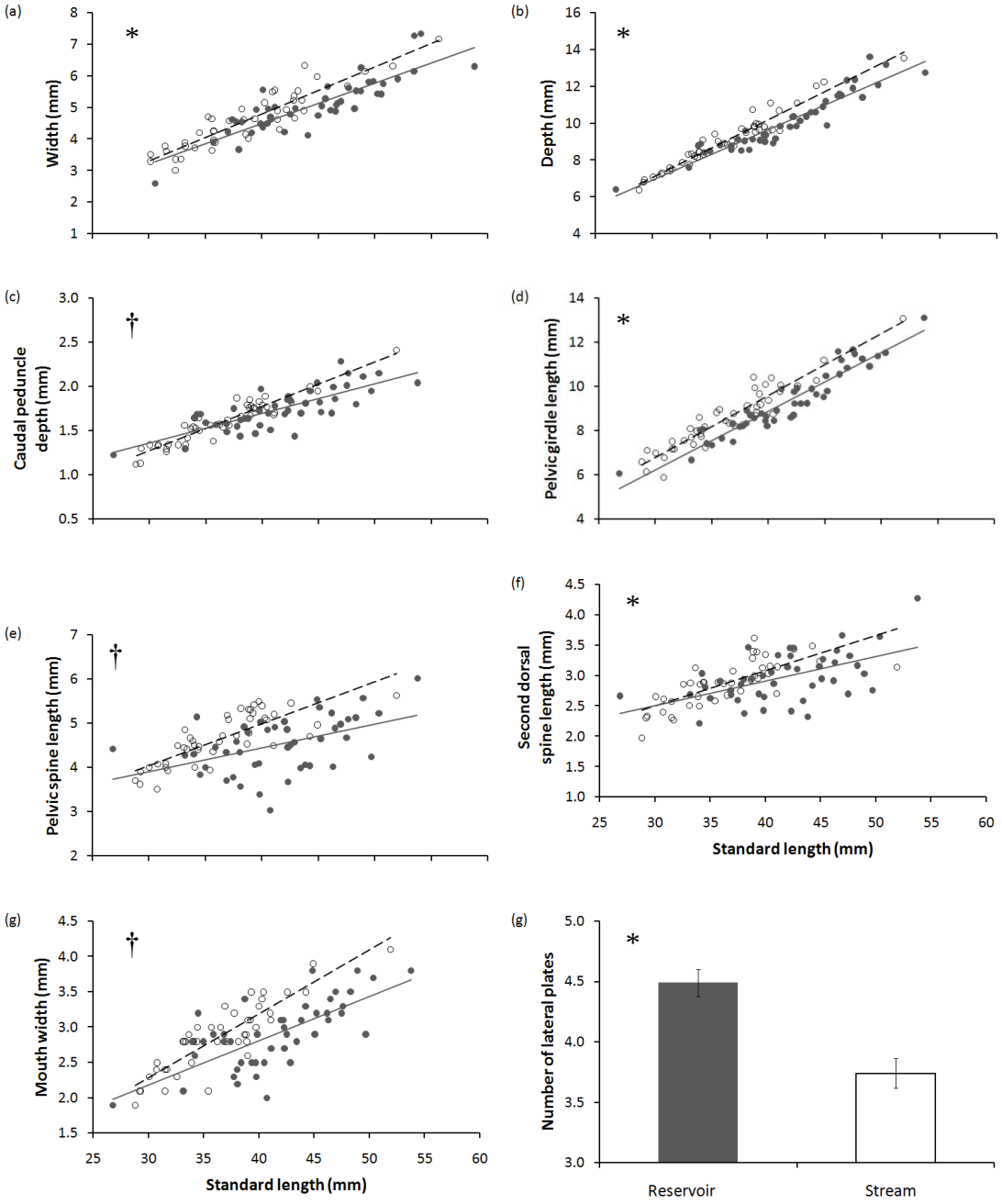


Figure 2.24 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle depth,(d) pelvic girdle length and (e) pelvic spine length, (f) second dorsal spine length and (g) mouth width in sticklebacks collected from the Cefni system (reservoir samples —●—, stream samples --○--). (h) Mean (±SE) number of lateral plates for fish from the reservoir (filled bar) and stream (open bar). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

Table 2.8 Percentage classification results of sticklebacks from the Cefni reservoir-stream system using a discriminant function analysis of shape.

	Predicted group membership	
Habitat	Stream	Reservoir
Stream	97.6	2.4
Reservoir	2.2	97.8

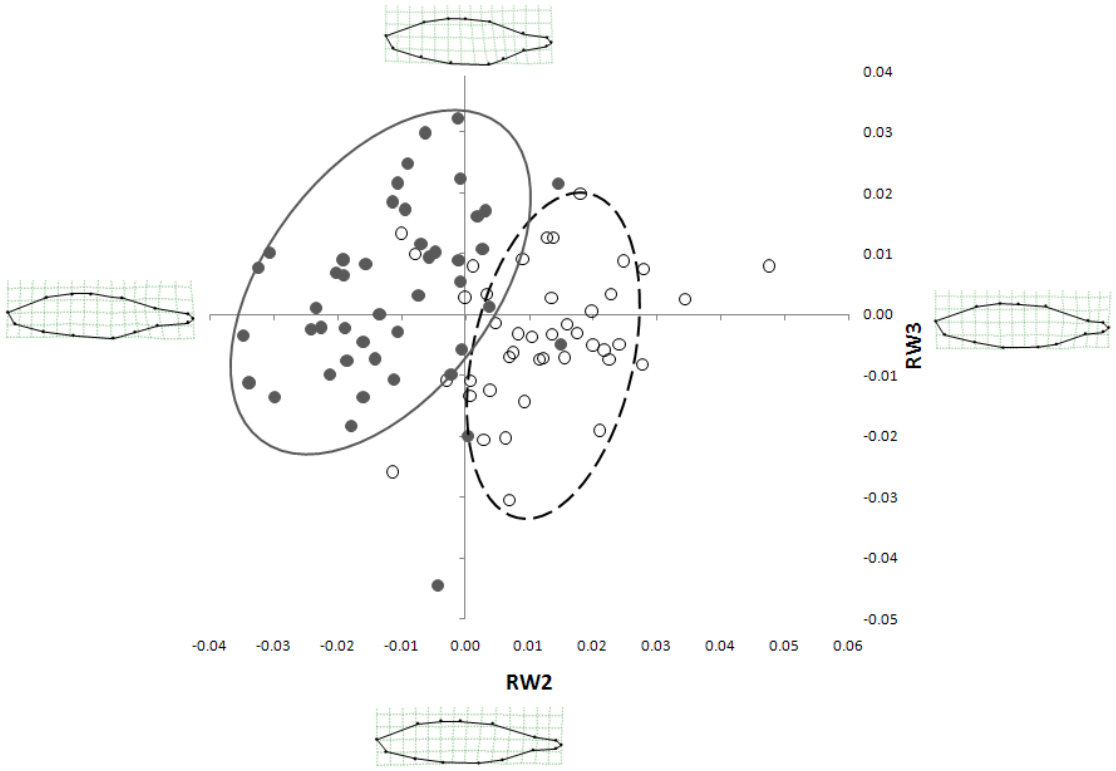


Figure 2.25 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Cefni** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

2.3.2.4. Carsington system

Principal components analysis revealed the presence of three components with eigenvalues exceeding 1, explaining 76% of the variance in the morphological traits measured. Component 1 was primarily related to overall size and shape of specimens, whereas component 2 appeared to be more closely related to spine lengths. The final component was associated with jaw angle and gill raker morphology (Table 2.9).

Table 2.9 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Carsington reservoir-stream system.

Trait	Component 1	Component 2	Component 3
Mouth width	0.868	0.310	0.125
Depth	0.854	0.464	0.116
Width	0.836	0.406	0.116
Caudal peduncle depth	0.826	0.394	0.051
Standard length	0.814	0.528	-0.022
Pelvic girdle length	0.736	0.562	0.168
Caudal peduncle length	0.549	0.445	-0.084
Average number of lateral plates	0.510	-0.196	-0.019
First dorsal spine length	0.317	0.842	0.159
Second dorsal spine length	0.180	0.831	-0.026
Pelvic spine length	0.262	0.797	0.166
Gill raker length	0.168	0.677	-0.259
Jaw angle	0.446	0.159	0.736
Number of fill rakers	0.117	0.099	-0.904

In comparison to reservoir-caught sticklebacks, fish from the stream had wider (U = 78.5, N_S = 19, N_R = 17, p = 0.001) and deeper ($F_{1,37}$ = 21.38, p < 0.001) bodies in addition to a longer pelvic girdle ($F_{1,37}$ = 5.41, p = 0.026). In terms of trophic morphology, stream fish had a wider mouth (Z = 89.0, N_S = 19, N_R = 21, p = 0.003), a more forwards pointing snout ($F_{1,37}$ = 4.85, p = 0.034) and fewer gill rakers (Z = 234, N_S = 19, N_R = 17, p = 0.018) (Figs. 2.26a-b, d-g). There was a significant interaction between SL and habitat type in caudal peduncle depth ($F_{1,36}$ = 5.13, p = 0.030) so that at a smaller size, stream fish had deeper caudal peduncles than reservoir fish but at larger sizes, this difference was negligible (Fig. 2.26c).

Two of the 26 RW scores were able to significantly differentiate between reservoir-caught and stream-caught individuals (Fig. 2.27). RW2 (23.6%; $F_{1,37}$ = 20.42, p < 0.001) was associated with body depth, the ventral fin and the caudal peduncle. Positive scoring fish were deeper bodied and had a longer ventral fin. They also had a

deeper and shorter caudal peduncle. Individuals scoring more positively in RW8 (2.2%; $F_{1,37} = 6.40$, $p = 0.016$) had a deeper head and body and a deeper caudal peduncle.

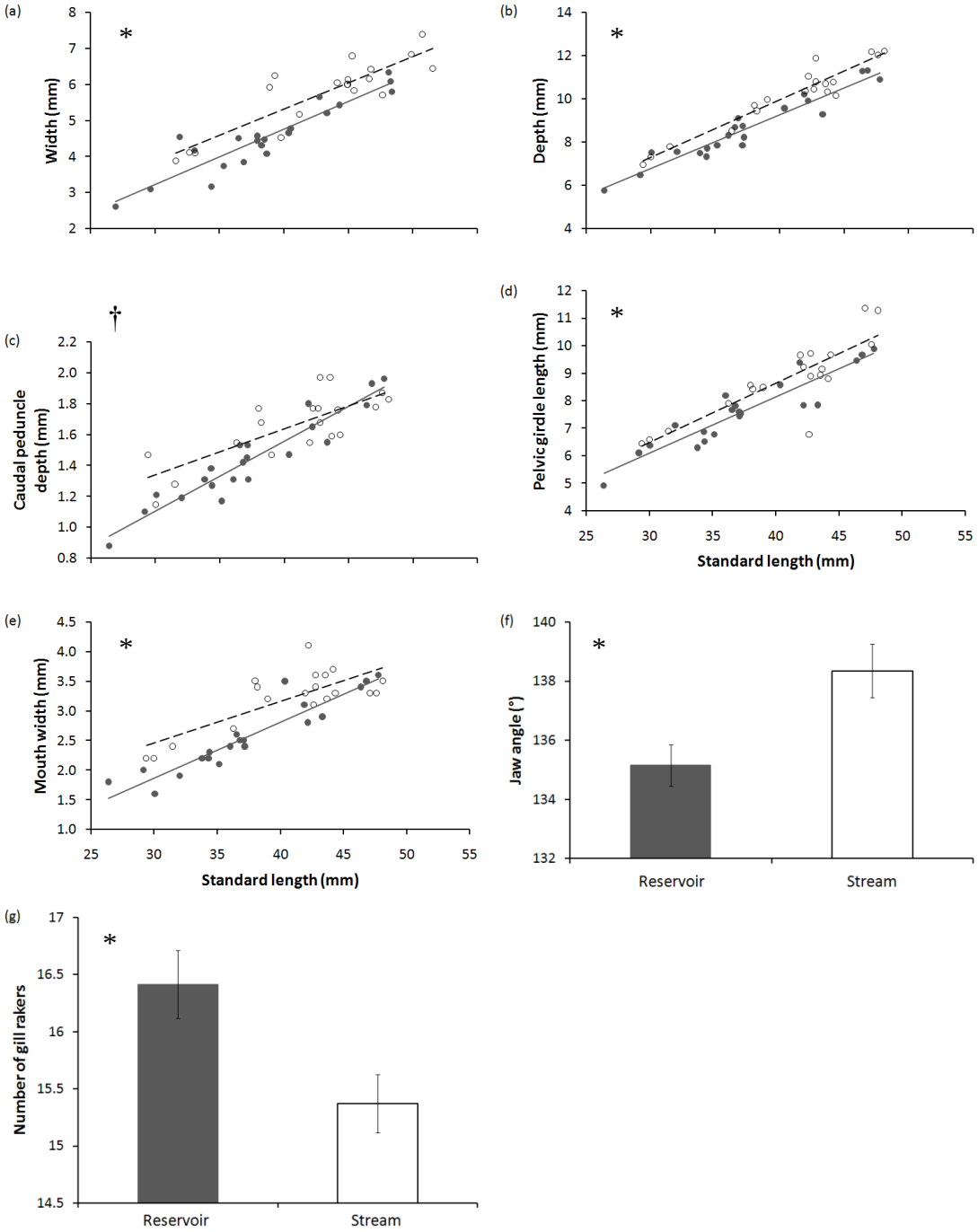


Figure 2.26 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle depth,(d) pelvic girdle length and (e) mouth width in sticklebacks collected from the **Carsington** system (reservoir samples —●— , stream samples --○--). (f) Mean (±SE) angle of the jaw and (g) mean (±SE) number of gill rakers for fish from the reservoir (filled bars) and stream (open bars). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

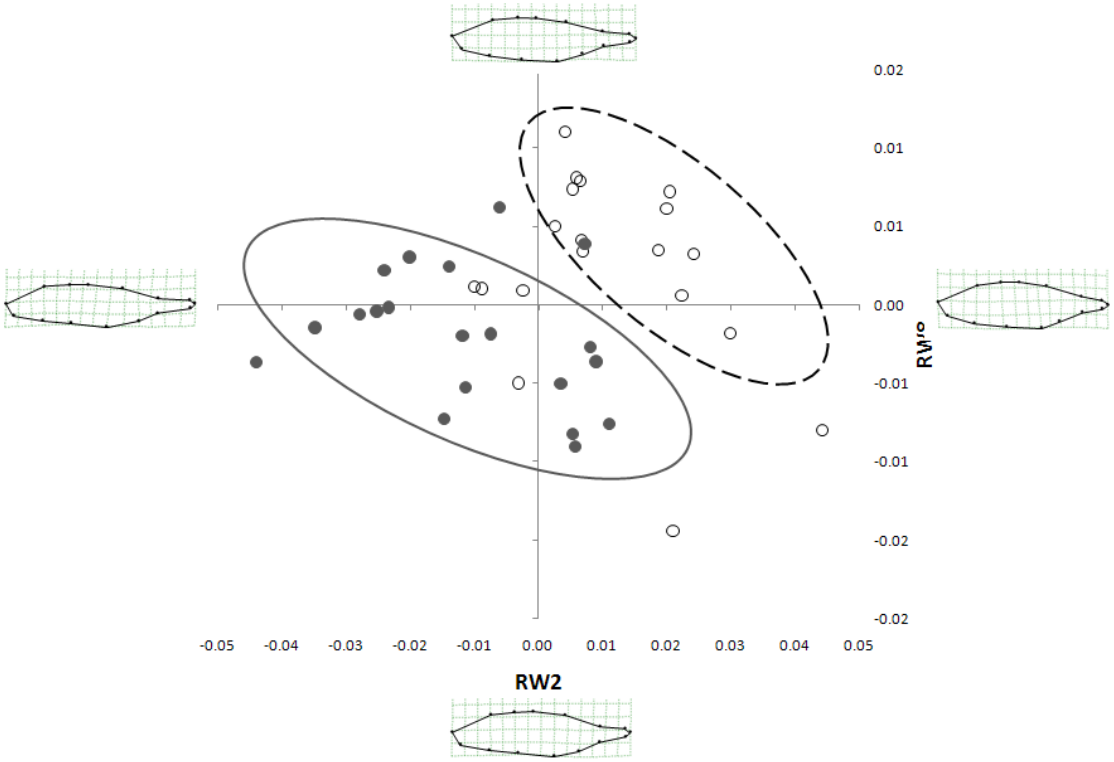


Figure 2.27 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Carsington** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.957, χ^2 (26) = 59.3, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 100% of the samples.

2.3.2.5. Kendoon system

Principal components analysis revealed the presence of four components with eigenvalues exceeding 1, explaining 72% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 55% of the variance. Component 1 was primarily related to overall size and shape of specimens, in addition to feeding morphology whereas component 2 appeared to be more closely related to spine length, but also closely related to standard length and depth as well (Table 2.10).

Table 2.10 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Kendoon reservoir-stream system.

Trait	Component 1	Component 2
Mouth width	0.850	0.217
Depth	0.743	0.607
Standard length	0.719	0.585
Caudal peduncle depth	0.640	0.442
Number of gill rakers	0.623	-0.008
Width	0.621	0.342
Gill raker length	0.584	0.137
Jaw angle	0.531	-0.276
Pelvic girdle length	0.474	0.497
Caudal peduncle length	0.381	0.329
Second dorsal spine length	0.195	0.889
First dorsal spine length	0.204	0.819
Pelvic spine length	0.040	0.797
Average number of lateral plates	0.036	0.320

There were significant differences in the width ($F_{1,49} = 14.38$, $p < 0.001$) and depth ($F_{1,48} = 8.95$, $p = 0.004$) of sticklebacks from reservoirs and streams with stream individuals being wider and deeper bodied than reservoir fish (Figs. 2.28a-b). Further, there were statistically significant differences in mouth width ($F_{1,48} = 17.63$, $p < 0.001$), length of the pelvic girdle ($F_{1,48} = 6.03$, $p = 0.018$), length of the gill rakers ($F_{1,29} = 11.88$, $p = 0.002$) and depth of the caudal peduncle ($F_{1,48} = 9.11$, $p = 0.004$) (Figs. 2.28c-f).

Shape analyses yielded just 2 significant RW: RW2 (20.5%, $F_{1,50} = 20.70$, $p < 0.001$) and RW7 (3.3%, $F_{1,50} = 5.56$, $p = 0.022$) (Fig. 2.29). Positive scores in RW2 were associated with a longer jaw and head, a deeper body and a longer ventral fin. Positive scores in RW7 were associated with a more posterior placement of the ectocoracoid and the dorsal spines, in addition to a longer head.

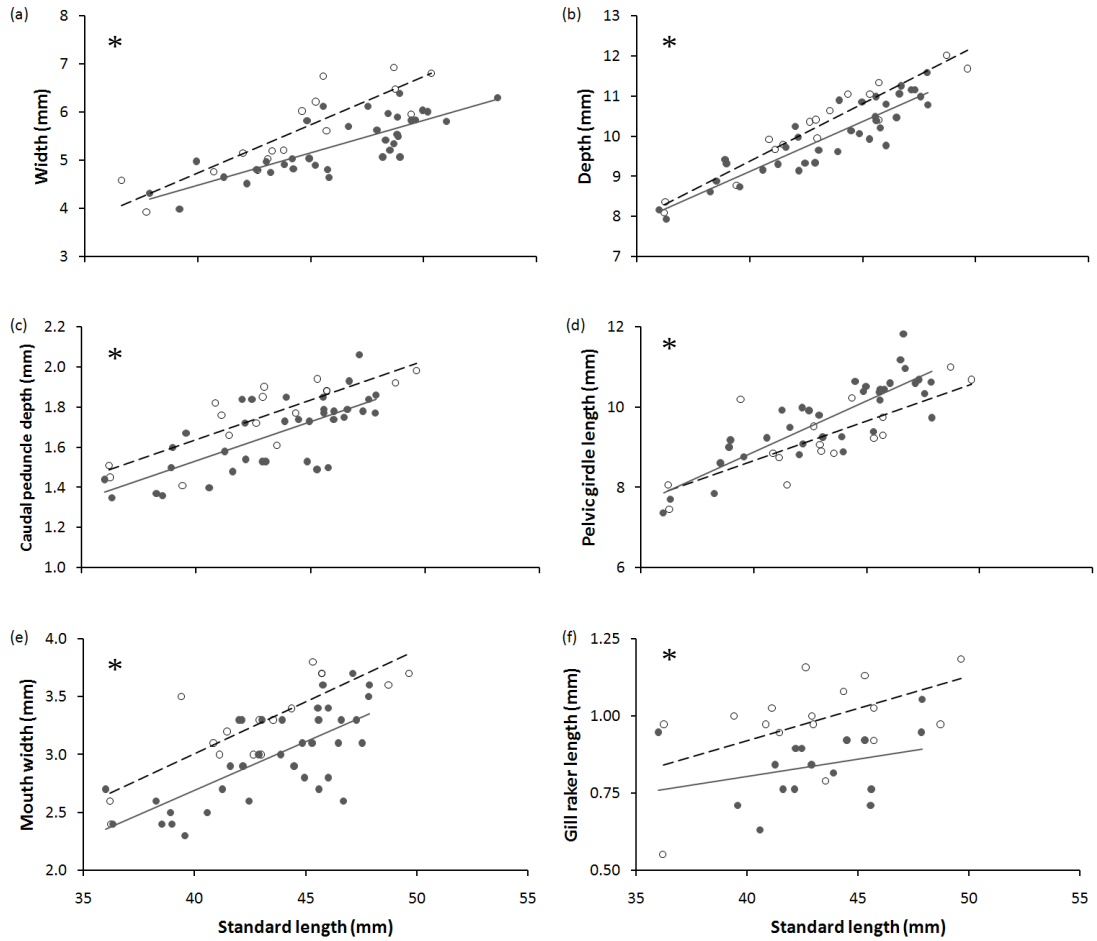


Figure 2.28 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle depth,(d) pelvic girdle length and (e) mouth width and (g) gill raker length in sticklebacks collected from the **Kendoon** system (reservoir samples —●— , stream samples --○--). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.817, χ^2 (26) = 59.3, p = 0.032). The discriminant function was correctly able to predict habitat membership for 96.2% of the samples (Table 2.11).

Table 2.11 Percentage classification results of sticklebacks from the Kendoon reservoir-stream system using a discriminant function analysis of shape.

	Predicted group membership	
Habitat	Stream	Reservoir
Stream	87.5	12.5
Reservoir	0	100

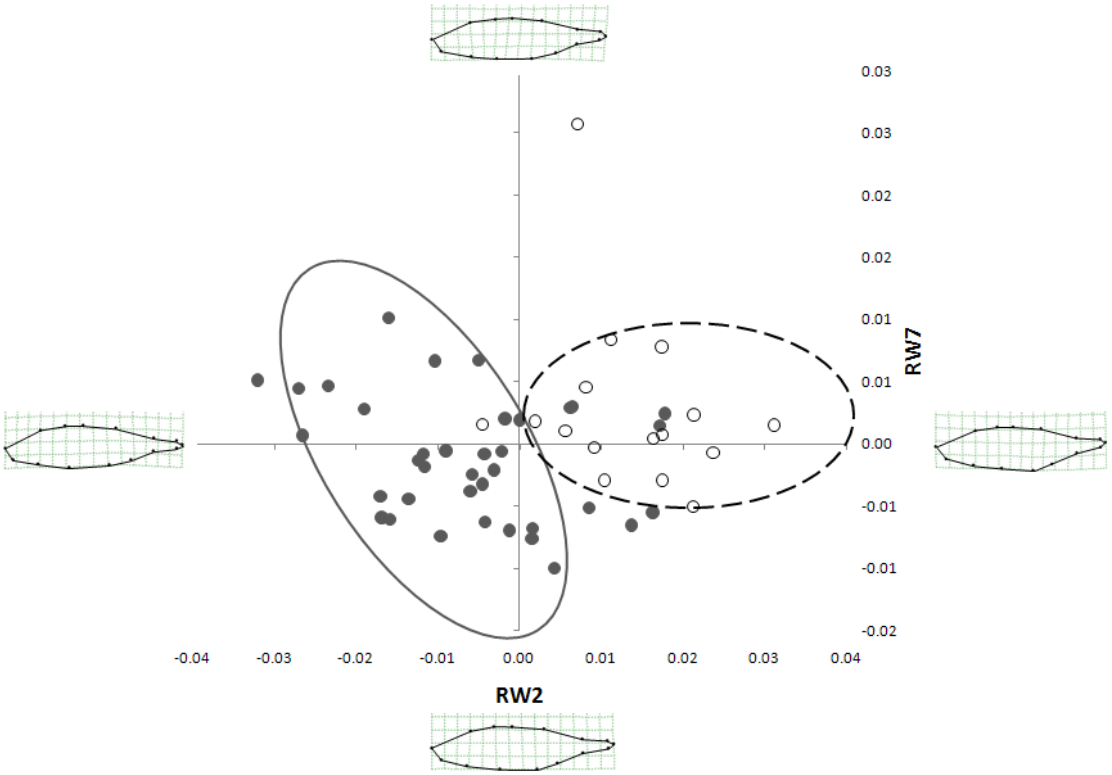


Figure 2.29 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Kendoon** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

2.3.2.6. Stithians system

Principal components analysis revealed the presence of four components with eigenvalues exceeding 1, explaining 72% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only three components should be retained and explained a total of 64% of the variance. Component 1 was primarily related specimen width and posterior morphology whereas component 2 appeared to be most closely related to spine length. Component 3 only explained 15% of the variance and was related to standard length and depth, in addition to the number of gill rakers (Table 2.12).

Table 2.12 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Stithians reservoir-stream system.

Trait	Component 1	Component 2	Component 3
Width	0.882	0.044	0.106
Mouth width	0.874	0.154	0.097
Pelvic girdle length	0.804	0.358	-0.081
Caudal peduncle depth	0.786	0.431	-0.151
Caudal peduncle length	0.568	0.360	0.314
Second dorsal spine length	0.210	0.903	0.034
First dorsal spine length	0.204	0.893	0.004
Pelvic spine length	0.147	0.839	-0.006
Gill raker length	0.303	0.603	-0.032
Depth	0.052	-0.230	-0.909
Standard length	0.426	0.357	0.784
Number of gill rakers	-0.118	0.259	-0.530
Average number of lateral plates	-0.049	-0.044	0.322
Jaw angle	0.191	0.246	0.173

Reservoir and stream sticklebacks from the Stithians system differed in depth ($F_{1,64} = 17.98$, $p < 0.001$), jaw angle ($F_{1,64} = 7.71$, $p = 0.001$), mouth width ($F_{1,64} = 11.93$, $p = 0.001$) and caudal peduncle depth ($F_{1,64} = 16.93$, $p < 0.001$) (Figs. 2.30b-e). There was a statistically significant interaction between SL and habitat type in body width ($F_{1,63} = 5.43$, $p = 0.023$). For the most part, reservoir fish were less wide than stream fish; however differences in depth appeared to decrease as fish from both sites increased in overall length (Fig. 2.30a).

A total of 24.3% of the variation in shape was significantly associated with differences between reservoir and stream sticklebacks (Fig. 2.31). Fish scoring highly on RW2 (15.1%, $F_{1,66} = 12.16$, $p = 0.001$) had a deeper body, longer dorsal fin and shorter and wider caudal peduncle. Fish scoring more positive on RW3 (9.2%, $F_{1,66} = 13.05$, $p = 0.001$) were slimmer along the entire length and had a noticeably more narrow caudal peduncle.

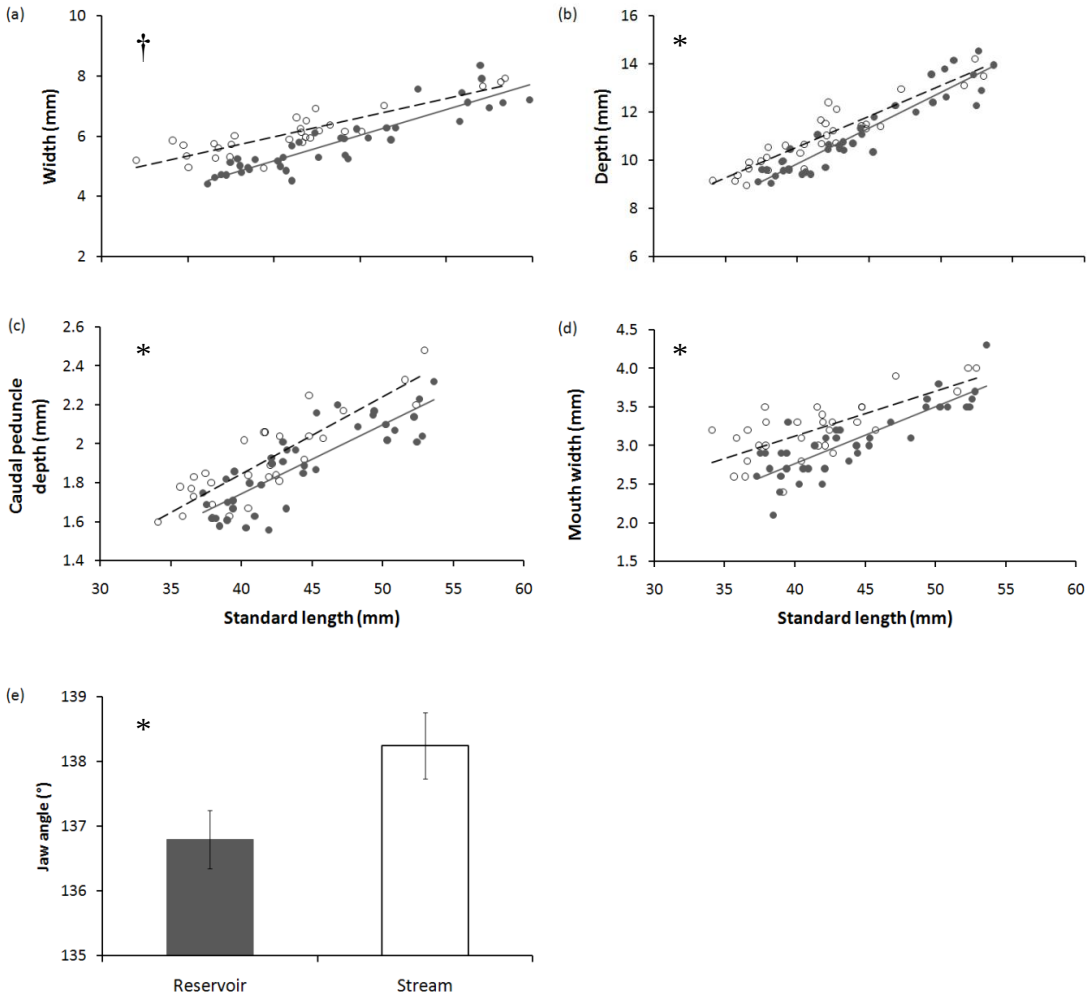


Figure 2.30 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle depth and (d) mouth width in sticklebacks collected from the **Stithians** system (reservoir samples —●— , stream samples --○--). (e) Mean (±SE) angle of the jaw for fish from the reservoir (filled bar) and stream (open bar). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.826, χ^2 (26) = 60.8, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 91.2% of the samples (Table 2.13).

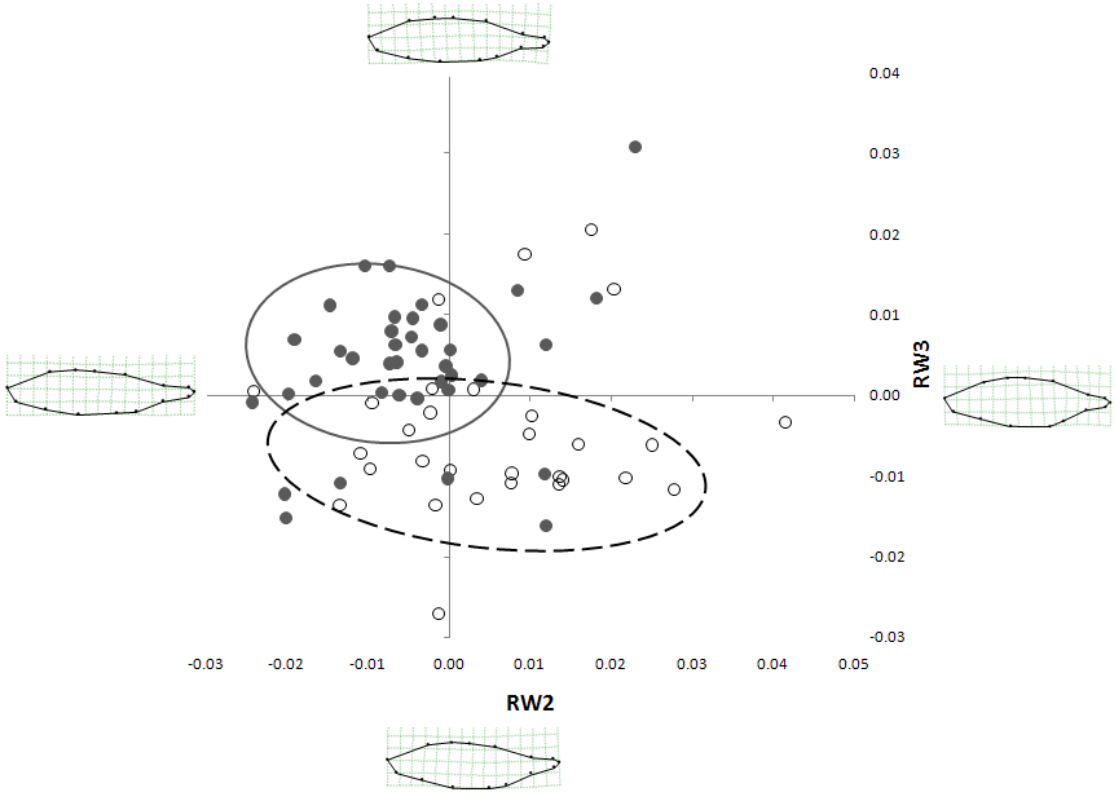


Figure 2.31 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Stithians** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

Table 2.13 Percentage classification results of sticklebacks from the Stithians reservoir-stream system using a discriminant function analysis of shape.

	Predicted group membership	
Habitat	Stream	Reservoir
Stream	86.7	13.3
Reservoir	5.3	94.7

2.3.2.7. Thornton system

Principal components analysis revealed the presence of four components with eigenvalues exceeding 1, explaining 80% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 72% of the variance. Component 1 was primarily related to overall shape of specimens whereas component 2 appeared to be most closely related to spine length and gill raker morphology (Table 2.14).

Table 2.14 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught from the Thornton reservoir-stream system.

Trait	Component 1	Component 2
Width	0.904	0.250
Mouth width	0.902	0.137
Depth	0.872	0.426
Standard length	0.866	0.452
Pelvic girdle length	0.804	0.435
Caudal peduncle length	0.782	0.448
Caudal peduncle depth	0.768	0.526
Jaw angle	0.510	-0.400
Second dorsal spine length	0.427	0.823
First dorsal spine length	0.459	0.790
Pelvic spine length	0.541	0.722
Gill raker length	0.085	0.794
Number of gill rakers	0.229	0.607
Average number of lateral plates	0.071	0.291

Fish caught from the stream had a wider body ($F_{1,42} = 6.035$, $p = 0.018$) than those from the reservoir (Fig. 2.33a). They also had a shorter ($F_{1,42} = 6.61$, $p = 0.014$) and narrower ($F_{1,42} = 12.81$, $p = 0.001$) caudal peduncle (Figs. 2.33c-d). Stream fish also had a wider mouth ($F_{1,42} = 12.77$, $p = 0.001$) and shorter gill rakers ($F_{1,42} = 71.94$, $p < 0.001$) (Figs. 2.32h-i). There was a significant interaction between SL and habitat type in the length of each of the spines: first dorsal spine ($F_{1,41} = 7.64$, $p = 0.009$), second dorsal spine ($F_{1,41} = 5.32$, $p = 0.026$), pelvic spine length ($F_{1,41} = 13.82$, $p = 0.001$). In all of these cases, stream fish had longer spines at smaller age lasses, but as fish increased in size (approx. 33mm+) reservoir fish had longer spines (Figs. 2.33e-g).

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.956, χ^2 (26) = 73.3, $p < 0.001$). The discriminant function was correctly able to predict habitat membership for 100% of the samples.

Significant differences between sits of capture were found in 2 of the 26 principal components of shape and explained a total of 35.8% of the differences observed (Fig. 2.32). Positive scores in RW1 (25.5%, $F_{1,43} = 18.49$, $p < 0.001$) were associated with fish that were more streamlined. They also had a shorter jaw and head length, in addition to a lengthened caudal peduncle. Many of the landmarks forward of the caudal peduncle (including the spines) seemed to be shifted more anterior as a result of its increased length. Positive scores in RW4 (10.3%, $F_{1,43} = 11.53$, $p = 0.001$) were related to a smaller head, a more upturned snout and slimmer bodies.

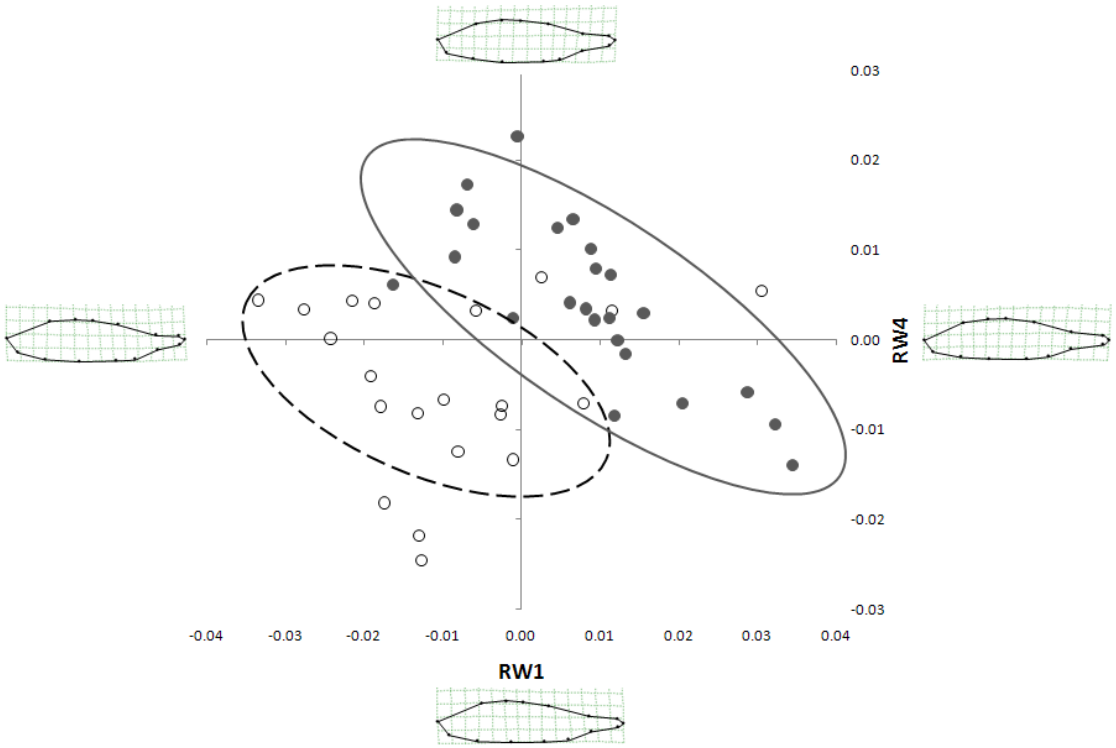


Figure 2.32 Plot of the relative warp scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks from the **Thornton** system. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

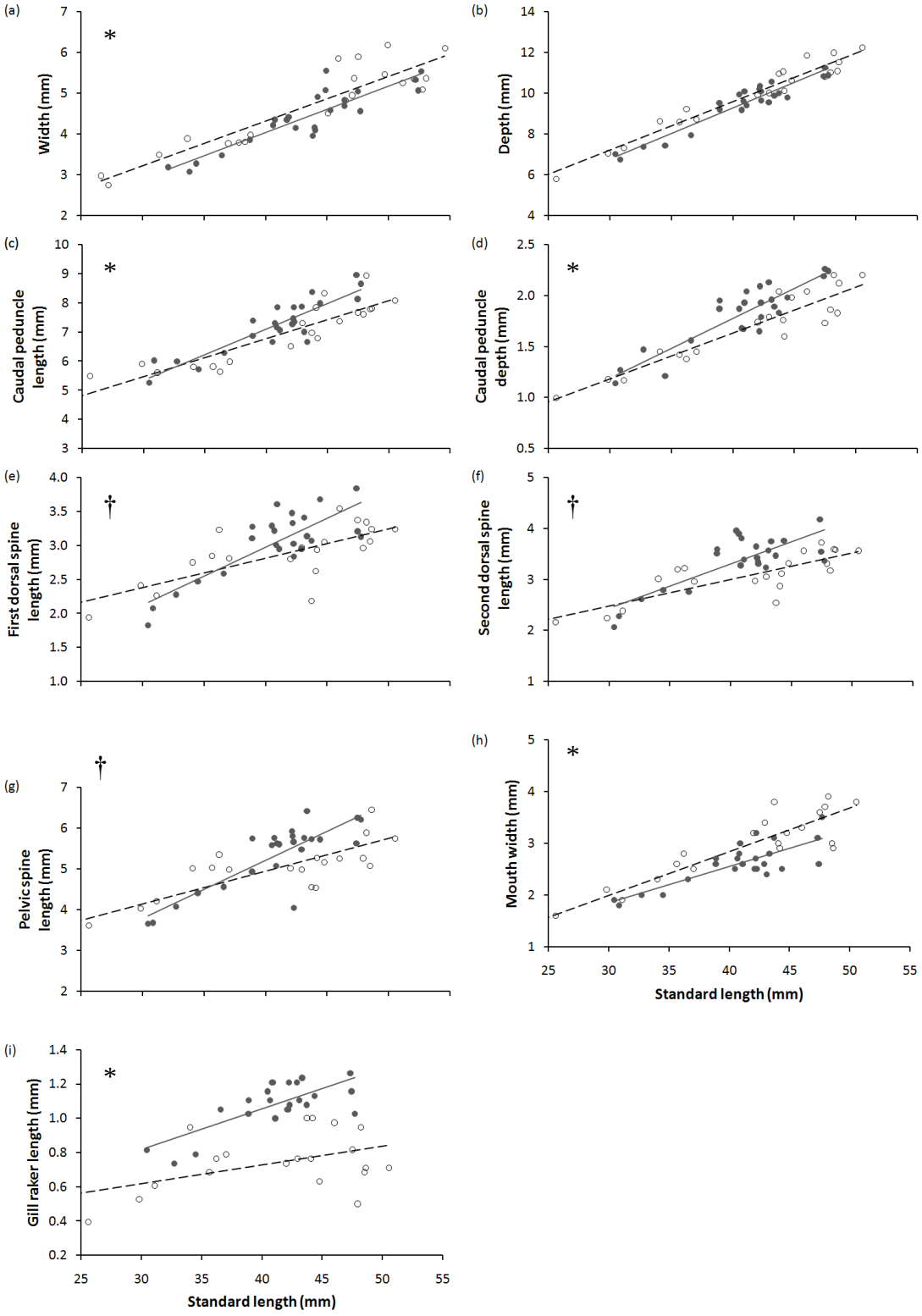


Figure 2.33 The relationship between standard length (SL) and (a) width, (b) depth, (c) caudal peduncle length, (d)caudal peduncle depth, (e) first dorsal spine length, (f) second dorsal spine length, (g) pelvic spine length, (h) mouth width and (i) gill raker length in sticklebacks collected from the **Thornton** system (reservoir samples —●—, stream samples -- ○ --). *indicates a significant difference at the 5% level. †indicates a significant interaction with SL.

2.3.3. Overall patterns of morphological variation between reservoir and stream sticklebacks across all systems

A summary of the morphological differences between reservoir and stream fish across systems is given in Table. 2.15. Overall, stream fish were wider (t = 4.65, df = 6, p = 0.003; Fig. 2.34a) deeper bodied (t = 4.44, df = 6, p = 0.004; Fig. 2.34b) and wider mouths (t = 9.12, df = 6, $p < 0.001$; Fig. 2.34j) than those from the reservoir.

Whereas for nearly all of the systems, mean caudal peduncle depth was greater in stream samples than it was in reservoir samples, the opposite was true for the Thornton reservoir-stream system (Fig. 2.34c). There was greater variability in the direction of difference for average length of the caudal peduncle: whereas it was longer in stream fish from the Blackbrook, Cefni and Kendoon systems, it was longer in reservoir fish from the Alaw, Carsington, Stithians and Thornton systems (Fig. 2.34d).

Similarly length of the dorsal and pelvic spines were not consistent across systems (Figs. 2.34e-g); whereas sticklebacks from the Blackbrook, Cefni Kendoon and Stithians systems all showed longer spines in the reservoir than in the stream, the opposite was true for fish from the Alaw, Carsington and Thornton systems. The average number of lateral plates differed little between habitats types within a system (Fig. 2.34m).

Average length of the pelvic girdle was less in reservoir fish than it was in stream fish in 6 of the 7 systems, although this difference was significant only in Alaw, Blackbrook, Cefni and Carsington. The difference in pelvic girdle length between reservoir and stream sticklebacks was also significant in the Kendoon system ($F_{1,49}$ = 5.87, p = 0.019), but it was longer in reservoir fish (Fig. 2.34h).

There were no consistent patterns in jaw angle (Fig. 2.34i), number of gill rakers (Fig. 2.34k) or gill raker length (Fig. 2.34l).

Table 2.15 A summary of the morphological differences observed between sticklebacks in seven reservoir-stream systems across the UK

Morphological trait	Alaw		Blackbrook		Cefni		Carsington		Kendoon		Stithians		Thornton	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Body width	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Body depth	+	-	+	-	+	-	+	-	+	-	+	-		
Caudal peduncle depth	-	+			-	+	-	+	-	+	-	+	+	-
Caudal peduncle length	+	-											+	-
First dorsal spine length	+	-	-	+									+	-
Second dorsal spine length	+	-			-	+							+	-
Pelvic spine length	+	-			-	+							+	-
Pelvic girdle length	-	+			-	+	-	+	+	-				
Jaw angle							-	+			-	+		
Mouth width	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Gill raker number							+	-						
Gill raker length	+	-	+	-					-	+			+	-
Plate number					+	-								

+ and – signs indicate whether the trait was significantly greater or lesser in the reservoir (R) or stream (S) at the 5% significance level. Those given in **red** indicate that there was a significant interaction between the trait and standard length (SL); in these cases, the signs refer to the direction of the result for adult individuals (i.e. high SL).

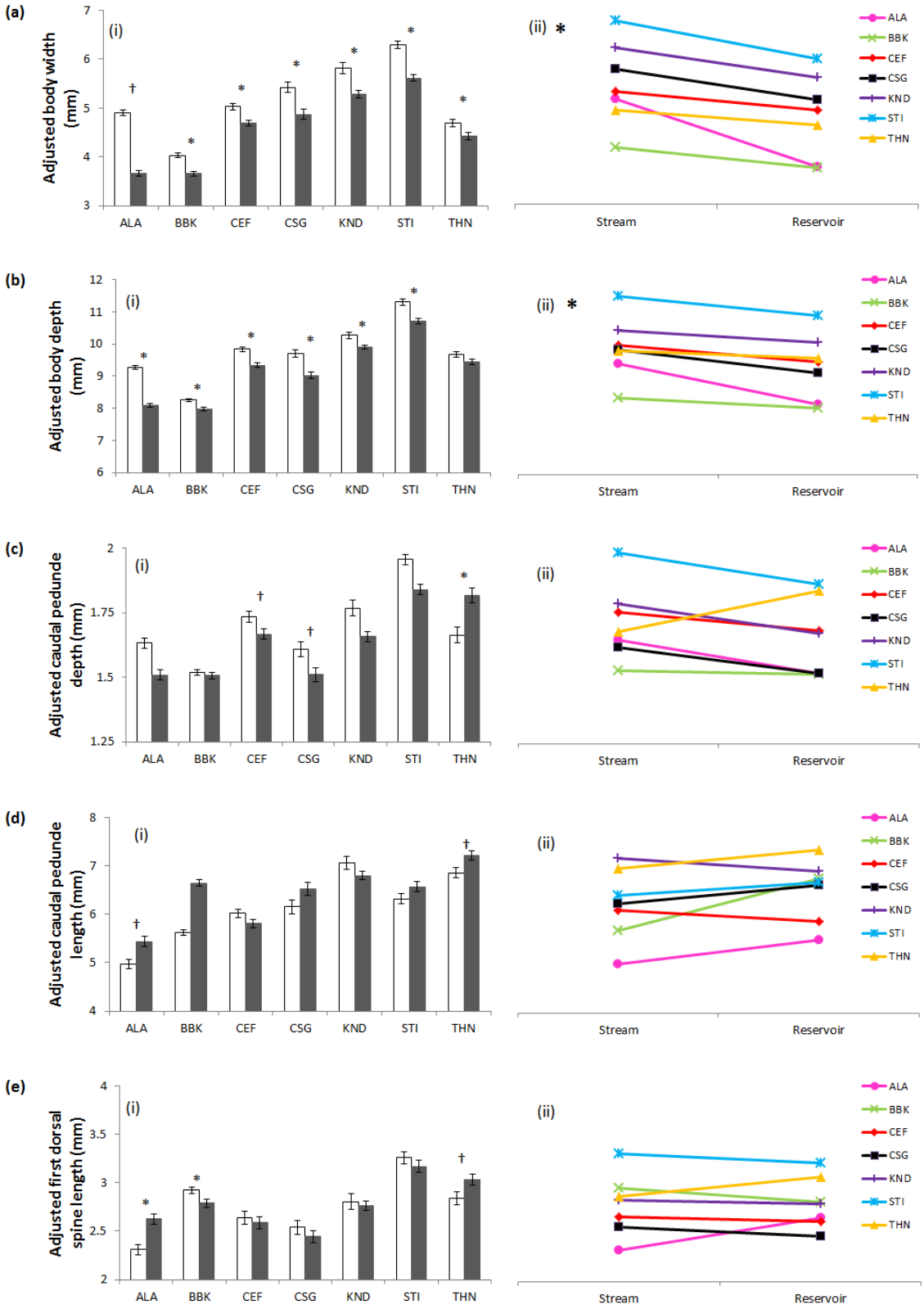


Figure 2.34 Mean ±SE (a) width, (b) depth, (c) caudal peduncle depth, (d) caudal peduncle length and (e) first dorsal spine length of reservoir- and stream-caught sticklebacks from geographically distinct systems. Data for each morphological trait is presented as (i) a bar chart where * indicates a significant difference between habitats and † indicates that there was a significant interaction with SL; (ii) a reaction norm plot where * indicates a significant difference across systems between reservoir and stream samples.

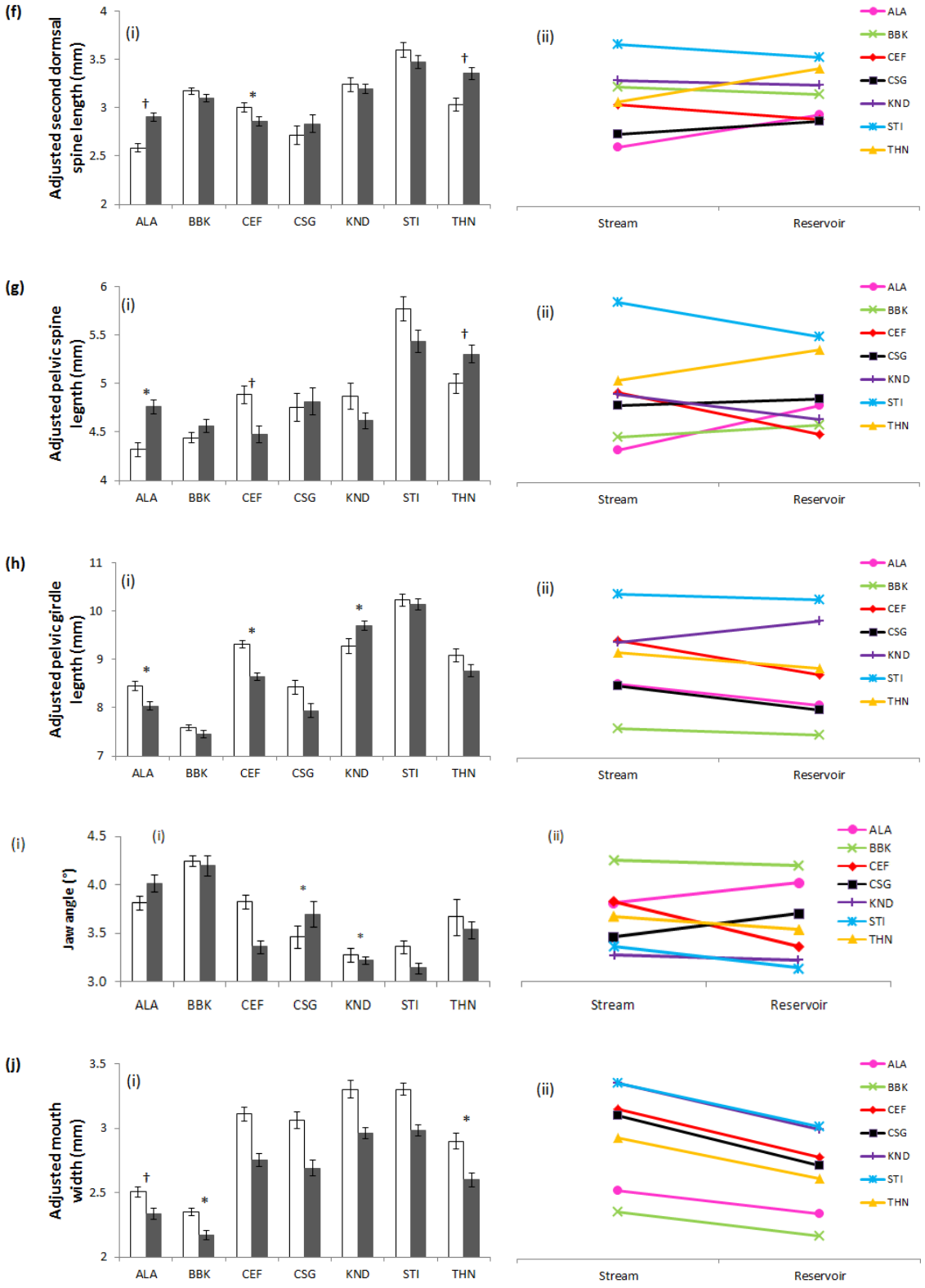


Figure 2.34 Mean ±SE (f) second dorsal spine length, (g) pelvic spine length, (h) pelvic girdle length, (i) jaw angle and (j) mouth width of reservoir- and stream-caught sticklebacks from geographically distinct systems. Data for each morphological trait is presented as (i) a bar chart where * indicates a significant difference between habitats and † indicates that there was a significant interaction with SL; (ii) a reaction norm plot where * indicates a significant difference across systems between reservoir and stream samples.

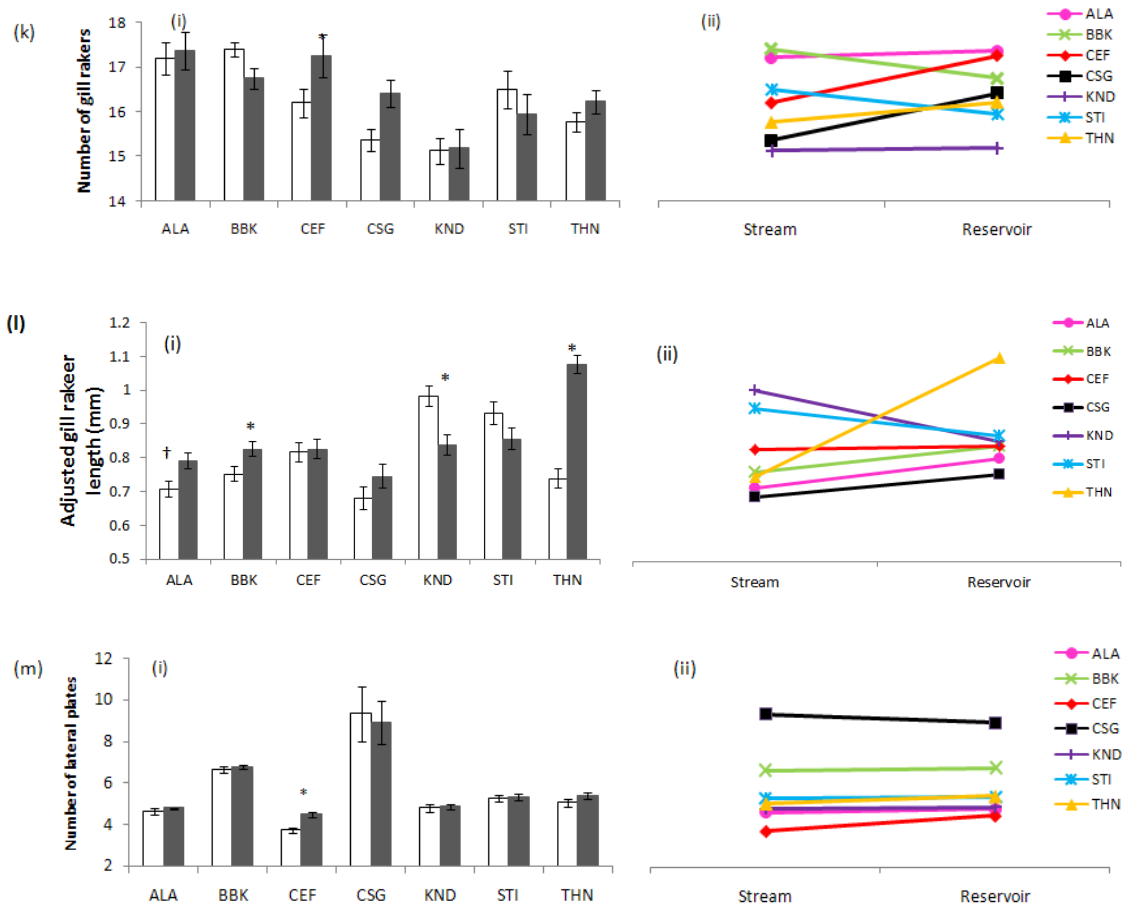


Figure 2.34 Mean ±SE (k) number of gill rakers, (l) gill raker length, and (m) number of lateral plates of reservoir- and stream-caught sticklebacks from geographically distinct systems. Data for each morphological trait is presented as (i) a bar chart where * indicates a significant difference between habitats and † indicates that there was a significant interaction with SL; (ii) a reaction norm plot where * indicates a significant difference across systems between reservoir and stream samples.

Of the 26 RW scores used to describe shape variation across geographically distinct systems, just 11 were able to significantly differentiate between reservoir and stream samples ($p < 0.05$), explaining 57.3% of the variation. Although RW2 (19.7%, $F_{1,416} = 31.41$, $p < 0.001$) and RW3 (12.0%, $F_{1,416} = 4.76$, $p = 0.030$) described the most variation (Fig. 2.35), RW2 and RW6 (3.6%, $F_{1,416} = 24.95$, $p < 0.001$) were the most significant and thus better able to discriminate between reservoir and stream samples (Fig. 2.36). Positive scores in RW2 were associated with fish that had a deeper body and longer and deeper head, which then caused a more posterior placement of the dorsal

spines. They also had longer dorsal and ventral fins in addition to a shorter and thicker caudal peduncle. Fish with high positive scores in RW3 had a shorter and deeper caudal peduncle and longer dorsal and ventral fins (Fig. 3.34b). Positive scores in RW6 were linked to a longer head and shorter pelvic girdle.

Overall morphological divergence between reservoir and stream fish within systems was not significantly correlated with reservoir age (r_s = -0.571, p = 0.180; Fig. 2.37).

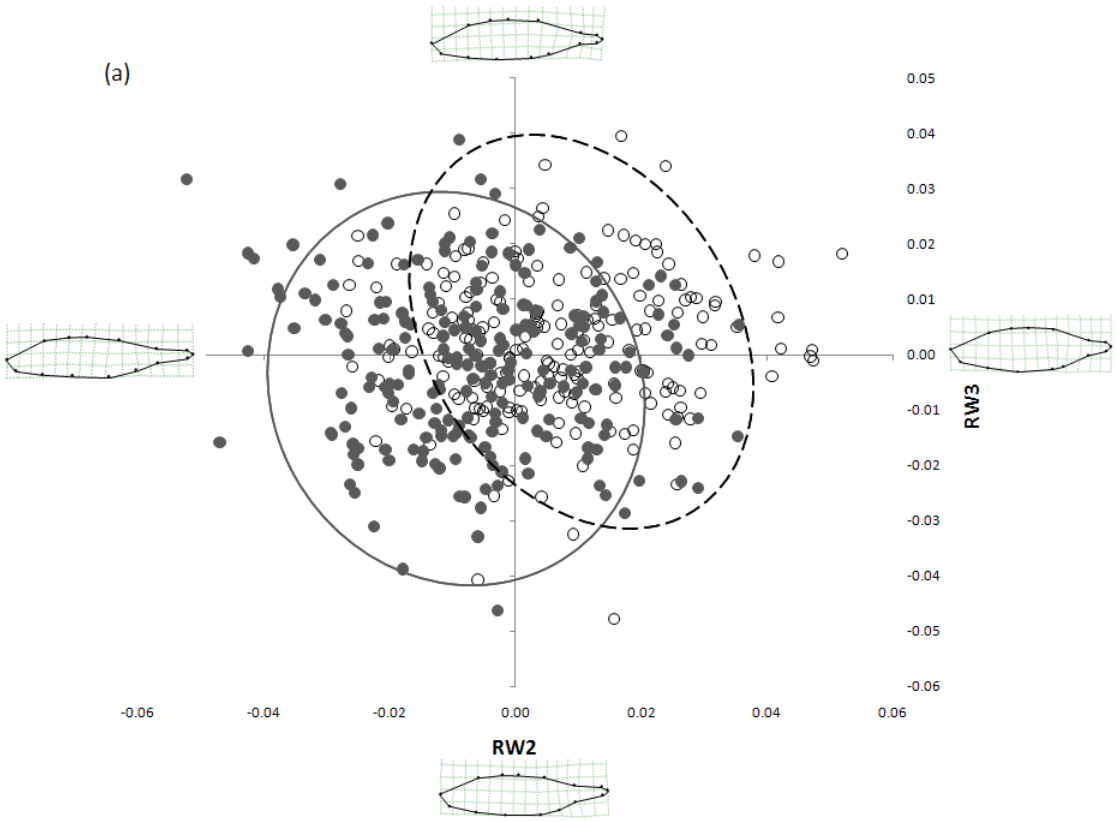


Figure 2.35 Plot of the relative warp scores significantly differentiating sticklebacks from reservoirs (filled circles) and streams (open circles) in the UK. Putative clusters are given (reservoir, unbroken line; stream, dashed line). Deformations associated with the minimum and maximum relative warp scores for each axis are given. The data refer to significant scores that describe the **most variation** (31.64%).

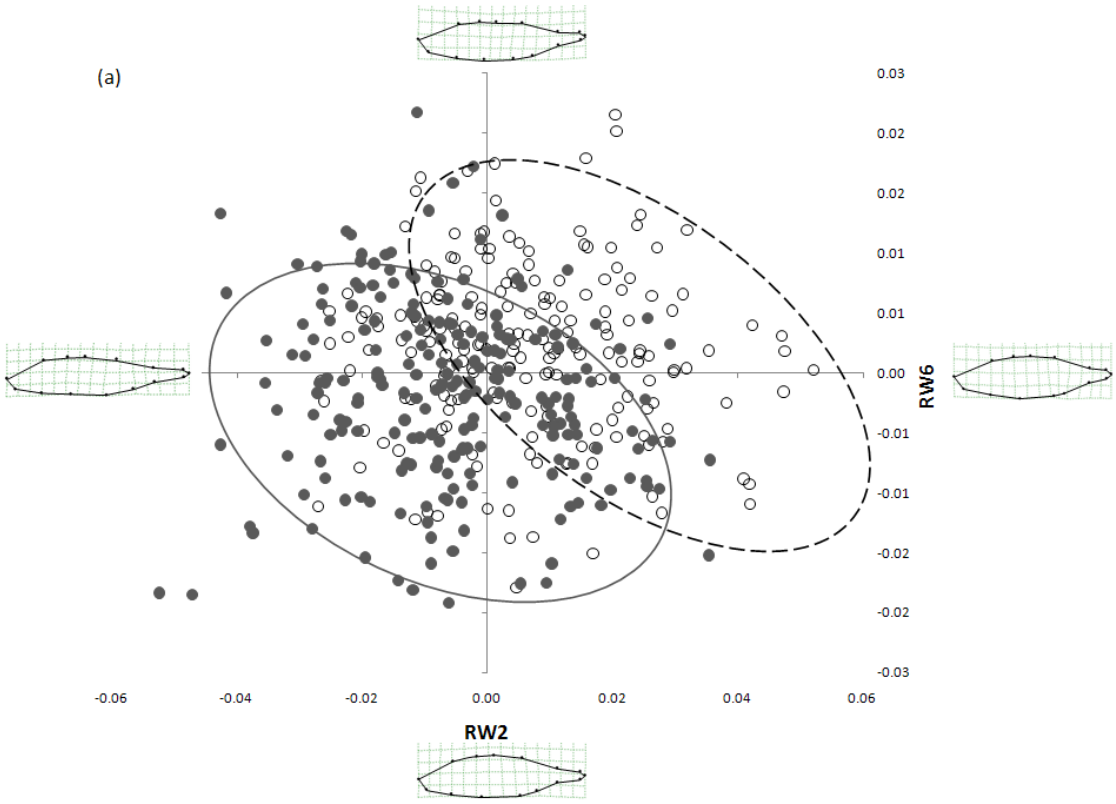


Figure 2.36 Plot of the relative warp scores significantly differentiating sticklebacks from reservoirs (filled circles) and streams (open circles) in the UK. Putative clusters are given (reservoir, unbroken line; stream, dashed line). Deformations associated with the minimum and maximum relative warp scores for each axis are given. The data refer to the **most significant** relative warp scores and describe 23.21% of the variation.

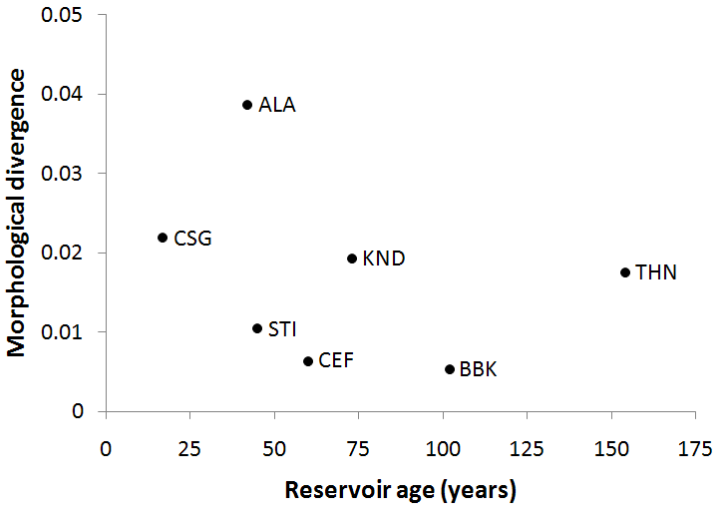


Figure 2.37 Relationship between reservoir age and morphological divergence in stickleback pairs from seven reservoir-stream systems in the UK. Reservoir age is based on the time since impoundment and gives an indication of the maximum time that fish have had to adapt to a lake-type habitat. Morphological divergence is calculated as the mean difference in shape between reservoir and stream fish based on geometric morphometric analyses of shape using RW2 and RW6.

2.4. DISCUSSION

2.4.1. Shape and swimming performance

Among sticklebacks from the reservoir-stream systems under investigation, stream fish were significantly wider and deeper bodied than those from reservoirs. In four of the seven systems, stream fish also displayed a significantly deeper caudal peduncle than fish sampled from reservoirs; and of the remaining three systems, stream fish in two systems showed a (non significant) pattern in the same direction. However, in the Thornton system, stream fish had narrower caudal peduncles than reservoir fish. Shape analyses showed that the greatest differentiation between reservoir and stream fish across sites was achieved by discriminating between individuals based on body depth and caudal peduncle morphology. The results mirror those observed in lake-stream systems, suggesting that these differences are adaptive.

Previous work has shown that sticklebacks in lakes tend to have slender and more streamlined bodies when compared to stream populations (Moodie 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993). Similar patterns of divergence across continents (Europe and North America) further suggest that these traits are adaptive (Berner *et al.* 2010). There is also evidence to suggest that differences in the body depth of sticklebacks from lakes and streams have a genetic component (Hendry *et al.* 2002; Sharpe *et al.* 2008); both common garden and reciprocal transfer experiments demonstrate that habitat of origin impacts morphology. Furthermore, gene flow is negatively associated with morphological divergence and has been shown to have a constraining influence on adaptation (Moore *et al.* 2007).

Body shape differences in fish are correlated with variation in swimming modality (Blake 2004), which is often dependent on foraging methods and predator-prey interactions (Blake 2004). Differences in hydrodynamic performance are

associated with both body form and swimming style (Tytell *et al.* 2010). For example, the shallow and streamlined body of mackerel, *Scombridae* sp. minimises drag and makes it suited to sustained, open water swimming (Tytell *et al.* 2010); whereas increased body depth is associated with muscle mass (Walker 1997) and advantageous to species inhabiting flowing environments, such as some populations of rainbow trout, *Oncorhynchus mykiss* (Keeley *et al.* 2005).

Within species variation has also been related to differences in environmental conditions, and in three-spined sticklebacks, the superior sustained and prolonged swimming performance associated with a more streamlined body has been linked to pelagic habitats and extensive migrations (Taylor & McPhail 1986). The primary role of the caudal peduncle on the other hand, is to provide propulsion and thrust, which are particularly important during fast-start manoeuvres and in flowing water (Bone & Moore 2008). In stream samples from the current study, in addition to a deeper caudal peduncle, the dorsal and/or ventral fins were more caudally placed increasing surface area at the rear, which further increases thrust (Law & Blake 1996).

2.4.2. Trophic morphology and feeding

In all of the reservoir-stream systems analysed, stream fish had a wider mouth than reservoir fish, and in three of the systems, stream sticklebacks also showed shorter gill rakers than reservoir sticklebacks. In general, the number of gill rakers did not differ between sites except in the Carsington system, where reservoir fish had a markedly greater number of gill rakers than those from the stream. The observed morphological patterns are consistent with functional morphological principles of feeding and locally available prey.

Stomach content analyses show that lake stickleback consume a substantial proportion of pelagic prey such as copepods and cladocerans, whereas stream stickleback consume primarily benthic prey, such as dipteran larvae and caddis fly larvae (Berner *et al.* 2009). However stickleback are opportunistic feeders, consuming whatever prey is convenient at the time (Wootton 1976). This means that their intake is strongly affected by seasonal availability (Hynes 1979). For example, lake-dwelling sticklebacks tend to consume more zooplankton during autumn and winter than during spring and summer, whereas stream-dwelling populations show less temporal variation in diet (Gross & Anderson 1984).

A number of studies have looked at gill raker diversity and found that gill rakers are longer and more numerous in sticklebacks that have been feeding primarily on zooplankton in comparison to those feeding on larger, benthic prey (Gross & Anderson 1984; Lavin & McPhail 1986; Schluter 1993; Berner *et al.* 2008). As such, the number of gill rakers also varies according to habitat type, with stream-dwelling populations having fewer than those from parapatric lakes (Moodie 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993).

Mouth gape can be a limiting factor for determining the maximum sized prey an individual can ingest, particularly for species like stickleback, that consume their prey whole. In juvenile red drum *Sciaenops ocellatus*, prey consumption is limited by gape, but only during the earliest stages of development (Krebs & Turingan 2003). However, the degree to which gape size predicts the upper limit of ingestible prey sizes between species varies, suggesting that there are additional factors contributing to prey selection (Schael *et al.* 1991). Often prey of intermediate size are most profitable and so the largest available prey are ignored in favour of slightly smaller prey (Wanzenbock 1995). In stickleback however, a prey width:mouth width ratio of 0.6 has been

associated with the best energy return per unit cost, thus the actual optimum gape width is also dependent on prey type and abundance (Gill & Hart 1994). In most stream habitats, the stickleback diet is dominated by benthic invertebrates (Berner *et al.* 2008) and may explain the observed larger mouth when compared to reservoir fish.

2.4.3. Armour morphology and predator avoidance

Spines differed significantly in length between habitats in four systems; Alaw, Blackbrook, Cefni and Thornton. However, patterns were not consistent, and in some systems, spines were longer in fish from the reservoir whereas in others they were longer in fish from the stream.

Given that the spines of three-spined stickleback are considered an adaptation against gape-limited predators (Hoogland *et al.* 1957), it is perhaps not surprising that differences between reservoir and stream habitats were not consistent across systems. Although spine length has been associated with habitat type in some cases (e.g. Lavin & McPhail 1993), in general, populations subjected to the highest levels of predation have the largest or most robust spines (Hagen & Gilbertson 1972; Moodie 1972a; Moodie & Reimchen 1976; Gross 1978). Although precise information on predation levels in the systems under investigation is not available, it is likely that the systems sampled varied tremendously in levels and type of predation. However, the fact that within individual systems the direction of divergence was the same for all spines indicates that these differences are population-specific. Spine length is considered to be under genetic control and the location of quantitative trait loci for spine length have been mapped to four separate linkage groups making it unlikely that they are inherited together by chance (Peichel *et al.* 2001).

Differences in plate morph (low, intermediate or complete) are most closely associated with divergence between marine and freshwater populations, with marine fish being primarily fully plated (Wootton 1976; Colosimo *et al.* 2004; Olafsdottir *et al.* 2007b). However, within freshwater populations there have also been various studies which have associated fine scale variation in the number of lateral plates with a change in predation level (for a review, see Reimchen 1994). In the current study, only the Cefni system showed a significant difference in the plate phenotype of reservoir and stream fish, with sticklebacks from the reservoir having more lateral plates than those from the stream. Water in the reservoir may have been calcium rich in comparison to the stream, thus increasing the availability to fish for skeletal plate development without incurring costs associated with a low ion environment (Giles 1983). Unfortunately, the calcium ion content of water was not quantified in the present study, so it is not possible to clarify this.

However, it is worth noting that in contrast to all other systems in which fish from the reservoir and stream sites were caught within a few weeks of each other, the sample from Cefni stream was caught two years before that from the reservoir. Previous studies have shown that stickleback populations can show dramatic changes in plate phenotype over a relatively short period of time. For example, an Alaskan lake cleared of fish was re-colonised by marine individuals that were almost monomorphic for the complete plate morph. However, in just 12 generations, three-quarters of the population were of the low plated morph (Bell *et al.* 2004). Further work has shown that experimentally transferred fish from marine tidal pools to isolated freshwater ponds show a reduction in plate number in just one generation (Kristjánsson 2005). Thus it is possible that had the reservoir been sampled two years earlier or the stream two years later, these differences may not have been apparent.

2.4.4. Morphological divergence in relation to reservoir age

It is clear from the results here and in previous studies (e.g. Moodie 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1993) that several aspects of stickleback morphology are modified in reservoir populations in ways that may be expected to provide a selective advantage in still water environments. Specifically, the results indicate that these modifications may be brought about as a result of ecological changes and selection pressures that arise from a change (directly or indirectly) in flow regime.

What is less obvious from my results is whether or not these differences reflect evolutionary adaptations in the populations, or individual plasticity. Under a hypothesis of adaptive evolutionary divergence, greater differences between reservoir and stream caught fish should be apparent in older reservoir than in younger ones because age of the reservoir gives an indication of the maximum time available for adaptations. However, this did not seem to be the case. The rate of adaptation is often related to the strength of divergent selection (Bernatchez *et al.* 1999; Lu & Bernatchez 1999), hence variation between systems may confound the effects of age. Under a theory of adaptive plasticity, differentiation between habitats would be unrelated to reservoir age as individuals modify their morphology as a direct and immediate response to changes in environmental selective pressures.

Most of the studies on lake-stream morphological divergence in stickleback have been conducted in North America. A study comparing lake-stream divergence across continents found that although European systems showed patterns of divergence in the same direction as North American systems, it was much lower in magnitude (Berner *et al.* 2010). The authors have attributed this to records indicating that the sticklebacks in their particular study were not native to European watersheds but were introduced around 150 years ago. Several studies have suggested that there is an

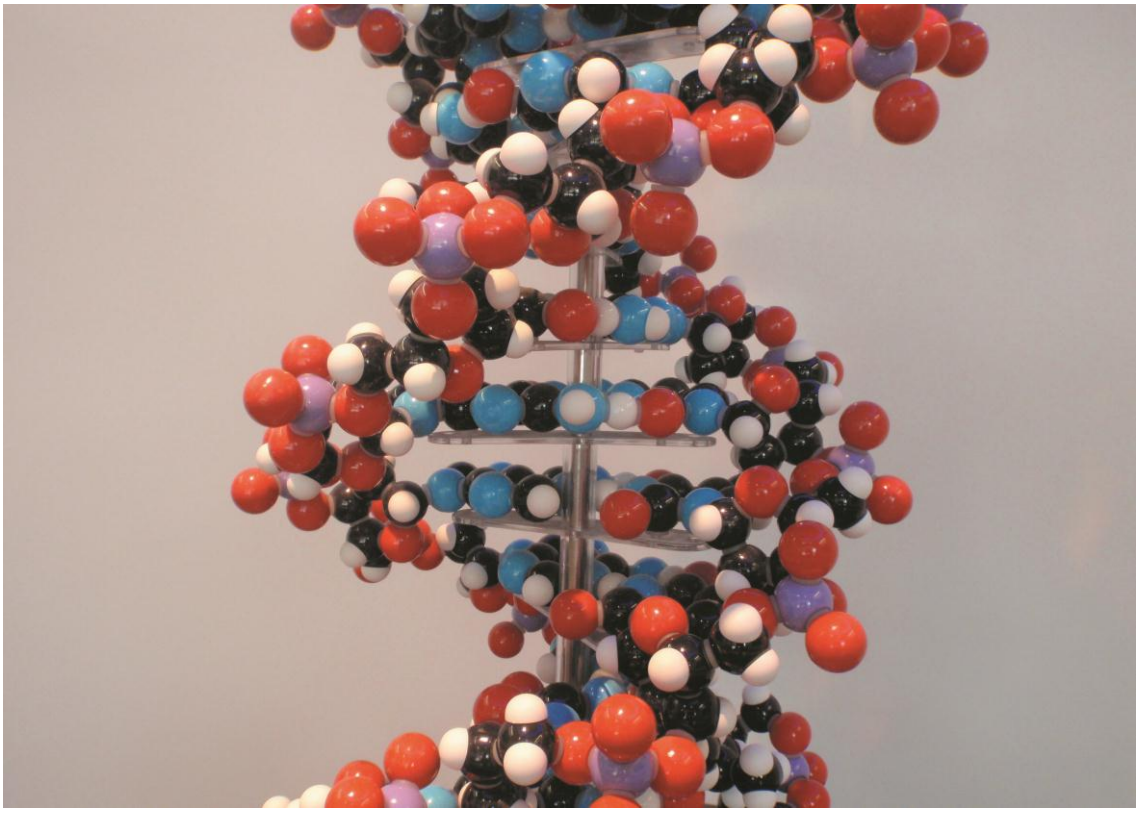
underlying genetic basis to differences in body shape (Lavin & McPhail 1993; Albert *et al.* 2007; Sharpe *et al.* 2008) which is constrained by gene flow (Hendry & Taylor 2004; Moore & Hendry 2005; Moore *et al.* 2007). However, body depth is also subject to strong plastic effects associated with rearing environment (Sharpe *et al.* 2008).

In the current study, body depth and width were actually most divergent between reservoir and stream fish in the two youngest reservoir-stream systems: Alaw, which was 42-years-old at the time of sampling, and Carsington, that had existed for only 17 years. Both of these systems also showed the greatest overall morphological differentiation, based on shape. In fact, fish from Thornton reservoir, which was the oldest reservoir sampled at 154-years-old, displayed only a non-significantly shallower body depth when compared to fish from Thornton stream. Together, these results suggest that the observed differences are either due to trait plasticity or that each system generates varying levels of selective pressures so that the degree of divergence is not related directly to the age of the reservoir. Similarly, habitat-appropriate foraging morphology was once again observed in the youngest systems, but not necessarily in older systems. There is an indication that variation in gill raker number is partly under genetic control (Gross & Anderson 1984; Lavin & McPhail 1986, 1993; Hendry *et al.* 2002) although there is also evidence of plasticity in external trophic morphologies (Day & McPhail 1996). This highlights the importance of the strength of selection in different ecological and environmental habitats.

In general, age of the reservoir seemed to have little association with the degree of differentiation and the greatest divergence was observed in the Alaw system. It is difficult to draw any firm conclusions about whether the results were the product of phenotypic plasticity or adaptive divergence. However, taken together with other studies, it seems most probable that there may be a role for both (Wund *et al.* 2008).

Chapter 3

Genetic population structure of three-spined sticklebacks in UK reservoir-stream systems



3.1. INTRODUCTION

Adaptive radiation "is the differentiation of a single ancestor into an array of species that inhabit a variety of environments and that differ in traits used to exploit those environments." (Schluter 2000). A central component of the process of ecological adaptive radiation is that divergent selection, i.e. selection that acts in contrasting directions in different populations, for utilising alternative resources causes divergence between populations and ultimately species (Benkman 2003). For populations living in sympatry, the ability to exploit different resources (e.g. food), has often been key to their parallel survival and in some cases, the cause of their ultimate divergence into separate species (McPhail 1993; Adams *et al.* 2003; Gow *et al.* 2008).

Although divergent selection acts on phenotypic traits by promoting their divergence, if those traits have a genetic basis, it can also indirectly promote differentiation at the molecular level (Schluter *et al.* 2010). A genetic basis to traits is crucial if species are to evolve and speciate as natural selection acts on traits that are heritable, that is, those that have a genetic component. Ecological speciation occurs when divergence in phenotypic traits leads to pre-mating reproductive isolation causing barriers to gene flow (Rundle & Nosil 2005). It can also facilitate genome-wide neutral divergence by random changes in the gene frequencies of a population (i.e. genetic drift) and is particularly relevant to populations that are geographically separated (Nosil *et al.* 2009). Non-ecological speciation can also occur as a result of drift or founder events, where a population is founded by only a small number of individuals and thus the genetic variation in the newly established population is less than that of the original population. In addition, alternative advantageous alleles may become fixed in separate populations experiencing the same selection pressures (Schluter 1996).

Habitat isolation refers to a situation where two species live in the same general area but occupy different habitats or ecological niches in that same area. It is often a precursor to ecological speciation and local adaptations to different environments can lead to genetic differentiation (reviewed in Rice & Hostert 1993). Reproductive barriers may evolve due to selection against less fit immigrants, termed 'immigrant inviability' and against intermediates formed from matings between populations (Nosil *et al.* 2005). If individuals show a preference for their own habitat, between-population matings and therefore gene flow may further decrease, facilitating neutral drift and reinforcing divergence. However, even in the face of immigrant inviability, reproductive isolation leading to speciation does not always evolve (reviewed in Rice & Hostert 1993) as gene flow between adjacent populations can inhibit genetic evolution (e.g. Hendry *et al.* 2002).

One of the predictions of ecological speciation is that the rate of evolution of reproductive isolation correlates with the strength of divergent selection (Schluter 2001). Based on this supposition, populations subjected to environments that differ in factors such as competition, resource availability, levels of predation and/or structural habitat differences, are predicted to evolve reproductive isolation quicker than those in more similar environments. Differing levels of phenotypic divergence and trophic niche partitioning in sympatric populations of lake whitefish *Coregonus clupeaformis* in two similarly aged lakes in the same river drainage system, suggest different levels of speciation by ecotypes in each lake (Bernatchez *et al.* 1999). Variation in trophic specialisation is congruent with that of prey availability in each lake and a correlation between genetic divergence and trophic specialisation supports a theory that reproductive isolation has evolved (at least in part) as a consequence of resource availability. Comparisons of the same river drainage system across six lakes showed

that gene flow is more restricted in sympatric populations that showed greater phenotypic specialisations for occupying distinct trophic niches (Lu & Bernatchez 1999).

The widespread colonisation of freshwater habitats by three-spined stickleback *Gasterosteus aculeatus*, across the northern hemisphere has made it a remarkable model organism for studying intra-specific diversity (Bell & Foster 1994). Furthermore, the publication of a genome-wide linkage map (Peichel *et al.* 2001) followed by the full genome sequence has made it possible to use population structure at neutral marker loci to infer patterns of genetic divergence (Slatkin 1987). In sticklebacks, research has shown that adaptive phenotypic variation has a genetic basis that is related to habitat type (Colosimo *et al.* 2004; Shapiro *et al.* 2004; Miller *et al.* 2007). Several studies have shown that the genetic divergence and structure of stickleback populations, as surmised from studies employing microsatellite analyses, is associated with habitat types (Reusch *et al.* 2001a; Leinonen *et al.* 2006; Makinen *et al.* 2006).

3.1.1. Stickleback divergence in lake-stream systems

Previous work has shown that stream-resident fish are deeper bodied and have shorter and fewer gill rakers whereas lake-resident fish tend to be more streamlined with long and more numerous gill rakers (reviewed in McPhail 1994). Research has also shown that several of these features also have an additive genetic basis whereby lake-stream hybrids show an intermediate phenotype (Gross & Anderson 1984; Lavin & McPhail 1993; Hendry *et al.* 2002) and that these are likely to have arisen as an adaptive response to divergent selection (Hendry & Taylor 2004).

Population structure analyses using neutral genetic markers (microsatellites) across several distinct lake-stream systems in Canada (Berner *et al.* 2009) and Europe

(Reusch *et al.* 2001a) have been able to differentiate individuals based on system and habitat type. The results are consistent with the hypothesis that divergent lake and stream populations have been partially maintained by reproductive isolation in parapatry. However, analyses on more recently diverged European lake-stream populations, which are thought to have diverged no more than 150 generations ago, did not find the same pattern of genetic differentiation (Berner *et al.* 2010).

In diverse but physically connected environments like some lakes and streams, morphological divergence has been positively correlated with genetic divergence but negatively correlated with gene flow (Hendry & Taylor 2004; Moore *et al.* 2007). Gene flow occurs through population mixing but its effects can be constrained by the physical structure of the environment. For example, physical barriers such as weirs or a steep decline may prevent gene flow from the reservoir into the inflowing stream, but do little to prevent gene flow from the stream into the reservoir. This example highlights why similar patterns of genetic differentiation may not always be found between studies of different populations.

3.1.2. Aims

The results of Chapter 2 clearly demonstrated that, for some morphological traits, patterns of divergence between man-made reservoirs and inflowing streams are consistent with those between streams and natural lakes. This is despite the fact that fish inhabiting reservoirs have had far less time diverge from their hypothesised ancestral stream-dwelling populations than those in lakes. The aim of the current chapter was to test the hypothesis that the morphological divergence observed in reservoir-stream systems is the result of reproductive isolation and paralleled by divergence at the genetic level. The fast mutation rate of DNA microsatellites makes

them particularly suited for inferring population structure in relatively recently diverged systems (e.g. Van Oppen *et al.* 1997). Since morphological divergence was not significantly correlated with reservoir age among the samples under investigation, the level of genetic differentiation was not expected to be correlated with time since impoundment. However, given the potential adaptive significance of the divergence in traits observed, systems showing the greatest morphological divergence were expected to show the greatest genetic divergence.

3.2. METHODS

3.2.1. Samples

DNA samples for genotyping were obtained from the pectoral fin tissue of 422 sticklebacks from seven geographically isolated reservoir-stream systems in the UK (Fig. 3.1): Alaw (ALA), Blackbrook (BBK), Cefni (CEF), Carsington (CSG), Kendoon (KND), Stithians (STI) and Thornton (THN). Reservoir samples are followed by the letter 'R' (i.e. ALA**R**) whereas stream samples are followed by the letter 'U' (i.e. ALA**U**). Sample sizes ranged from 16 (KNDU) to 45 (CEFR). All tissue had previously been stored at room temperature in 100% ethanol since collection.

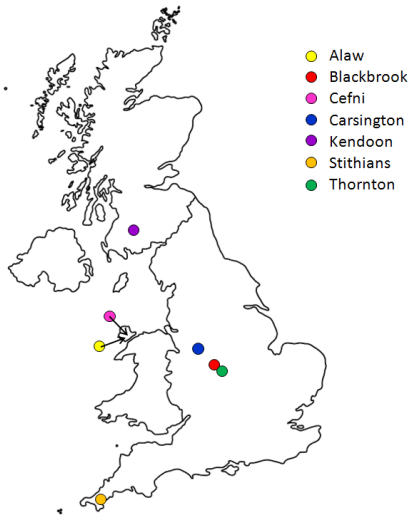


Figure 3.1 Map of the UK showing the location of the seven reservoir-stream systems where sticklebacks were successfully caught

3.2.2. DNA extraction and amplification

Genomic DNA was extracted from pectoral fin clippings using an ammonium acetate precipitation method (Nicholls *et al.* 2000) and visualised on a 0.8% agarose gel stained with SYBRsafe (Fisher Scientific, Leicestershire, UK). DNA quantity was assessed using a BMG LABTECH FLUOstar OPTIMA fluorometer (Imgen Technologies, Virginia, USA) and then diluted to 10ng/µl.

All samples were amplified and genotyped at 18 microsatellite loci, using markers that have previously been used in European sticklebacks (Makinen *et al.* 2006) and one sex-linked marker (*Idh*) (Peichel *et al.* 2004), in seven multiplex reactions that were designed using Multiplex Manager 1.0 (Holleley & Geerts 2009). Markers were from 14 different linkage groups in positions not closer than 0.25cM from any mapped QTL area. The aim was to cover genome-wide variability whilst avoiding the effect of selection via hitchhiking, whereby neutral alleles spread through the gene pool by virtue of being linked to a gene which is positively selected (Barton 2000). The composition of each multiplex and the properties of the loci amplified are given in Table 3.1. Fragments were amplified using Qiagen Multiplex PCR kits (Qiagen Inc., West Sussex, UK) using 2µl reactions (Kenta *et al.* 2008). Each reaction contained 1µl Qiagen Q-mix, 0.2µM of each primer and approximately 10ng of template DNA. Forward primers were labelled with a fluorescent dye (6-FAM or HEX) and the 5'end of the reverse primer was modified with a GTTT tail to enhance the 3' adenylation (Brownstein *et al.* 1996).

An initial touchdown PCR profile was used to assess the suitability of the markers chosen for these samples: 95˚C for 15 minutes, followed by a touchdown cycle of 94˚C for 30 seconds, 65˚C for 90 seconds, then 72˚C for 60 seconds with the annealing temperature being dropped by 1˚C every cycle to 51˚C, followed by a further

25 cycles at 50˚C. The final cycle incorporated an extended period of 30 minutes at 72˚C. Based on the results of the PCR, a profile with an annealing temperature of 56˚C was chosen and run for 36 cycles for all subsequent PCR reactions.

Multiplexed products (1:270 dilutions) were genotyped with GeneScan ROX-500 size standard using automated genotyping on an ABI3730 DNA analyser (Applied Biosystems, California, USA) and alleles were scored manually using GeneMapper v3.7 (Applied Biosystems, California, USA).

Table 3.1 Composition of multiplexes and genetic characteristics of 19 microsatellite loci amplified from 422 samples of Gasterosteus aculeatus DNA from seven reservoir-stream systems in the UK and used to infer population structure.

Locus	*MP*	N_A	*Size range (bp)*	H_O	H_E
Stn163	a	23	134-199	0.649	0.806
Stn34	a	12	167-198	0.392	0.752
Stn46	a	7	235-244	0.438	0.75
Stn57	b	25	101-156	0.52	0.903
Stn79	b	3	113-117	0.164	0.19
Stn19	c	30	162-245	0.666	0.918
Stn3	c	9	144-164	0.519	0.688
Stn110	d	13	164-190	0.684	0.863
Stn21	d	33	142-210	0.796	0.937
Gac1125	e	25	155-211	0.646	0.887
Stn122	e	24	182-245	0.602	0.877
Stn174	e	9	100-116	0.583	0.766
Stn12	f	13	135-161	0.642	0.847
IdhSex	f	2	273-304	0.432	0.339
Stn195	f	11	169-198	0.573	0.81
Stn38	f	3	211-215	0.159	0.233
Gac7033	g	18	193-242	0.556	0.854
Stn132	g	14	111-148	0.508	0.821
Stn135	g	10	104-118	0.261	0.672

Stn loci are described in Peichel *et al*. (2001); Gac loci are described in Largiader *et al*. (1999). Multiplex PCR (MP), number of detected alleles (N_A), range of allele sizes, observed (H_O) and expected (H_E) heterozygosity are given for each locus

3.2.3. Data analysis

Microsatellite genotype frequencies were checked for deviations from expected proportions under Hardy-Weinberg equilibrium and linkage disequilibrium for each locus and population combination using default settings (1000 dememorizations, 100 batches, 1000 iterations per batch) in GENEPOP 4.0 (Raymond & Rousset 1995). Statistical significance was evaluated both before and after correcting for multiple tests using the false discovery rate control (Verhoeven *et al.* 2005). Samples were also checked for the presence of null alleles using Cervus 3.0 (Marshall *et al.* 1998) and 1 bp jumps using the Microsoft Excel Microsatellite Toolkit add-in (Park 2001). Of the 18 markers, three (*Stn34*, *Stn135* and *Stn163*) showed significant deviations from Hardy-Weinberg in over half of the reservoir-stream systems. Two of these (*Stn34* and *Stn163*), together with another marker (*Gac7033*) also showed a high prevalence (over 20%) of null alleles, so these four markers were removed from further analyses (Dakin & Avise 2004). Although tests for linkage disequilibrium showed significant linkage across several markers, this was only seen in one or two populations. Given that loci are located on separate linkage groups (Makinen *et al.* 2006) it is unlikely to be caused by physical linkage and is probably a sampling artefact.

F_{ST} values describe the proportion of total genetic variation contained in a subpopulation, relative to the total genetic variation. Weir and Cockerham (1984) pairwise F_{ST} values were calculated between habitat types in all seven reservoir-stream systems using FSTAT v2.9.3 (Goudet 2001). The correlation between F_{ST} values and reservoir age was calculated and tested for significance using Spearman's rank correlation coefficient. The test was repeated for correlations between F_{ST} and morphological divergence. Morphological divergence between habitat types was calculated separately for each system using the relative warp score, RWS (obtained

from geometric morphometric analyses of shape, Chapter 2) that was best able to discriminate between reservoir and stream fish in that system. The difference in the average RWS between reservoir and stream fish was calculated and used as a proxy for morphological divergence. Global F_{ST} values were also calculated across all putative populations and separately for each individual system. FSTAT was also used to calculate gene diversity and allelic richness (Table 3.2) within each system.

Table 3.2 Genetic diversity estimates for stickleback samples collected and genotyped at 14 microsatellite loci, from seven reservoir-stream system in the UK.

System	N	h	A_R	PA
ALA	70	0.562	5.011	18
BBK	60	0.352	3.553	5
CEF	89	0.453	3.957	11
CSG	40	0.7	6.524	6
KND	51	0.615	5.24	6
STI	68	0.644	4.74	3
THN	44	0.581	4.687	5

Number of individuals (N), gene diversity (h), allelic richness (A_R), and number of private alleles (PA) are given for each reservoir-stream system.

Population structure was examined using two Bayesian clustering methods: STRUCTURE v 2.3.3 (Pritchard *et al.* 2000) and BAPS (Bayesian Analysis of Population Structure) v5 (Corander *et al.* 2003; Corander *et al.* 2004).

The programme STRUCTURE implements a model-based clustering method for inferring population structure using genotype data from unlinked markers by estimating the most likely number of clusters (K). Individuals are probabilistically assigned to a population in such a way as to minimise linkage and Hardy-Weinberg disequilibria within populations. The programme thus assumes that deviations from Hardy-Weinberg are due to population genetic structure. Ten independent simulations of K=1-20 were performed with all 422 samples, followed by a further ten independent simulations of K=1-5 for individuals in each reservoir-stream system. Iterations were

performed with 500,000 Markov Chain Monte Carlo (MCMC) repetitions preceded by 100,000 burnin repetitions using the admixture and correlated allele frequencies models with a separate alpha inferred for each population (Pritchard *et al.* 2000). The most probable number of clusters (*K*) was determined by examining the log likelihood scores and the *ad hoc* statistic ΔK, which is based on the rate of change in the log probability of data between successive *K* values (i.e. successive number of putative populations) (Evanno *et al.* 2005). The programme was run initially without *a priori* assumptions of population structure but later repeated with a model that uses this information (Hubisz *et al.* 2009). This model makes use of location information to assist clustering when the amount of data available is limited. However, running the programme with *a priori* assumptions of population structure has the advantage that it does not tend to find structure when none is present and it is able to ignore sampling information when the ancestry of the individual is uncorrelated with sampling location (Pritchard *et al.* 2000).

BAPS was also used to assess population structure, although the computational approach is somewhat different to STRUCTURE. It is based on identifying populations with different allele frequencies rather than partitioning individuals into clusters in Hardy-Weinberg equilibrium. It is considered better able to identify distinct clusters when F_{ST} estimates between subpopulations are small (Latch *et al.* 2006). Weak stochastic fluctuations in allele frequencies thus are taken as evidence of genetic structure. However, it has the disadvantage that it tends to create more populations when cluster analysis is based on individuals (Frantz *et al.* 2009); hence the greatest confidence in results is attained when results from STRUCTRE and BAPS arrive at the same conclusion (Latch *et al.* 2006).

An individual level clustering mixture analysis was implemented to identify the number of clusters in the population. The number of clusters (*K*) was set to 2, 4, 7, 14

and 20 for the analysis on all 422 samples and K=1-5 for each individual reservoir-stream system, each with ten repetitions. The results from the mixture analyses were then used to conduct admixture analyses using the same parameters, which would allow reliable identification of admixture events in the ancestry of sampled individuals (Corander & Marttinen 2006).

STRUCTURE and BAPS both test for population structure based on genotype alone. In cases where genetic structure is the result of geographical isolation, rather than adaptive divergence, populations that are geographically close to one another are expected to be less differentiated than those which are further apart, a phenomenon known as isolation by distance (IBD) (Wright 1940, 1943). Tests for patterns of IBD were performed using SPAGeDi (Spatial Pattern Analysis of Genetic Diversity) v1.3 (Hardy & Vekemans 2002). SPAGeDi uses geographical coordinate information to compute a linear regression of F_{ST}/1-F_{ST} on the log of geographical distances. The slopes of these regressions can be used as a measure of spatial structuring. A formal assessment of the regression was then assessed using the Mantel Test in GenePop, using 1000 permutations. Initially, analyses of IBD were computed between all 14 putative populations. However, results were skewed as a result of the very close pairs of 'populations' within a system and so the analysis was repeated between just the seven reservoir-stream system by assigning individuals within a system to one geographical coordinate, regardless of the habitat type they were caught from. The significance of the slopes generated with and without amending the geographical information was tested for significance using 10,000 permutations.

3.3. RESULTS

3.3.1. Genetic differentiation

The overall genetic differentiation across all systems across all loci was substantial (F_{ST} = 0.317, 95% CI = 0.217-0.364). Pairwise statistically significant F_{ST} values were also detected between all system pairs (p = 0.00238) after standard Bonferroni correction using a nominal level of 5% (Table 3.3). The greatest level of genetic differentiation was between BBK and CEF (F_{ST} = 0.4282). In fact, BBK showed a high degree of genetic differentiation when compared to all sites ($F_{ST} \geq$ 0.2919). The least differentiation was between CSG and KND (F_{ST} = 0.1613).

Separate analyses investigating the level of genetic differentiation between reservoir and stream samples for each system showed a significant F_{ST} in all systems apart from KND and STI (F_{ST} = -0.0044 and 0.0019, respectively), where negative values indicate large differences between individuals from the same habitat type rather than between individuals from different habitat types (Yang *et al.* 2007). The highest levels of genetic differentiation between reservoir and stream habitats was at CEF (F_{ST} = 0.1055) and the lowest significant genetic differentiation between habitats was at THN (F_{ST} = 0.0272).

Results from SPAGeDi showed no evidence for IBD. Although pairwise F_{ST} was significantly correlated with "as the crow flies" geographic distance when the data was considered to have come from 14 separate populations (r_p = 0.045, p = 0.0077), closer inspection showed that this was caused primarily by samples from within a reservoir-stream system being less genetically differentiated than those from geographically distinct locations (Fig. 3.2). Once removed from the analysis, pairwise F_{ST} was not correlated with distance (r_p = -0.009, p = 0.8316).

Table 3.3 Matrix of pairwise F_{ST} values calculated using FSAT between samples from seven reservoir-stream systems; Alaw (ALA), Blackbrook (BBK), Cefni (CEF), Carsington (CSG), Kendoon (KND), Stithians (STI) and Thornton (THN). The final row gives F_{ST} values for genetic differentiation between reservoir and stream habitats within a system.

	ALA	BBK	CEF	CSG	KND	STI	THN
ALA							
BBK	0.3939*						
CEF	0.2995*	0.4282*					
CSG	0.1852*	0.3131*	0.2848*				
KND	0.2429*	0.3747*	0.3250*	0.1613*			
STI	0.2510*	0.3378*	0.3345*	0.1814*	0.2372*		
THN	0.2529*	0.2919*	0.3236*	0.1783*	0.2713*	0.2606*	
R Vs S	0.0412‡	0.0573‡	0.1055‡	0.0071‡	-0.0044	0.0019	0.0272‡

Significance levels for comparisons between systems were subject to correction using the standard Bonferroni method and *indicates a significant difference at an overall 5% level. ‡ indicates a significant difference at the 5% level for within system comparisons between habitat types.

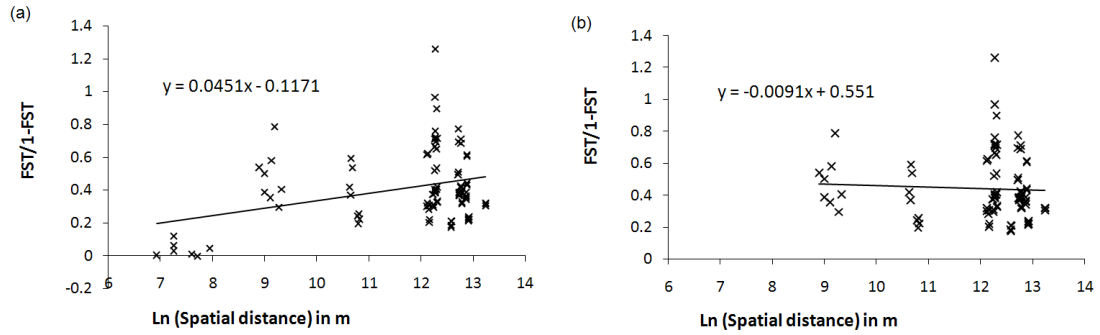


Figure 3.2 Relationship between "as the crow flies" geographic distance and genetic differentiation in sticklebacks sampled from seven distinct reservoir-stream systems. (a) includes relationship data from reservoir-stream pairs within a system (r_p – 0.045, p = 0.0077); (b) within-system relationship data points have been removed (r_p = -0.009, p = 0.8316)

Levels of genetic differentiation, based on F_{ST} values, did not show any correlation with either reservoir age (r_s = 0.143, p = 0.760) or measures of morphological divergence (r_s = -0.429, p = 0.337) (Fig. 3.3).

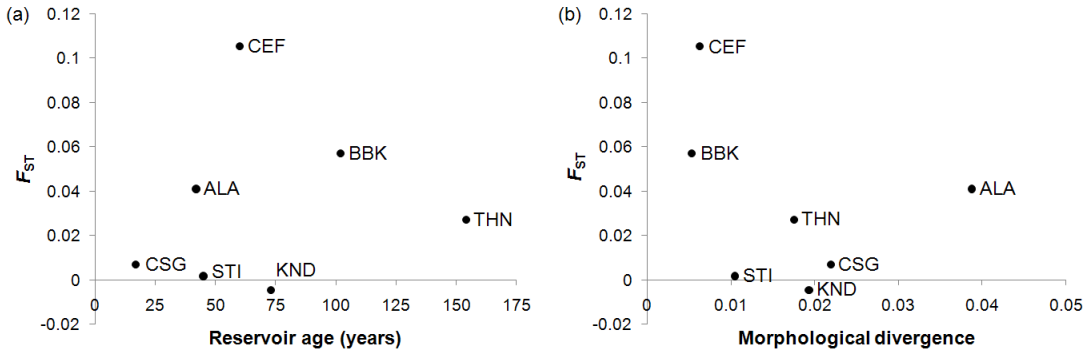


Figure 3.3 Relationship between genetic differentiation and (a) reservoir age and (b) measures of morphological differentiation in stickleback pairs from seven reservoir-stream systems in the UK. Reservoir age is based on the time since impoundment and gives an indication of the maximum time that fish have had to adapt to a lake-type habitat. Morphological differentiation is calculated as the average difference in shape between reservoir and stream fish based on geometric morphometric analyses of shape.

3.3.2. Population structure

Initial analyses in STRUCTURE revealed that the entire stickleback dataset clustered into 8 populations. The highest and most convergent log likelihood values were obtained for *K*=8 (Fig. 3.4a). However, convergent and similarly high log likelihood values were also obtained for *K*=7 and *K*=9 and in addition, values began to plateau for larger values of *K*. The highest value for Δ*K* was achieved *K*=7 with an additional peak at *K*=8, suggesting that the dataset most likely clustered into 7 or 8 populations (Fig. 3.4b). Bar plots of these assignments show a clear pattern between STRUCTURE inferred clusters and geographic location with very little admixture (Fig. 3.5).

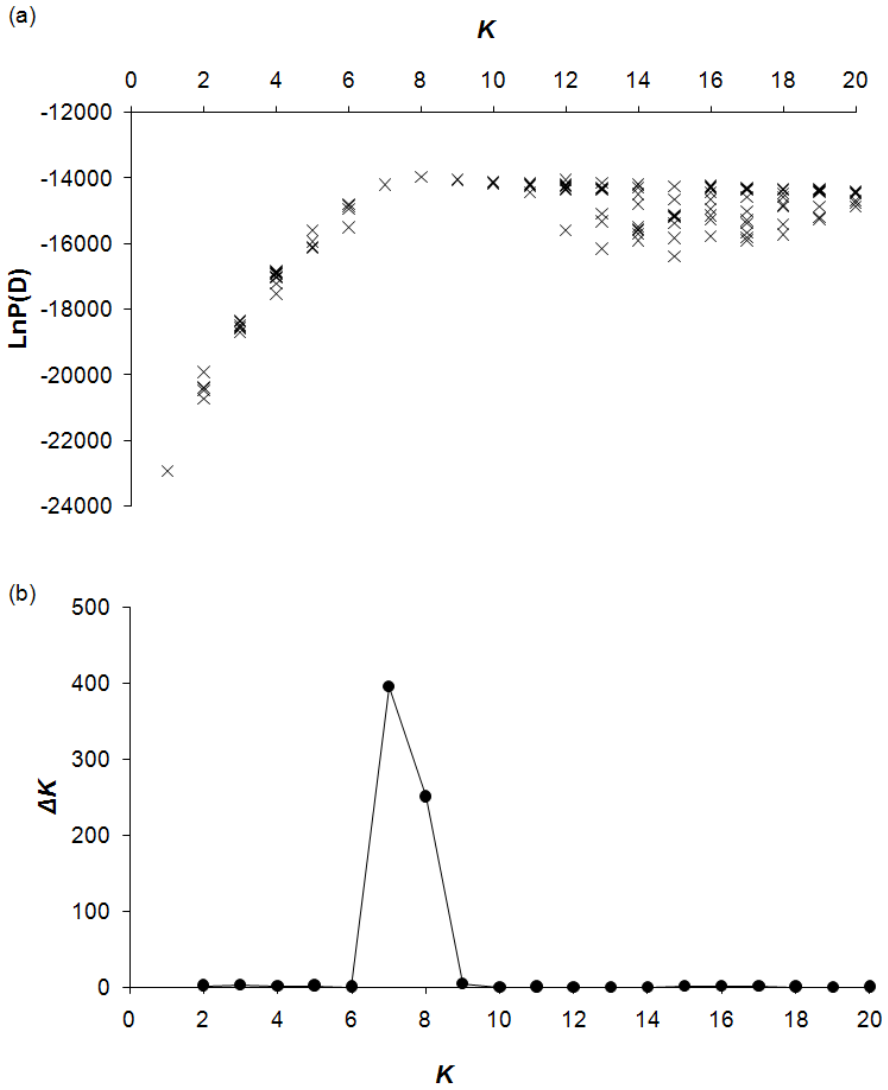


Figure 3.4 Results from analyses using STRUCTURE to infer the number of putative populations (K) in a dataset of 422 stickleback samples genotyped at 14 microsatellite loci. (a) Log likelihood scores for each of the 10 STRUCTURE runs at each value of K. There was no clear peak for any value of K; hence (b) the Evanno *et al.* (2005) calculation of ΔK was used to determine the most likely number of clusters.

For *K*=7, each system is considered as a cluster; for *K*=8, as well as each system being considered a separate population, CEF was additionally differentiated into CEFR and CEFU. Using *a priori* location information did not appear to improve the assignment of individuals to any particular population and thus the results are not presented here.

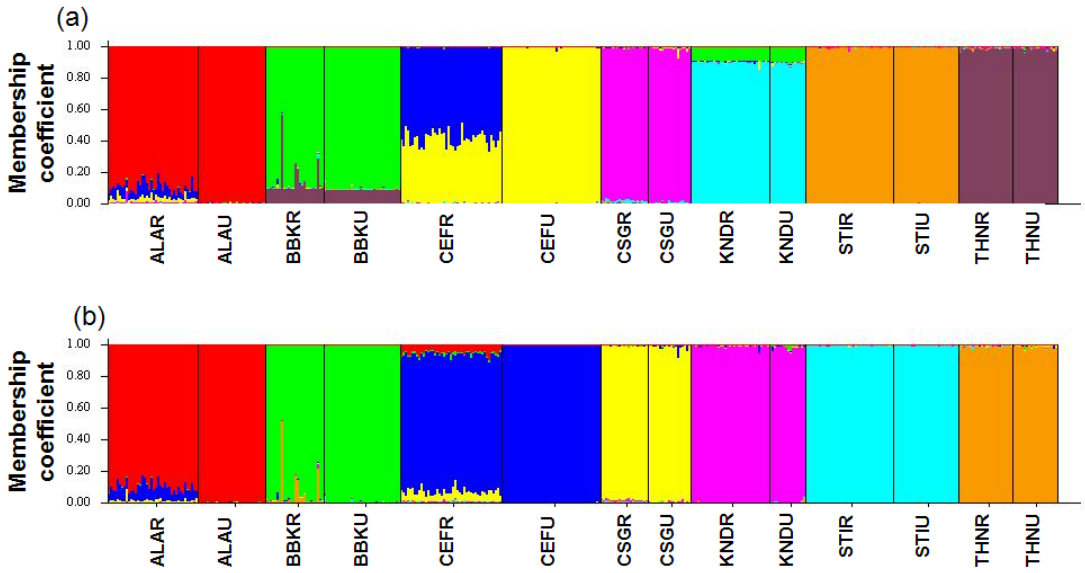


Figure 3.5 STRUCTURE-inferred assignment bar plots for sticklebacks from seven reservoir-stream system when the putative number of populations (*K*) is (a) 8 and (b) 7. Clusters are based on 422 individuals genotyped at 14 microsatellite loci where the width of the bar represents the number of samples from each sampling location.

Analysis of the full dataset in BAPS also suggested the optimal population structure was obtained with *K*=8 (probability = 1). Similar to the STRUCTURE analyses for *K*=8, BAPS partitioned the dataset into clusters which related to the geographic location of capture, additionally separating CEF into CEFR and CEFU (Fig. 3.6).

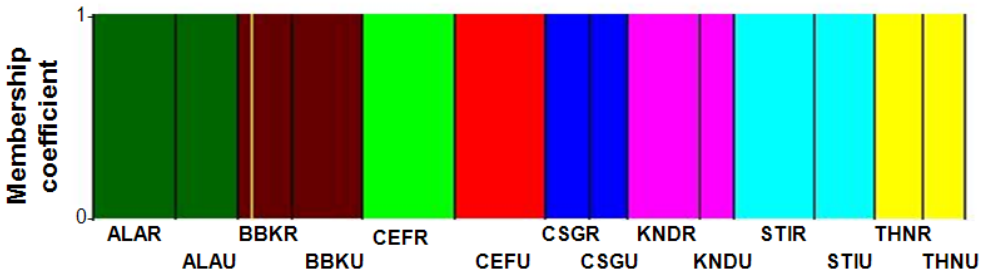


Figure 3.6 BAPS-inferred assignment bar plots for sticklebacks from seven reservoir-stream system when the putative number of populations (*K*) is 8 and populations are allowed to be admixed. Clusters are based on 422 individuals genotyped at 14 microsatellite loci where the width of the bar represents the number of samples from each sampling location.

Based on the data calculated in BAPS, a neighbour-joining phylogenetic tree using Nei's distances (Takezaki & Nei 1996) was computed and clearly showed that the two systems located on the island of Anglesey (ALA and CEF) were separated from the other systems in England and Scotland at an early stage (Fig. 3.7). Furthermore, THN and BBK, which are geographically close and share the same drainage system, were relatively closely related.

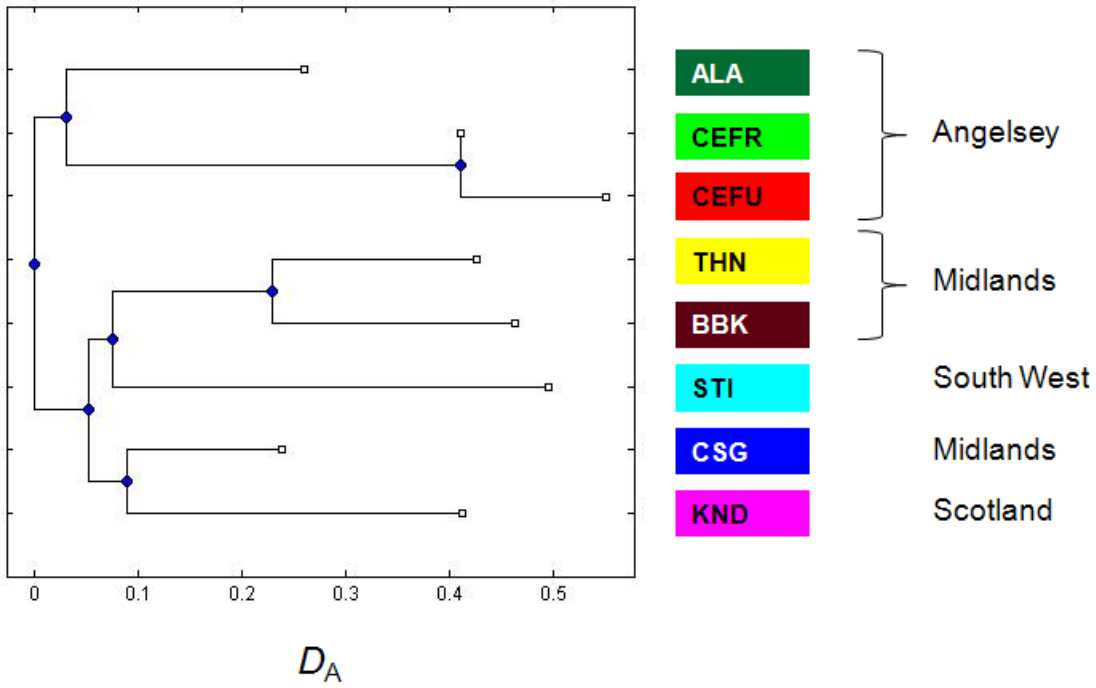


Figure 3.7 Neighbour-joining phylogenetic tree based on 422 stickleback samples from seven reservoir-stream systems. 'Populations' are based on the structure of the dataset inferred by the programme BAPS using samples genotyped at 14 microsatellite loci. Branch length indicates Nei's standard genetic distance.

3.3.3. System-specific population structure analyses

Analyses using STRUCTURE and BAPS were also undertaken for each individual system. The purpose of this was to investigate fine-scale genetic structure, i.e. if samples from within a system could be differentiated into separate reservoir and stream samples.

Using log likelihood values calculated in STRUCTURE, only ALA and CEF could be statistically differentiated into two populations (K=2). For all other systems,

the highest log likelihood values calculated were for K=1. The method employed for calculating ΔK means that it is not possible to determine any more accurately whether K=1 or K=2 (Evanno *et al.* 2005). Even so, bar plots of the assignments were not clear and individuals looked admixed. Thus the analyses were re-run using *a priori* information about location. This noticeably improved the assignment of individuals in ALA and CEF to the 'correct' population. Using location in the model also seemed to suggest that BBK could additional be partitioned into 2 distinct clusters. No obvious changes were noted for any of the other systems, nor did it create structure where there was none initially (Fig. 3.7)

Once again, results from BAPS were in accordance with those obtained using STRUCTURE, partitioning ALA, BBK and CEF into two clusters but leaving CSG, KND, STI and THN defined most likely as one.

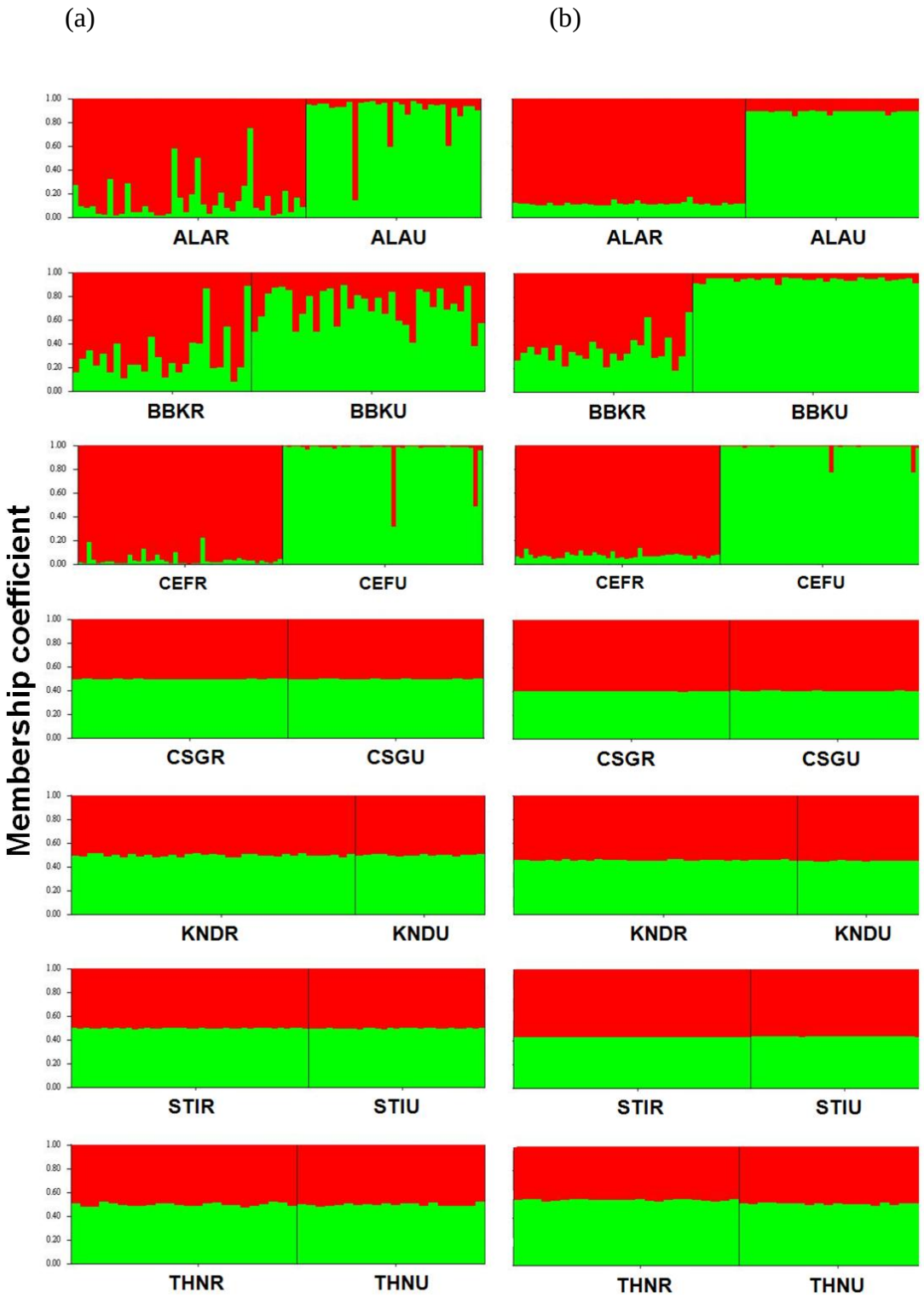


Figure 3.7 STRUCTURE-inferred assignment bar plots for individuals in each reservoir-stream system when the putative number of populations (*K*) for each system is 2. (a) shows the assignment of individuals without information on sampling location whereas (b) gives the assignment of individuals using a model that utilises *a priori* location information in the computation.

3.4. DISCUSSION

3.4.1. Genetic differentiation and population structure across geographically distinct systems

This study clearly shows that neutral genetic variation in stickleback populations across the UK is sufficient to differentiate between geographically distinct reservoir-stream systems but that divergence is not the result of isolation-by-distance. Construction of a phylogenetic tree using Nei's measure of genetic distance indicated that two systems located on the island of Anglesey off the North Wales coast (Alaw and Cefni) diverged earliest from the rest of the UK samples. The tree also suggests that the Blackbrook and Thornton systems, both of which are located in the East Midlands and in the same drainage system (Water Framework Directive, available online at http://www.wfduk.org/implementation/RBDmapfiles/view), diverged from one another most recently. However, it also suggested that they were more closely related to the Stithians system in Cornwall than they were to the Carsington system, despite being part of the same basin. In fact, it appears that fish from Carsington had more similarities with fish from the Scottish system, Kendoon, than they did with neighbouring systems.

The Alaw and Cefni systems, based at the northwest tip on the island of Anglesey are both close to the coast and at altitudes of less than 50m above sea level. Marine sticklebacks are considered to be the living ancestors of freshwater populations (Walker & Bell 2000) and studies have shown that divergence from a marine ancestor into a freshwater morph can occur in a little as 40 years (Klepaker 1993; Gelmond *et al.* 2009) and that specific morphological adaptations with a genetic basis can occur in just one generation (Kristjánsson 2005). Taken together these studies suggest that a possible reason for the Anglesey samples to appear as though they diverged early from

the other samples may be due to the migration of marine and anadromous forms into the system. The Stithians system, located in the southwest corner of England, is also physically close to the sea but it located approximately 160m above sea level, potentially making it more difficult for marine stickleback to colonise the system. Similarly, all of the other reservoirs are also more upland: Blackbrook, 120m; Carsington, 230m; Kendoon, 160m and Thornton, 130m. It is also possible that these patterns are the result of separate colonisation events with the Anglesey populations colonising inland freshwaters separately to the rest of the UK populations.

Whereas some earlier work has suggested that population clusters occur based on geographical or water system origins (Makinen *et al.* 2006), the results here found that population differences were not the result of isolation and distance and that stickleback in separate river basin districts were often more closely related than those in the same river basin district. This suggests that there may be ecological similarities between genetically related systems and that differentiation represents adaptive divergence rather than drift, or that the pattern of genetic diversity amongst UK sticklebacks is the result of multiple colonisation events. Parallel changes at the molecular level result in genetic similarities and suggests adaptive evolution (Zhang & Kumar 1997). Previous work has shown that geographically isolated stickleback populations in distinct habitats evolve similar traits independently and that this reflects parallel evolution (Thompson *et al.* 1997). Independent parallel evolution has also been suggested as the origin of normal and dwarf 'morphotypes' in rainbow smelt, *Osmerus mordax* (Taylor & Bentzen 1993).

3.4.2. Genetic differentiation and population structure within systems

Overall, fish within a reservoir-stream system were genetically more similar than fish that were geographically separated but living in similar habitat types. At a macro-geographical scale this suggests that habitat has little or no effect on genetic differentiation. However, system-specific individual analyses indicated that the Alaw, Blackbrook and Cefni systems showed greater genetic diversity between reservoir and stream habitats, than they did within habitats, and so fish inhabiting the different habitats within each system might therefore be considered as separate populations. Furthermore, genetic differentiation in the Cefni system was such that it separated into two populations even when included in the UK-wide analyses.

The levels of genetic differentiation detected here are particularly interesting because they do not correlate with either the observed levels of morphological differentiation in these systems or reservoir age, which gives the maximum time available for adaptation. Fish from the reservoir and stream habitats of the Thornton system have had up to 154 years to diverge but genetic differentiation, although statistically significant, was around one-quarter of what it was in the Cefni system, where fish have had just 60 years to diverge. Additionally, Alaw, one of the more recently created reservoir-stream systems (42 years), showed the greatest degree of morphological differentiation when compared to other systems, but only an intermediate level of neutral genetic differentiation.

Principally, genetic diversity at functionally important loci is the primary driver of a population's ability to respond to selection. Microsatellite markers are used to infer genome-wide genetic diversity because they are considered to be unaffected by selection (Slatkin 1987) and correlate with genomic variability (Hansson & Westerberg

2002; Thibert-Plante & Hendry 2010). However, microsatellite marker heterozygosity does not always correlate with nucleotide diversity (Vali *et al.* 2011). Whereas divergent selection may keep allele frequencies distinct for ecologically relevant loci, the same may not be the case for neutral loci (Berner *et al.* 2010). Here, variation in the magnitude of genetic and morphological divergence between reservoir and stream sticklebacks across systems strongly suggests that phenotypic differences are the result of strong divergent selection, but that microsatellite variability is the result of drift (Wu 2001).

Throughout this study, habitats type has been classified as 'stream' or 'reservoir' with flow regime assumed as the main surrogate for divergent selection. However, there are likely to be several other factors that are likely to impede or encourage divergence that vary between systems. Site descriptions given in section 2.2.1 highlight differences in substratum, flow, depth, size, predation levels and reservoir use, which may also affect the strength of divergent selection. Barriers to fish movement, such as weirs for example, may constrain gene flow from the reservoir into the stream whilst permitting gene flow from the stream into the reservoir (e.g. Wofford *et al.* 2005). Gene flow is a particularly important factor for heritable adaptations, as previous research has shown that it can limit adaptive divergence (Hendry & Taylor 2004; Moore *et al.* 2007). However, under conditions of high gene flow, selection may favour the evolution of plasticity, thus levels of genetic differentiation may also differ from levels of morphological differentiation (Lind *et al.* 2011).

Differing patterns of neutral and morphological differentiation may also be attributable to variation in effective population sizes in the different systems (Leinonen *et al.* 2006). Smaller populations are more sensitive to the effects of drift; hence under these circumstances, the effect of selection may be counteracted by drift (Wright 1931).

Similarly, knowing some historical facts about the populations, such as details of any rare events can also be valuable when drawing conclusions regarding population structure. For example, an extreme pollution event causing a population bottleneck may result in a reduction in genetic variation. In the current study, sampling the stream habitat in the Cefni system took place 2 years after sampling at the reservoir whereas in all other systems, sampling at both habitats was completed within a few weeks of one another. The stronger differentiation seen in the Cefni system could therefore be the result of different sampling years. Ecological changes between sampling dates may have caused high fish mortality causing founder effects in subsequent generations.

3.4.3. Conclusions

This study, which addressed the neutral genetic divergence of stickleback populations from across the UK, has provided evidence to support the notion that geographically separated populations are genetically distinct. These genetically distinct populations most likely represent the adaptive divergence of the populations to their system-specific environmental conditions. However, together with results from earlier research (Chapter 2), the results of this chapter also suggests that genetic variation at neutral loci is not always a reliable measure for differentiating relatively recently separated parapatric populations. It has highlighted the importance of considering genetic divergence in combination with divergence in functionally important traits, particularly for younger systems, in addition to the life-history and specific environmental conditions encountered by the populations.

Chapter 4

Morphological differences between three-spined sticklebacks from lakes, reservoirs and streams

4.1. INTRODUCTION

Ecological speciation occurs when divergent selection acting on populations in contrasting environments leads to reproductive isolation (Schluter 2001). One of the ways in which divergent selection can arise is from the exploitation of alternative resource environments and resource competition, which leads to niche-specific adaptations by the population (Schluter 1996). Therefore, reproductive isolation is not always a direct consequence of different environments but can sometimes occur as a result of natural selection in other traits of morphology, physiology and behaviour (reviewed in Rice & Hostert 1993). Phenotypic differentiation in adaptive radiations is a likely outcome from natural selection (e.g. Schluter 1988, 1994) where the rate of adaptation and diversification is often related to the strength of divergent selection (Bernatchez *et al.* 1999; Lu & Bernatchez 1999). Studying phenotypic differences within species where populations inhabit contrasting environments is the first step in identifying ecological adaptations that may lead to reproductive isolation and thus ecological speciation.

Fish in recently glaciated rivers and lakes are ideal for undertaking such a study because there are a number of species that show high levels of morphological differentiation but low levels of genetic divergence (for a summary, see Schluter 1996). Lakes across the temperate northern hemisphere were generally formed as the ice sheets of the last glacial period retreated, some 12-15,000 years ago, leaving behind 'islands' of water bodies. Initial colonisations were mostly from glacial refugia; but with only a few passage routes for dispersion, competition for resources may have been a causal factor in the divergence seen between closely related species pairs. For example, in the North Pacific, genetically distinct freshwater kokanee salmon *Oncorhynchus nerk*, are thought to have arisen from anadromous sockeye salmon independently in several

unconnected ‘island’ river systems (Taylor *et al.* 1996). Similarly, freshwater populations of the three-spined stickleback *Gasterosteus aculeatus* are thought to have been derived independently several times from the anadromous form (Hagen 1967; Reusch *et al.* 2001a).

The small size, ubiquitous nature and variation in form of the three-spined stickleback across the northern hemisphere has made it an ideal species for studying the effects of local selective regimes (Bell & Foster 1994; McKinnon & Rundle 2002). Within freshwater environments, sticklebacks, in addition to several other species (e.g. see Swain & Holtby 1989; Brinsmead & Fox 2002; Keeley *et al.* 2005) show habitat-specific adaptations to lake and stream environments (Moodie 1972a, 1972b; Gross & Anderson 1984; Reimchen *et al.* 1985; Lavin & McPhail 1993; Thompson *et al.* 1997). In particular, lake-dwelling sticklebacks are usually more streamlined and have more gill rakers than those from streams; traits considered adaptive to the foraging environment (McPhail 1994). Slimmer bodies are better suited for sustained swimming in open water whereas more robust bodies are better suited to burst swimming, presumed to be more typical in flowing water (Webb 1984; Taylor & McPhail 1986). Gill rakers act as a sieve preventing the loss of food from the buccal-pharyngeal cavity; more gill rakers are advantageous when foraging on small zooplankton prey typically found in lakes whereas fewer gill rakers are better suited for feeding on larger, benthic macro-invertebrates more typically included in the diet of stream and river dwelling sticklebacks (Gross & Anderson 1984; Berner *et al.* 2010). Research has shown that stickleback populations residing in the same habitat are more similar than those which are in close geographical proximity or that share the same watershed (Reusch *et al.* 2001a), further suggesting that the traits are adaptive.

Consistent differences between lake-dwelling and stream-dwelling sticklebacks in replicate systems across the northern hemisphere are compelling evidence for suggesting that differentiation is the result of divergent selection. Genetic studies across Europe suggest that allopatric river and lake populations resulted from multiple colonisations rather than sharing a common ancestry (Makinen *et al.* 2006). Based on this supposition, parapatric lake-stream populations may have evolved in allopatry with secondary contact creating a parapatric system (Lavin & McPhail 1993). However, the existence of geographically distinct parapatric lake-stream systems with strikingly similar traits across habitats also highlights the possibility of independent and habitat-specific parallel evolution (Lavin & McPhail 1993).

Regardless of whether differentiation has been the result of divergence in allopatry or parapatry, the level of gene flow (Hendry *et al.* 2002; Moore *et al.* 2007) and strength of divergent selection (Hendry & Taylor 2004) are important factors for maintaining differentiation in currently parapatric systems. I have already shown that sticklebacks in newly created (<200 year old) man-made lakes, which are presumed to have been colonised by sticklebacks from the inflowing stream, show morphological features consistent with sticklebacks from natural lakes (Chapter 2). I have also shown that in some cases, reservoir and stream populations are genetically distinct and suggested that different levels of differentiation could be related to the strength of divergent selection acting in each system (Chapter 3). However, it is unclear if sticklebacks occupying reservoirs are on a continuum towards becoming more lake-like in their morphology, and if so, exactly how far along that continuum they have moved. Information of this kind would be valuable for making estimates of the rate of adaptation in parapatric populations which experience gene flow.

4.1.1 Aims

The aim of the current study was to compare the morphology of three-spined stickleback from a range of stream-, reservoir- and natural lake-resident populations. The hypothesis under investigation was that the morphological divergence between reservoir-stream pairs of sticklebacks is adaptive. Under this hypothesis, stream samples were expected to show stream-typical morphological adaptations (e.g. a wide and deep body with a wide mouth and short, thick caudal peduncle) whereas lake samples were expected to show patterns of divergence in the opposite direction from streams (e.g. a slender and narrow body with a more narrow mouth and longer, narrower caudal peduncle). Given that natural lakes in the UK were formed at the end of the last ice age, sticklebacks inhabiting those lakes could potentially have had thousands of years to adapt to a freshwater lake habitat. Reservoirs, on the other hand, are much more recent, so fish colonising them from inflowing streams and rivers have had considerably less time to adapt to their environment. Under a hypothesis of adaptive divergence, with strong divergent selection acting on fish in reservoirs, sticklebacks may look completely lake-like in their morphology and would show significant morphological differences when compared to stream fish but be indistinguishable from sticklebacks caught in natural lakes. Intermediate phenotypes in traits would suggest lower selection pressures and reservoir fish would represent an interim stage in the divergence between lake- and stream-adapted fish.

4.2. METHODS

4.2.1. Fish supply

All fish were caught using the standard sampling methods described in section 2.2.2. The stream and reservoir samples were the same seven systems as those used in

earlier work (Chapter 2); however reservoir samples were supplemented by sampling at six additional reservoirs that were not paired with inflowing stream samples. Lake samples were obtained by sampling at new sites. Site details are given in Fig. 4.1 and Table 4.1.

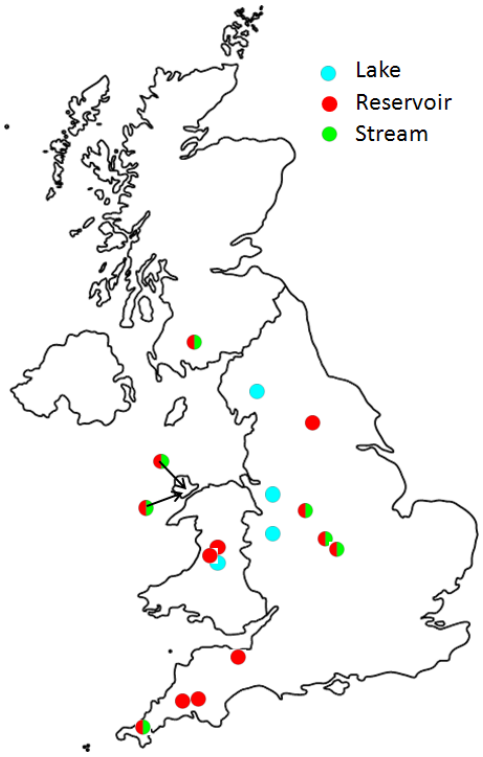


Figure 4.1 Map of the UK showing the locations where sticklebacks from lakes (blue), reservoirs (red) and streams (green) were successfully caught

4.2.2. Statistical analyses

A Principal Components Analysis was undertaken to detect which traits best differentiated reservoir and stream fish. Several linear measures correlated with standard length (SL) and so were regressed onto SL and the residuals compared between habitat types using a nested ANOVA, with habitat type (lake, reservoir, stream) as a fixed factor and population as a random factor. Trait that did not correlate with SL were compared using a nested ANOVA on square-root transformed data. Post-hoc testing was conducted using the Tukey method to identify differences between habitat types. Landmark data were analysed as previously described in section 2.2.4.2.

Table 4.1 Description of sites sampled for the analysis of stickleback morphology

Site name	Habitat type	Code	Latitude (N)	Longitude (W)	Sample size
Alaw	Reservoir	ALAR	53°20’25”	4°26’20”	40
Alaw	Stream	ALAU	53°22’25”	4°22’33”	30
Aqualate	Lake	AQUL	52°46’45”	2°20’26”	11
Beaverdyke	Reservoir	BVRR	53°59’14”	1°39:56”	34
Blackbrook	Reservoir	BBKR	52°44’56”	1°19’05”	26
Blackbrook	Stream	BBKU	52°44’49”	1°18’06”	40
Burrator	Reservoir	BURR	50°30’09”	4°01’38”	43
Carsington	Reservoir	CSGR	53°03’40”	1°38’00”	21
Carsington	Stream	CSGU	53°04’31”	1°36’06”	19
Cefni	Reservoir	CEFR	53°16’32”	4°19’43”	45
Cefni	Stream	CEFU	53°17’13”	4°20’05”	44
Clatworthy	Reservoir	CLTR	51°03’57”	3°22:42”	26
Eiddwen	Lake	EIDL	52°16’53”	4°02:40”	41
Frongoch	Reservoir	FROR	52°21’45”	3°52:43”	47
Kendoon	Reservoir	KNDR	55°11’54”	4°12’02”	36
Kendoon	Stream	KNDU	55°12’15”	4°13’01”	16
Oerfa	Reservoir	OERR	52°24’08”	3°52’17”	31
Siblyback	Reservoir	SIBR	50°30’27”	4°29’23”	47
Stithians	Reservoir	STIR	50°11’22”	5°12’34”	39
Stithians	Stream	STIU	50°11’33”	5°13’12”	30
Tatton mere	Lake	TATL	53°18’49”	2°22’03”	39
Thornton	Reservoir	THNR	52°40’01”	1°18’34”	24
Thornton	Stream	THNU	52°40’17”	1°19’01”	21
Ullswater	Lake	ULSL	54°34’24”	2°54’43”	29

4.3. RESULTS

4.3.1. Differences in the linear measurements of morphology between lake-, reservoir-, and stream-caught fish

Principal components analysis revealed the presence of three components with eigenvalues exceeding 1, explaining 80% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 70% of the variance. Component 1 was

primarily related to overall size and shape of specimens, whereas component 2 was related to spine lengths (Table 4.2).

Table 4.2 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each trait based on sticklebacks caught from the lakes, reservoirs and streams across the UK.

Trait	Component 1	Component 2
Depth	0.851	0.465
Standard length	0.846	0.458
Mouth width	0.829	0.353
Width	0.826	0.340
Caudal peduncle depth	0.762	0.493
Pelvic girdle length	0.722	0.551
Caudal peduncle length	0.719	0.353
Jaw angle	0.336	0.045
Average number of lateral plates	0.220	-0.044
Second dorsal spine length	0.195	0.925
First dorsal spine length	0.150	0.925
Pelvic spine length	0.851	0.465

A significant effect of habitat type was apparent in the body width ($p = 0.004$), depth ($p = 0.003$), and mouth width ($p < 0.001$) of sticklebacks (Table 4.3).

Table 4.3 Results from a two-way nested ANOVA to determine whether habitat type and population had a significant effect on the morphology of sticklebacks from lakes, reservoirs and streams.

	Habitat type			Population		
	F ratio	df	p	*F* ratio	df	p
Width	**7.23**	**2 , 20**	**0.004**	18.1	20 , 734	<0.001
Depth	**7.70**	**2 , 21**	**0.003**	21.4	21 , 747	<0.001
Caudal peduncle depth	3.24	2 , 21	0.058	13.7	21 , 747	< 0.001
Caudal peduncle length	1.02	2 , 21	0.376	12.5	21 , 747	< 0.001
First dorsal spine length	0.334	2 , 21	0.720	18.7	21 , 737	< 0.001
Second dorsal spine length	0.529	2 , 21	0.597	22.1	21 , 736	< 0.001
Pelvic spine length	0.147	2 , 21	0.864	18.7	21 , 746	< 0.001
Pelvic girdle length	1.69	2 , 21	0.209	17.6	21 , 746	< 0.001
Mouth width	**17.6**	**2 , 21**	**<0.001**	5.43	21 , 747	< 0.001
Jaw angle	0.09	2 , 21	0.913	11.9	21 , 747	< 0.001
Number of plates	0.28	2 , 21	0.758	24.2	21 , 715	< 0.001

Traits which show a significant effect of habitat are given in **bold**

Post-hoc testing using the Tukey method showed that there were significant differences in width and depth between all habitat types ($p < 0.001$ for all); stream fish had the deepest and widest bodies, followed by reservoir fish whereas lake fish had the least deep and narrowest bodies (Fig. 4.2a,b). In terms of gape, stream fish had a significantly wider mouth width than lake and reservoir fish ($p < 0.001$) but no significant differences were apparent between reservoir and lake fish ($p > 0.05$; Fig. 4.2c).

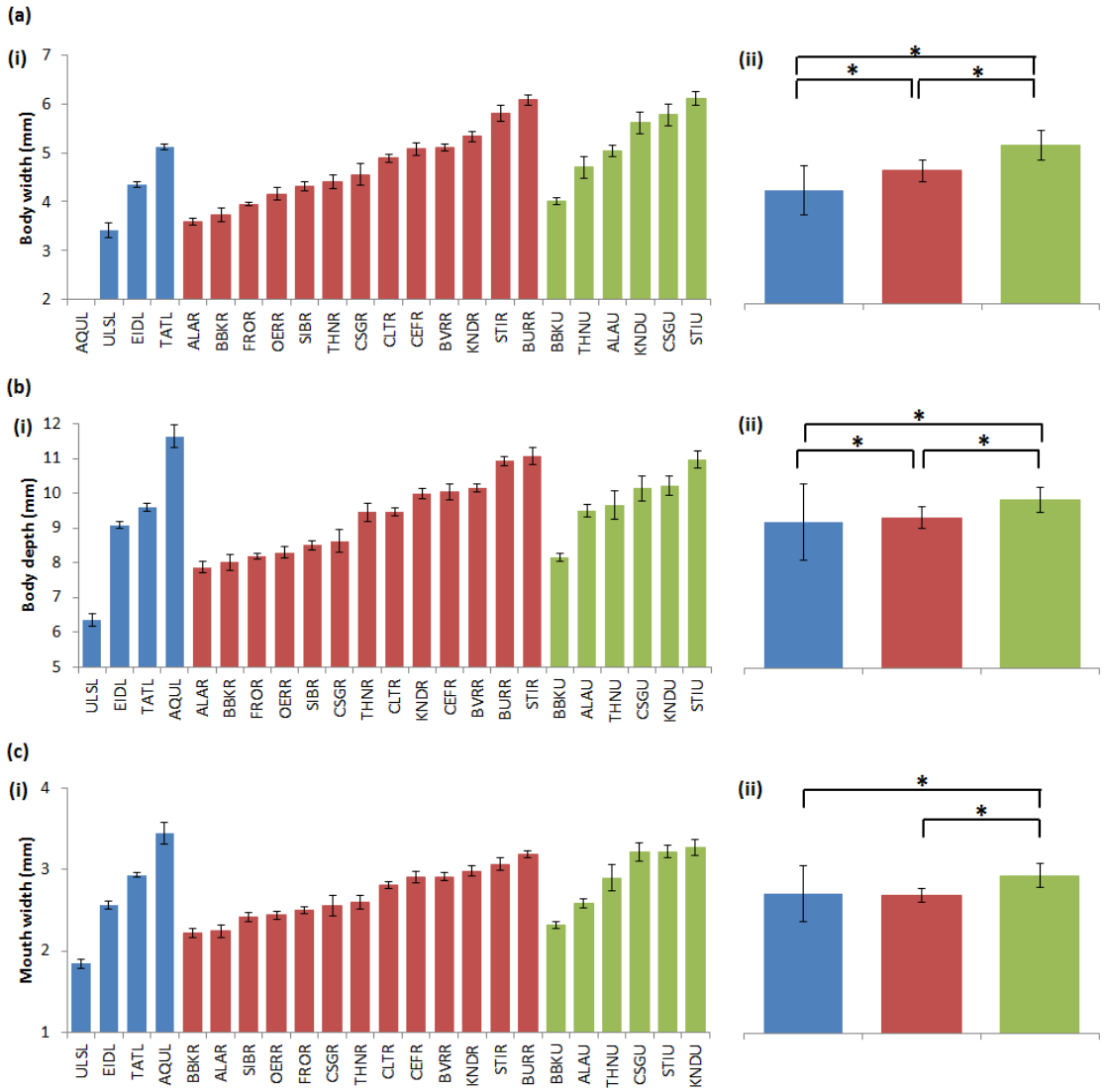


Figure 4.2 Differences in mean ±SE for (a) **body width**, (b) **body depth** and (c) **mouth width** of sticklebacks collected from lakes (blue fill), reservoirs (red fill) and streams (green fill). (i) represents data for each population sampled. (ii) shows the mean ±SE for all of the populations in that habitat. Significant differences based on post-hoc testing after a nested ANOVA are indicated by *($p < 0.001$).

4.3.2. Differentiation between stickleback caught from lakes, reservoirs and streams using geometric morphometric analyses of shape variation

Of the 26 relative warps (RW) calculated to describe variation in the overall shape of fish from lakes, reservoir and streams, the first 5 described 76.6% of the variation, with subsequent RW describing less than 5% each (Fig. 4.3). RW2 (17.36%; $F_{2, 21} = 5.45$, $p = 0.012$) and RW5 (7.58%; $F_{2,21} = 6.43$, $p = 0.006$) were able to significantly differentiate between samples (Fig. 4.4). Post-hoc testing using the Tukey method confirmed that scores in RW2 and RW5 were significantly different between all samples ($p < 0.01$). However, neither of these was significant at the 5% level after stringent multiple comparison corrections using the false discovery rate control.

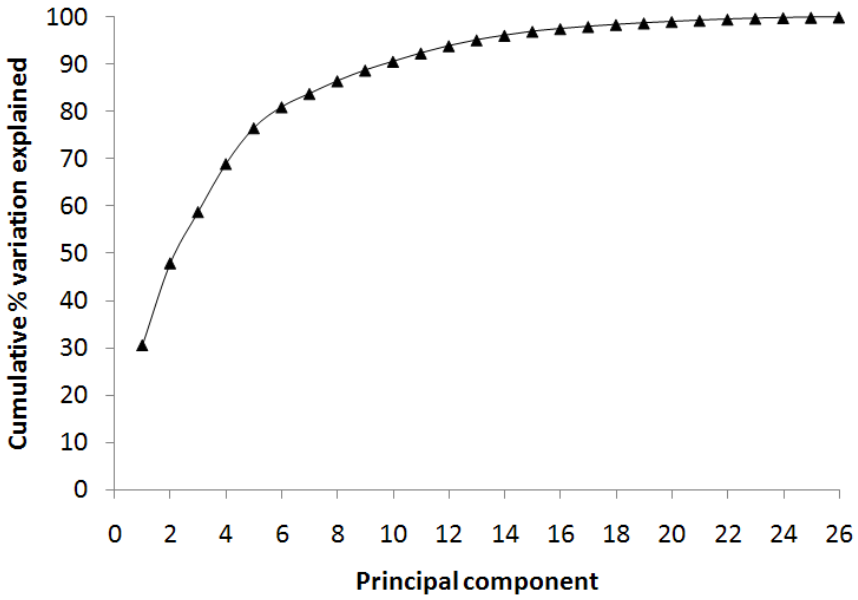


Figure 4.3 Cumulative percentage of variation in shape across all samples described by the 26 principal components of shape (relative warps)

Positive values in RW2 were associated with a much more narrow body, shorter ectocoracoid and a longer and narrower caudal peduncle. Positive scores in RW5 were associated with a narrower body depth, particularly in the middle and caudal region of the fish as well as a longer dorsal and ventral fin and a narrower caudal peduncle.

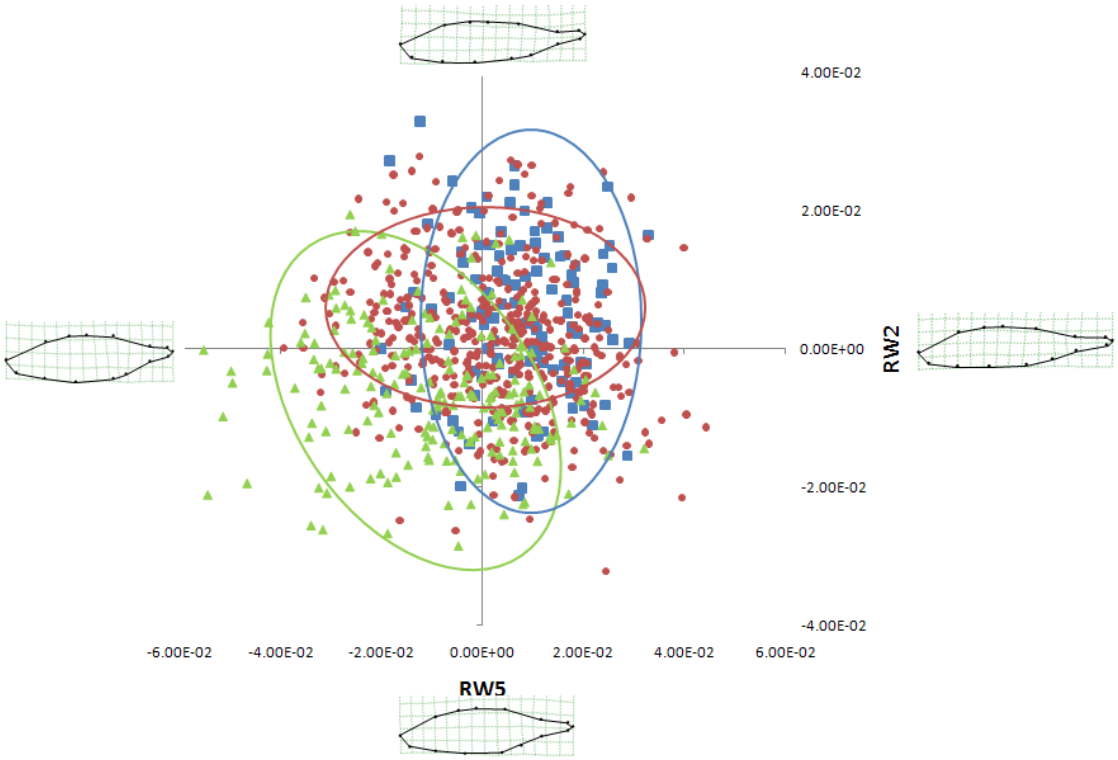


Figure 4.4 Plot of the relative warp (RW) scores significantly differentiating lake (blue squares), reservoir (red circles) and stream (green triangles) sticklebacks based on shape with putative cluster givens. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

Twenty-three canonical discriminant functions were calculated to predict system membership; of which 16 were statistically significant and together explained 98.1% of the variation ($p < 0.05$; Table 4.4). Together the discriminant functions were correctly able to predict group membership for 59.9% of the samples.

Table 4.4 Tests of canonical discriminant functions to assess suitability of partial warp scores for differentiating between sticklebacks from lakes, reservoirs and streams.

Function	Canonical correlation	χ^2	df	*p* value
1	0.772	3661.8	598	<0.001
2	0.695	2990.4	550	<0.001
3	0.654	2501.8	504	<0.001
4	0.604	2089.4	460	<0.001
5	0.582	1753.3	418	<0.001
6	0.534	1446.8	378	<0.001
7	0.499	1198.6	340	<0.001
8	0.479	986.4	304	<0.001
9	0.443	793.4	270	<0.001
10	0.402	632.0	238	<0.001
11	0.384	501.3	208	<0.001
12	0.331	383.2	180	<0.001
13	0.299	297.4	154	<0.001
14	0.266	228.1	130	<0.001
15	0.254	174.0	108	<0.001
16	0.221	124.8	88	0.006
17	0.199	87.8	70	0.074
18	0.179	58.0	54	0.330
19	0.144	33.9	40	0.742
20	0.105	18.3	28	0.918
21	0.096	10.2	18	0.926
22	0.064	3.3	10	0.973
23	0.019	0.3	4	0.991

4.4. DISCUSSION

This study has demonstrated that lake- and stream-dwelling sticklebacks in the UK show patterns of morphological divergence similar to those observed elsewhere in Europe (Gross & Anderson 1984) and North America (Thompson *et al.* 1997). Moreover, the results strongly suggest that the morphology of reservoir-dwelling sticklebacks is, on average, intermediate between lake and stream fish

Using morphometric analyses, differences in the shape of fish between different habitats were primarily related to the relative size around the body depth and caudal

peduncle. A clear pattern emerged, with stream fish being deep-bodied with short and wide caudal peduncles and lake fish being more narrow bodied and less wide, with longer and thinner caudal peduncles. The relevance of these traits is discussed below with specific reference to the linear measures recorded.

4.4.1. Variation in width and depth

The results indicated that the width of reservoir fish was equivalent to that of lake fish, both of which were significantly less wide than in stream fish, suggesting that slimmer bodies are better suited to still water environments. Increased width may point towards a greater muscle mass (Walker 1997); a trait particular useful for fast-start and powered turns and may also be associated with greater stability and inertia in flowing water conditions (Webb 1984).

Body depth, on the other hand, was significant different between fish from all three habitats; stream fish had the deepest body, followed by fish from the reservoir, whereas lake fish had the least deep bodies. An intermediate phenotype in reservoir fish for body depth, but not for width suggests that the importance of habitat-appropriate width may be more critical than that of depth. However, previous studies comparing the morphology of sticklebacks between lakes and streams (e.g. Reimchen *et al.* 1985; Lavin & McPhail 1993; Hendry *et al.* 2002) did not measure width, so it is difficult to reconcile these findings with other systems.

4.4.2. Caudal morphology in relation to swimming performance

In terms of caudal morphology, lake and reservoir fish tended to have a tail stem that was longer than stream fish, whereas caudal depth was greatest in stream fish, followed by reservoir fish and then those from the lake. Because stem length was

measured as length behind the dorsal and ventral fins, a longer caudal peduncle implies shorter fins whereas a shorter peduncle indicates longer fins, relative to standard length. Sustained swimming performance is optimised by small body and fin areas by minimising resistance and drag (Webb 1984; Blake *et al.* 2005), thus shorter fins may be adaptive for swimming performance in lakes and reservoirs. Selection for sustained swimming ability in the ancestral anadromous form is thought to be the result of extensive migrations (Hagen 1967). Whereas sticklebacks in natural lakes may have maintained this trait, selection pressure for previously stream-adapted fish to adopt this trait may be low, resulting in the non-significant difference between stream- and reservoir-resident fish (Langerhans 2009). Furthermore, sticklebacks are able to mitigate the effects of drag by collapsing their dorsal fin if necessary (Blake *et al.* 2005).

A large caudal depth is associated with increased thrust and improved inertia during feeding (Walker 1997) and is thus considered adaptive in a flowing water environment. Similar patterns of increased caudal depth in stream populations when compared to lake populations have been previously observed in sticklebacks (Sharpe *et al.* 2008) and also in rainbow trout, *Oncorhynchus mykiss* (Keeley *et al.* 2005).

4.4.3. Differences in the anti-predator morphology of lake-, reservoir- and stream-caught stickleback

Spine length did not vary significantly between fish from different habitats. Given the mixed pattern of spine length within reservoir-stream systems reported earlier (Chapter 2) and in other studies of parapatric populations (Reimchen *et al.* 1985), it is not surprising that no clear differentiation between habitats was found here. Long spines are considered an advantage against gape-limited predators (Gross 1978), hence

differences in spine length are expected to be related to predation regimes rather than habitat type diversity, *per se*. Similarly, the pelvic girdle and lateral plates are also associated with defence against predators (Reimchen 1992; Barrueto 2009; Lescak & von Hippel 2011). Detailed information about the predation regime at each of the sampling locations was not available, but it is likely that there was considerable variation among habitats and sites

4.4.4. Mouth gape width and its association with foraging

The results indicated that mouth width was indistinguishable between reservoir and lake sticklebacks, but that it was much wider in fish from stream. Mouth gape width has considerable implications for the maximum sized prey that can be consumed, particularly for fish like sticklebacks that eat their prey whole. Several studies have shown that fish feeding on large prey have a larger gape with than those feeding on smaller prey (Malmquist 1992; Keeley *et al.* 2005; Berner *et al.* 2008; Palkovacs & Post 2008). However, there is also evidence for phenotypic plasticity in trophic traits, such as those observed in arctic charr *Salvelinus alpinus* (Adams *et al.* 2003; Garduno-Paz & Adams 2010) and in sticklebacks (Day & McPhail 1996). Hence, if mouth width differences have a risen as a result of phenotypically plastic, then the differences observed may be a direct consequence of prey availability rather than being related to time available for adaptation.

4.4.5. Conclusions

The results of this study have shown that sticklebacks in streams and natural, ancient lakes show the same patterns of morphology in the UK as seen elsewhere in the northern hemisphere. It adds further support to the hypothesis that variation in traits

measured reflects the adaptive divergence of those traits, which is related to the selection regimes that characterise streams and lakes.

Although reservoirs share many characteristics with natural lakes (Moss 1988), there are still likely to be aspects which differ significantly and could affect stickleback evolution. For example, although it may be possible to obtain relevant information about the filling and draining of reservoirs, much remains unknown about the life-history of ancient lakes, and how they have been impacted by human activity (Moss 1988). Furthermore, although it would be possible to collect information on current predation regimes in lakes, reservoirs and streams, consistent and detailed information on historical patterns of predation are unlikely to exist.

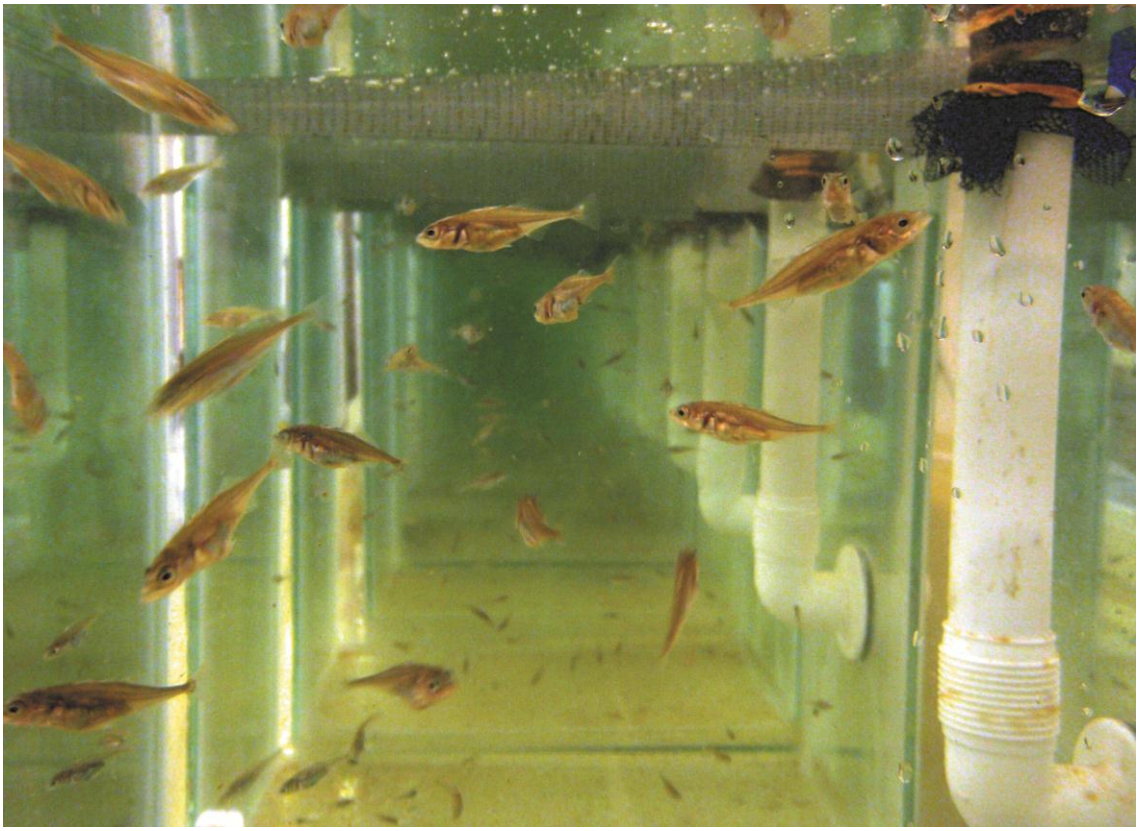
One key difference is that reservoirs are significantly younger than natural lakes, and fish caught from reservoirs in the current study showed morphology that was, on average, intermediate between streams and natural lakes. Given that the rate of adaptation is usually related to the strength of divergent selection (Bernatchez *et al.* 1999), the extent to which each trait in the reservoirs was lake-like gives an indication of the strength of selection acting on that trait. For example, caudal peduncle depth in reservoir fish showed an intermediate phenotype between lake fish and stream fish; whereas mouth width in reservoir fish was indistinguishable from that of fish from natural lakes. If variation in both of these traits is a result of environmental selective pressures, then it appears as though there has been greater selection pressure on trophic morphology than there has been on caudal morphology.

In earlier work (Chapter 2), reservoir fish were compared with that of parapatric stream fish to look at relative differences in various aspects of morphology. Reservoir age was not correlated with levels of divergence, which were thought to be more closely related to the specific characteristics of each individual reservoir-stream system.

Although fish from additional reservoirs that did not have a connected stream sample were included in the analysis of the current study, morphological traits continued to cluster by habitat type. This suggests that there are more than likely at least some characteristics of reservoirs which are shared across sites. As such, it also highlights the potential that reservoirs have to act as an important tool for understanding the process of divergence seen in lake-stream stickleback pairs. Nevertheless, it is also vital to keep in mind that some traits may be phenotypically plastic; and the extent to which they are habitat-appropriate is likely to reflect both the extent of their plasticity but also the strength of selection.

Chapter 5

The effect of rearing environment on the morphology of three-spined stickleback



5.1. INTRODUCTION

Population-level variation in phenotypic traits that perform specific functions in a given environment are often considered to have arisen as an adaptive response to different selective environments and thus to be the product of natural selection (e.g. Schluter 1988, 1994). The magnitude and speed of trait changes in response to divergent environments are determined by the strength of selection on a trait, in addition to the proportion of phenotypic variation of the trait that is genetically determined, i.e. the broad-sense the heritability of the trait (Donohue 2009). Thus, an evolved response to selection results in changes in the population mean trait value over successive generations towards a form that has higher fitness. For example, mean body weight and horn size of bighorn trophy rams *Ovis canadensis*, both traits which are highly heritable, have declined significantly over time, in response to trophy hunting targeting large rams with rapidly growing horns (Coltman *et al.* 2003).

However, *individuals* within a population may also alter their phenotype in response to the environment during their own lifetime and this is referred to as phenotypic plasticity (Agrawal 2001). If those phenotypic changes are beneficial, then they can also be said to be adaptive (Gotthard & Nylin 1995). Common jewelweed *Impatiens capensis* develop elongated stems in dense stands to increase light capture, but remain non-elongated when at low density. Dudley & Schmitt (1996) carried out an experiment where they manipulated phenotype by inducing or suppressing elongation using a light cue and then transplanted individuals into high or low density plots. Their work showed that elongated individuals had greater fitness in terms of lifetime reproduction (estimated as the number of flowers, fruits and pedicels at the census immediately prior to death) in high density plots whereas non-elongated individuals had greater fitness in low density plots. Similarly, crucian carp *Carassius carassius* show

phenotypic plasticity for body depth, which increases in response to predation by pike, *Esox lucius* (Brönmark & Miner 1992). A deep body serves as a deterrent against predation by gape-limited piscivores (Wahl & Stein 1988) and can enhance the fast-start escape response (Webb 1984; Domenici & Blake 1997; Godin 1997; Walker *et al.* 2005). An increase can also be induced in the laboratory by exposure to chemical cues from piscivorous fish (Holopainen *et al.* 1997).

In both of these examples, the ability to alter morphology by phenotypic plasticity results in increased fitness (i.e. increased lifetime reproduction and decreased vulnerability to predation for the common jewelweed and crucian carp, respectively) and supports a theory of *adaptive* plasticity (Pigliucci 2001). If plasticity is adaptive, then it is also expected to be under selection and artificial selection experiments have shown that plasticity is a trait that responds to selection (Waddington 1960). Models for the genetic basis of plasticity have been proposed and show how it may become an evolved trait (reviewed in Scheiner 1993). However, limited benefits or even a cost of plasticity may inhibit its evolution (DeWitt *et al.* 1998). Using the earlier example, deep-bodied carp suffer density dependent foraging costs when compared to shallow-bodied individuals due to increased hydrodynamic drag (Brönmark & Miner 1992). They also have a lower standard metabolic rate, which negatively impacts their stress tolerance and growth rate (Pettersson & Brönmark 1999). In addition to these direct fitness costs, there may also be costs associated with of maintaining the genetic and cellular machinery necessary to be plastic (Scheiner 1993).

Plasticity and genetic variation can be considered two, non-mutually exclusive ways of adapting to local environmental conditions (Crispo 2008). The advantages of either method depend on the level of environmental consistency, such that genetic adaptations may be more likely to evolve in constant, non-variable environments while

plasticity may be more beneficial in environments which are spatially or temporally heterogeneous (reviewed in Alpert & Simms 2002). However, there is evidence to suggest that moderate levels of plasticity are optimal for driving genetic evolution (reviewed in Price *et al.* 2003).

Arctic charr *Salvelinus alpinus* show phenotypically plastic foraging behaviours and is also characterised by discrete variation in foraging anatomy within single populations (Adams *et al.* 1998). Trophic morphology in charr has been shown to vary according to the type of prey consumed (Adams *et al.* 2003), leading to the conclusion that foraging anatomy is, at least in part, phenotypically plastic and is thought to arise through behavioural plasticity. Behavioural phenotypes, particularly those related to foraging and resource polymorphism, have a strong functional significance (Smith & Skúlason 1996) and are thus considered strong candidates for subsequent divergence (West-Eberhard 1989). However, although plasticity may drive initial phenotypic divergence, genetic changes in the direction of the plastic response may also result (e.g. Adams & Huntingford 2004). A possible mechanism by which this could occur is genetic assimilation, in which although the acquired adaptive character is initially a response to the current environment, it may also become canalised so that the response is adjusted in a way which brings about a single and definite end-result (Waddington 1942). Over several generations, the acquired character may become assimilated by the genotype and appear independently of any specific environmental influences (Waddington 1953).

Initial plasticity followed by genetic assimilation for a given trait is particularly relevant to adaptive radiations (Schluter 2000). Ancestral generalists are more likely to meet resource requirements in a novel environment than specialists, hence plasticity plays an important role for initial survival following displacement, whereas long-term

success may be linked more closely with genetic divergence and specialisation (Schluter 2000). For example, in recently impounded rivers, generalist fish with an opportunistic feeding behaviour were found to be most successful in colonising the first stage of reservoir formation (de Merona *et al.* 2003). Over time however, specialist species became more important and numerous in the assemblage, most probably by outcompeting generalist fish, highlighting the importance of specialisation for longer-term survival.

In the three-spined stickleback *Gasterosteus aculeatus*, variation in body form and morphology (Bell & Foster 1994) allied to a fully sequenced genome, has made it an ideal species for comparing the role of plasticity and genetic divergence on phenotypic traits. Marine sticklebacks are generally considered to be the ancestral phenotype and recent research has identified regions of the genome that are associated with traits important for freshwater adaptation (Hohenlohe *et al.* 2010). Previous work has ascertained that there are several morphological characteristics under genetic control, including gill raker length and number, spine length and body shape (Gross & Anderson 1984; McPhail 1992; Lavin & McPhail 1993; Peichel *et al.* 2001; Hendry *et al.* 2002; McKinnon & Rundle 2002; Leinonen *et al.* 2006; Sharpe *et al.* 2008). However, a number of these and other studies also suggest that traits may show some degree of plasticity (Gross & Anderson 1984; Day *et al.* 1994; Hendry *et al.* 2002; Sharpe *et al.* 2008; Olafsdottir & Snorrason 2009; Frommen *et al.* 2011).

One way in which the effects of phenotypic plasticity and genetic adaptation can be partitioned is by excluding the effects of rearing environment by raising the offspring of individuals from morphologically distinct populations in a common laboratory environment ("common-garden"). If the phenotypic divergence of the parental population is maintained in common-garden reared offspring, then observed

population differences can be considered to be the result of underlying genetic divergence. However, if common-garden rearing experiments result in phenotypically similar offspring, then differences in the parental population are likely to be the result of plasticity. Conover and Present (1990) raised Atlantic silversides *Menidia menidia*, which show latitude-dependent differences in growth rates, from three different locations in a common environment for several generations. Differences in growth rates were maintained, leading the authors to conclude that there is a genetic basis to the growth rate in this species. Experiments on the intertidal gastropod, gold ringer *Monetaria annulus* however, found no size differences between populations reared in common-garden conditions even though there are remarkable size differences in wild populations (Irie & Morimoto 2008). Thus, maximum size in gold ringer is considered to be phenotypically plastic.

Rearing the offspring of individuals from one population under different environments can also evidence for the role of environmental factors in phenotypic divergence. A decline in the survival rates of coho salmon *Oncorhynchus kisutch* reared in hatcheries and released into the Pacific Ocean when compared to wild marine coho salmon (Beamish *et al.* 2008) have been attributed to various differences in the physiology and behaviour of smolts (Chittenden *et al.* 2008). However, when pure hatchery-bred, pure wild-bred and hybrid offspring were divided so that a proportion of each could be raised in both a traditional hatchery environment and naturally in a contained side channel, there were only a few phenotypic differences in genetic groups reared in the same habitat (Chittenden *et al.* 2010). Rearing environment, on the other hand, played a significant role in smolt survival, size, swimming endurance, predator avoidance and migratory behaviour. Experiments of this kind provide an excellent

opportunity for assessing the relative genetic and environmental contributions to the phenotypes observed.

5.1.1. Aims

In Chapter 2, I compared the morphology of reservoir and stream stickleback pairs and showed that reservoir fish tended to have slimmer and shallower bodies, narrower caudal peduncles, narrower mouths and shorter gill rakers; differences that are considered adaptive for life in still water ecosystems. However, the extent to which those differences have arisen as a result of genetic changes in morphology through adaptive divergence, or as a result of adaptive plasticity remains unclear. The aim of the current chapter was to investigate this further, using a "common-garden" and "divergent rearing environment" experimental approach. Under a hypothesis of adaptive divergence, laboratory-bred fish were expected to show patterns of morphological variation similar to those observed in wild populations. However, if the observed variation in the wild population have arisen as a result of plasticity in response to environmental conditions, then offspring reared under the same conditions, were expected to be indistinguishable based on their morphology. To test these alternative hypotheses, fish from the Thornton reservoir-stream system, previously shown to have divergent morphology (details in section 2.5.2.7), were bred to produce F_1 progeny of reservoir and stream fish, which were reared under common conditions. In addition, reservoir and stream fish were also crossed to produce reservoir-stream 'hybrid' offspring, which were also reared under common conditions. To test the effect of rearing environment, additional fish from natural lakes and rivers were bred to produce F_1 progeny of lake and river fish, which were split so that some were reared in still and some reared under flowing water conditions.

5.2. METHODS

5.2.1. Fish supply and husbandry

Adult sticklebacks from Thornton reservoir and stream were caught between April-July during 2008 and 2009 using unbaited metal minnow traps (5 x 5mm mesh) left overnight. Adult sticklebacks from three additional lakes and rivers (Table 5.1) were caught between April-June 2009 using the same technique. On return to the University of Leicester, fish were housed in separate sex groups in stock aquaria, according to their habitat of capture. The temperature was set to 18°C and the photoperiod held constant at 16L:8D. Females were fed twice daily *ad libitum* with frozen bloodworm and regularly assessed for gravid status (indicated by a noticeable swelling of the abdomen). Males were fed once daily *ad libitum* and were assessed for sexual maturity based on the presence of red nuptial colouration.

5.2.2. IVF protocol

In the common-garden rearing study, a split-clutch IVF (SC-IVF) technique was used to generate reservoir and stream crosses (Barber & Arnott 2000). Each SC-IVF was carried out using two males and two females, one of each sex from the reservoir and the stream-caught samples to reduce the potential influence of population-specific maternal or paternal effects. Each cross thus generated 4 families (Fig. 5.1).

In the divergent rearing environment study, eggs were not split and males were used only to fertilise a single clutch of eggs. Only males and females from the same site were crossed.

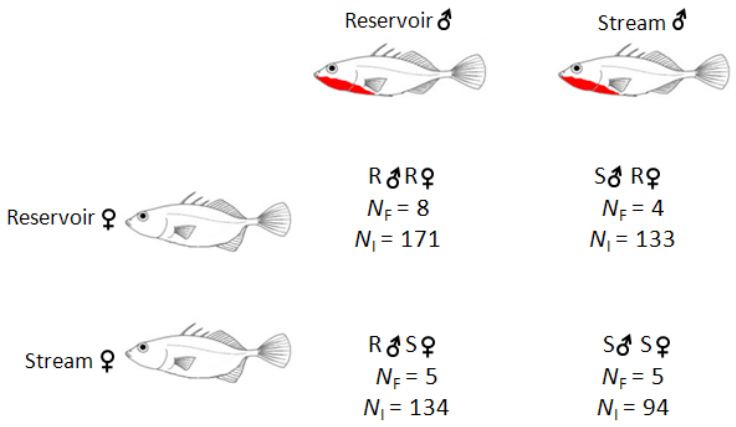


Figure 5.1 Schematic diagram describing the four cross types generated using a SC-IVF technique with a male and a female from the reservoir and the stream. N_F refers to the number of families in that cross and N_I refers to the number of individuals.

5.2.3. Conditions for fish reared under a common-garden approach

Each family was reared in an 8L aquarium using a re-circulatory and temperature controlled system. Families in which the number of fish exceeded 40 were split and housed in two separate aquaria. Fish were initially fed Liquifry for 7d before being provided with live *Artemia* sp. nauplii. After approximately 2 months, bloodworms (*Chironomus* sp. larvae) were also introduced into their diet. Fry were initially reared under summer conditions (18°C and 16L:8D) until November, when winter conditions (8°C and 10L:14D) were imposed. Summer conditions were re-established in February, allowing fish to come into breeding condition. On achieving adult size and morphology (approximately 1 year after hatching), fish were sacrificed and processed for morphological investigation (see section 2.2.3 for details).

5.2.4. Conditions for fish reared under different environments

Fish from three natural lakes (Aqualate Mere, Llyn Eiddwen and Tatton Mere) and three rivers (River Eye, River Welland and Afon Ystwyth) were bred to produce first generation lake- and river-fish (Table 5.1). Initially, all fish were reared in 5L aquaria and fed firstly on Liquifry for 7d, followed by live *Artemia* sp. nauplii. After approximately 2 months, offspring from each population were split; half were transferred to still-water aquaria and half were transferred to flowing water tanks (Fig. 5.2). Fish were maintained in these conditions on a mixed diet of live *Artemia* sp. nauplii and *Chironomus* sp. larvae. Fry were initially reared under summer conditions (18°C and 16L:8D) until November, when winter conditions (8°C and 10L:14D) were imposed. Summer conditions were re-established in February, allowing fish to come into breeding condition. On achieving adult size and morphology (approximately 1 year after hatching), fish were sacrificed using an overdose of 0.1% Benzocaine anaesthetic. Digital images of the right lateral view were taken (Fujifilm FinePix s9600) and measurements were made using ImageTool v3.0. Samples were originally intended for other purposes which did not require clearing and staining, hence only limited measures of morphology could be made.

Table 5.1 Description of sites sampled for the analysis of stickleback morphology

Site name	Habitat type	Latitude (N)	Longitude (W)	Sample size (flowing)	Sample size (still)
Aqualate Mere	Lake	52°46’52”	2°20’27”	31	19
Llyn Eiddwen	Lake	52°16’57”	4°02’42”	40	35
Tatton Mere	Lake	53°19’15”	2°22’09”	36	28
River Eye	River	52°45’06”	0°48’27”	21	21
River Welland	River	52°31’29”	0°52’24”	39	37
Afon Ystwyth	River	52°23’55”	4°05’08”	26	40

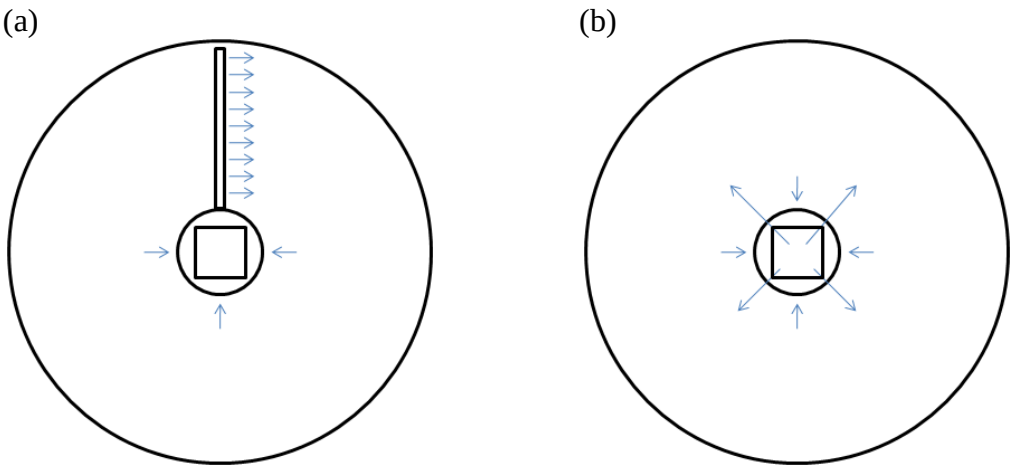


Figure 5.2 Schematic diagram of (a) flowing and (b) still water tanks (50cm diameter) used to rear stickleback for the divergent rearing environment study. A water current of ~8cm/sec was created and maintained using a pump housed in a central pipe. The pump pumped water into a spray bar which directed the water around the tank. The central pipe was perforated around the base (2cm from the bottom) and around the top (at water level) to allow water to re-enter the pipe for pumping. Non-flowing tanks were identical, except there was no spray bar and pumped water was instead allowed to flow over the top of the central pipe. Arrows indicate water flow direction.

5.2.5. Statistical analyses

5.2.5.1. Common-garden rearing experiment

A Principal Components Analysis was undertaken to detect which traits best differentiated reservoir and stream fish. Traits for which the linear measure correlated with standard length (SL), were regressed onto SL and the residuals compared between cross types (Reservoir x Reservoir, R♂R♀; Reservoir x Stream, R♂S♀; Stream x Reservoir, S♂R♀; Stream x Stream, S♂S♀) using a nested ANOVA with cross type as a fixed factor and family as a random factor. Size-corrections were not computed for measures which did not show a correlation with SL (jaw angle and number of lateral plates), which were compared between habitats using a nested ANOVA. Post-hoc testing was conducted using the Tukey method to distinguish between crosses that showed differences. Landmark data were analysed as described in section 2.2.3.3. It was not possible to reliably sex all of the fish produced hence sex was not included as a

factor. However, sample sizes were large and it is unlikely that there would be a skew in sex ratios.

5.2.5.2. Divergent rearing environment experiment

A two-way ANCOVA was conducted to test for the effect of habitat of origin (lake or river) and rearing conditions (flowing or still) on both body depth and depth of the caudal peduncle across all populations with SL as the covariate. Population-specific ANCOVAs, with SL as the covariate, were used to test if rearing environment had an effect on individual populations.

5.3. RESULTS

5.3.1. Common-garden rearing effects on the morphology of sticklebacks from the Thornton reservoir-stream system

5.3.1.1. Linear measurements of morphology

Principal components analysis revealed the presence of three components with eigenvalues exceeding 1, explaining 74% of the variance in the morphological traits measured. However, inspection of the screeplot suggested that only two components should be retained and explained a total of 65% of the variance. Component 1 included nearly all of the measures of morphology including those of overall size and shape and spine length. Component 2 was primarily related jaw angle and the average number of lateral plates, but also included width, mouth width and length of the second dorsal spine (Table 5.2).

Table 5.2 Rotated component matrix using a principal components analysis extraction method showing factor loadings for each variable based on sticklebacks caught bred from the Thornton reservoir-stream system

Trait	Component 1	Component 2
Standard length	0.936	0.014
Depth	0.900	0.291
Pelvic girdle length	0.853	-0.196
Caudal peduncle depth	0.835	0.266
Pelvic spine length	0.811	-0.109
Second dorsal spine length	0.745	-0.417
First dorsal spine length	0.743	-0.290
Caudal peduncle length	0.711	-0.288
Mouth width	0.706	0.445
Width	0.668	0.427
Jaw angle	-0.033	0.684
Average number of lateral plates	0.027	-0.315

The specific combination of male and female parent habitat of origin (i.e. cross type) had a significant effect on body depth ($p = 0.007$), length of the pelvic spine ($p = 0.037$) and mouth width ($p = 0.022$) (Table 5.3).

Results from post-hoc testing using the Tukey method are outlined in Table 5.4. In summary, fish from S♂R♀ crosses had the shallowest bodies, followed by R♂R♀ bred fish; whereas offspring arising from R♂S♀ and S♂S♀ crosses had the deepest bodies (Fig. 5.3a). Pelvic spine was longest in R♂S♀ crosses and shortest among those from R♂R♀ crosses. Offspring from S♂R♀ and S♂S♀ crosses were intermediate in pelvic spine length (Fig. 5.3b). In terms of mouth width, fish from R♂R♀ crosses had the narrowest mouths whereas those from S♂S♀ crosses had the widest mouths (Fig. 5.3c). There were no differences in the mouth width of fish arising from R♂S♀ or S♂R♀ crosses; both had intermediate mouth widths when compared to fish from R♂R♀ and S♂S♀ crosses.

Table 5.3 Results from a two-way nested ANOVA to determine whether cross types and family had a significant effect on the morphology of stickleback reared under common laboratory conditions.

	Cross type			Family		
	F ratio	df	p	*F* ratio	df	p
Width	0.275	3 , 18	0.843	20.6	18 , 510	<0.001
Depth	**5.23**	**3 , 18**	**0.007**	6.08	18 , 510	<0.001
Caudal peduncle depth	1.75	3 , 18	0.191	11.2	18 , 510	< 0.001
Caudal peduncle length	0.225	3 , 18	0.878	18.1	18 , 510	< 0.001
First dorsal spine length	0.954	3 , 18	0.433	7.46	18 , 510	< 0.001
Second dorsal spine length	0.469	3 , 18	0.707	8.72	18 , 510	< 0.001
Pelvic spine length	**3.47**	**3 , 18**	**0.037**	9.96	18 , 510	< 0.001
Pelvic girdle length	0.525	3 , 18	0.670	4.84	18 , 510	< 0.001
Mouth width	**4.03**	**3 , 18**	**0.022**	10.5	18 , 510	< 0.001
Jaw angle	0.095	3 , 18	0.962	10.29	18 , 510	< 0.001
Number of plates	0.170	3 , 18	0.915	15.08	18 , 510	< 0.001

Traits which show a significant effect of cross type are given in **bold**.

Table 5.4 Results from post-hoc Tukey tests to determine which cross types (R, reservoir; S, stream) showed significant differences in morphology when reared under common laboratory conditions.

	Body depth			Pelvic spine length			Mouth width		
	R♂R♀	R♂S♀	S♂R♀	R♂R♀	R♂S♀	S♂R♀	R♂R♀	R♂S♀	S♂R♀
R♂S♀	<0.001			<0.001			<0.001		
S♂R♀	<0.001	<0.001		<0.001	<0.001		<0.001	0.269	
S♂S♀	0.001	0.536	<0.001	0.002	<0.001	0.842	<0.001	<0.001	<0.001

Numbers indicate the *p* value of the test.

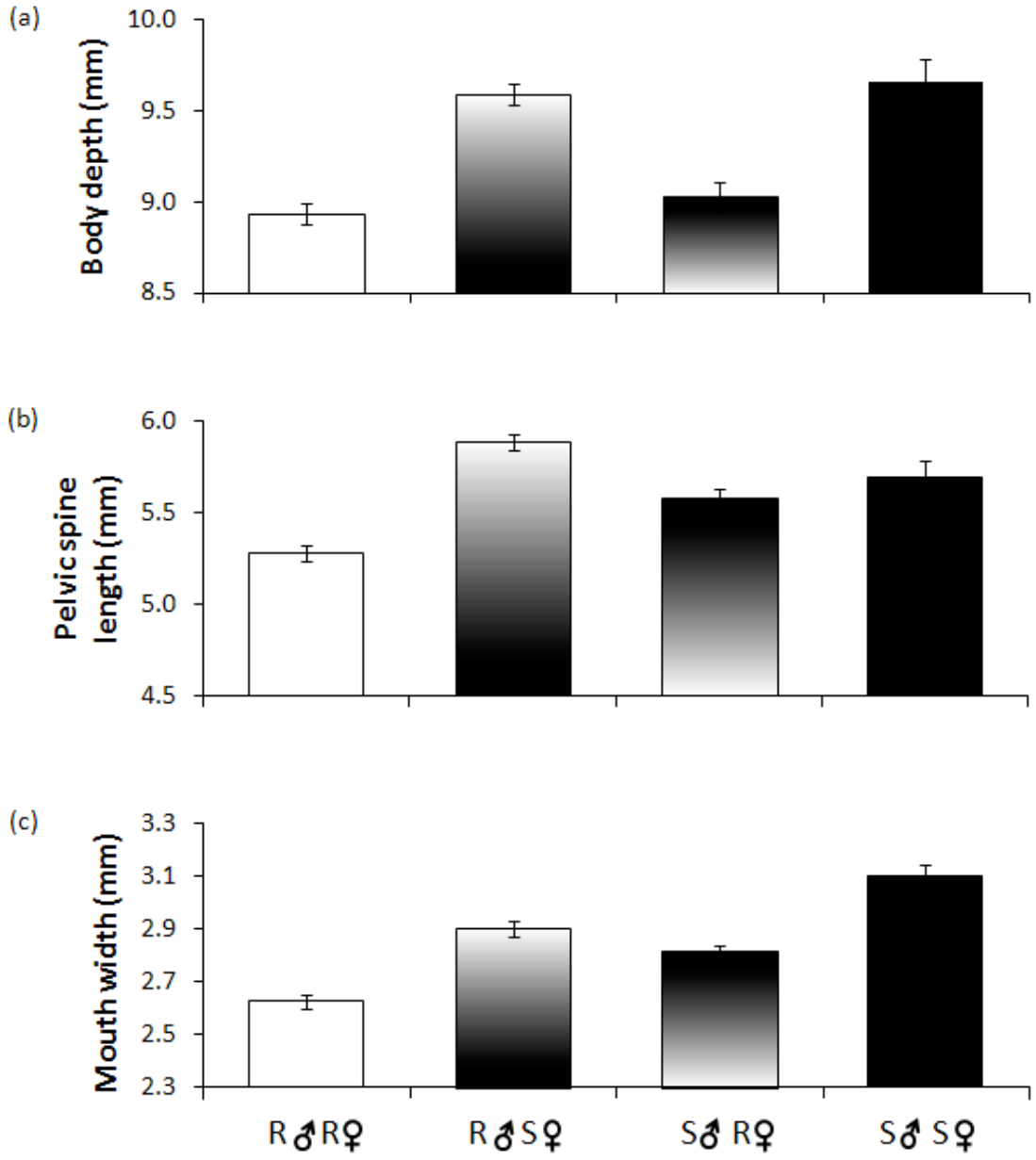


Figure 5.3 Key measures of morphology for reservoir (R) and stream (S) stickleback crosses, reared under a common environment in the laboratory: (a) body depth, (b) pelvic spine length and (c) mouth width. Bar heights indicate mean value of fish from each cross type and error bars indicate ±SE.

5.3.1.2. Geometric morphometric analyses of shape

Twenty-six principal components of shape (relative warps, RW) were calculated to describe the variation in shape across all samples. The first six RW scores described a total of 79.4% of the variation, with successive RW scores describing less than 5% each (Fig 5.4).

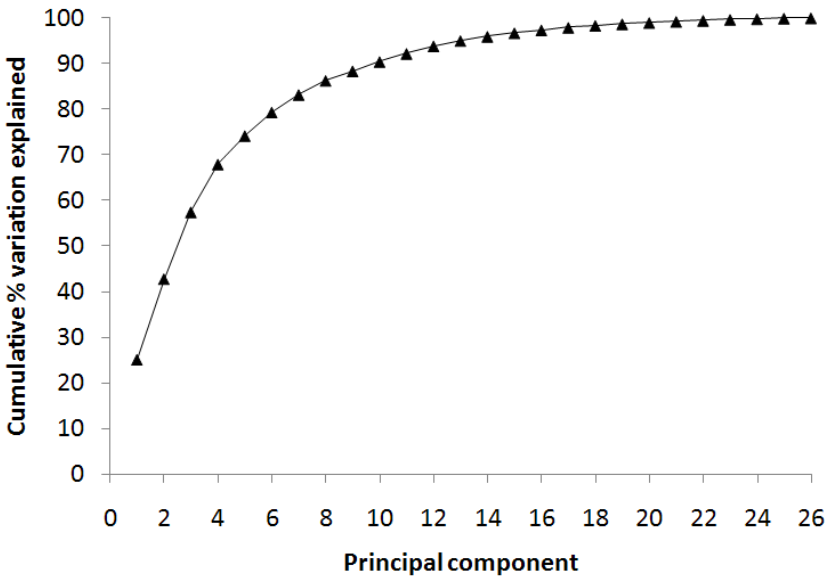


Figure 5.4 Cumulative percentage of variation in shape across all samples described by the 26 principal components of shape (relative warps).

Of the 26 RW scores calculated, 16 could significantly differentiate between cross type ($p < 0.05$) and explained a total of 93% of the variation. Although RW1 (25.2%, $F_{3,515} = 45.2$, $p < 0.001$) and RW2 (17.7%, $F_{3,515} = 4.35$, $p = 0.005$) described most of the variation in shape, RW4 (10.5%, $F_{3,515} = 25.3$, $p < 0.001$) and RW8 (3.1%, $F_{3,515} = 18.9$, $p < 0.001$) were the most significant and thus better able to discriminate between cross types (Fig. 5.5). RW4 was associated with body depth, particularly in the midsection and the posterior of the fish. Fish with positive scores in RW4 were deeper bodied and had a deeper and slightly longer caudal peduncle. RW8 was associated with anterior body depth and length of the head. Fish with positive scores in RW8 had a slightly shallower and smaller head.

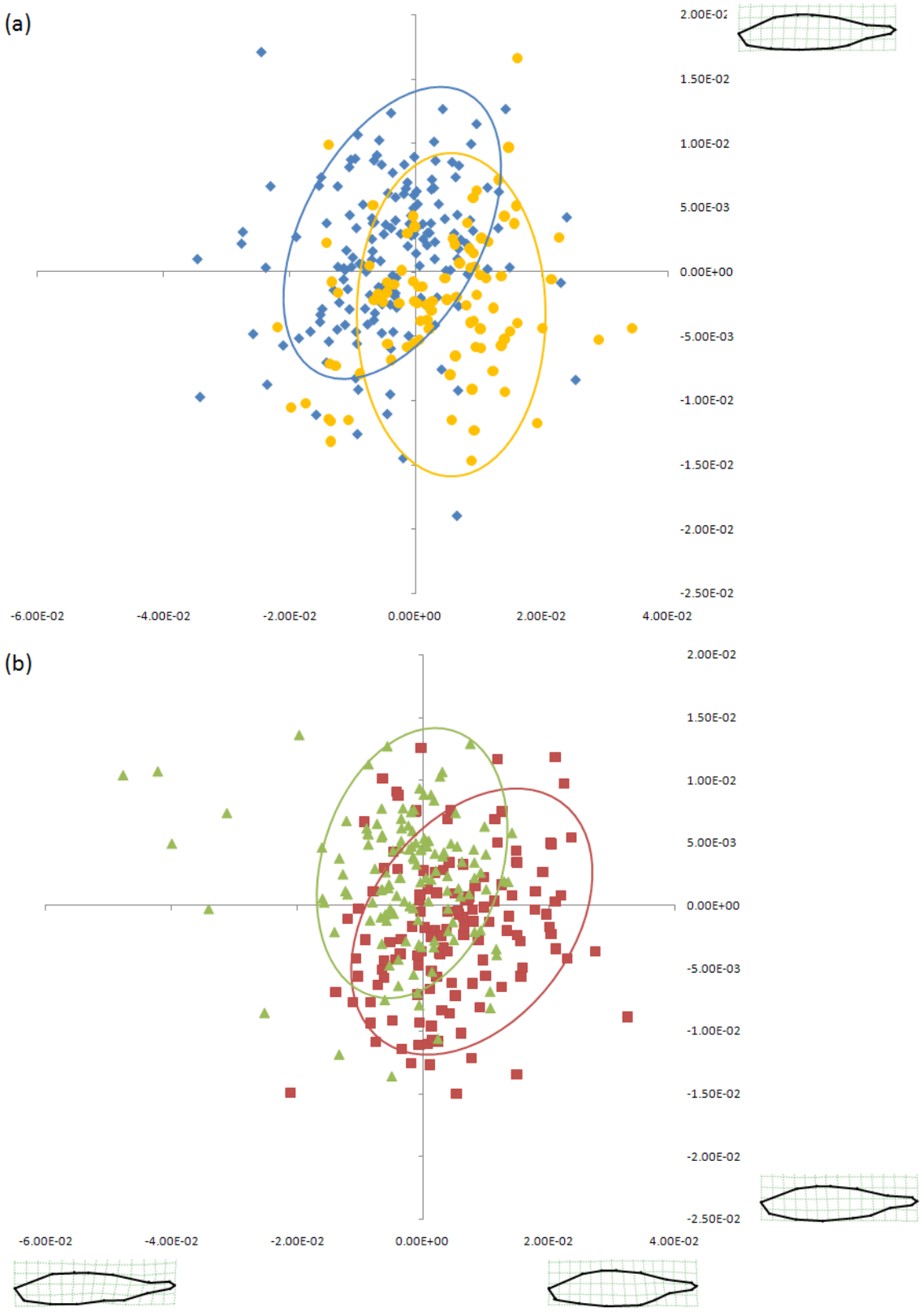


Figure 5.5 Plot of the relative warp scores significantly differentiating (a) R♂R♀ (blue diamonds) and S♂S♀ (yellow circles) sticklebacks, and (b) R♂S♀ (red squares) and S♂R♀ (green triangles) sticklebacks. Putative clusters are given (reservoir, unbroken line; stream, dashed line). Deformations associated with the minimum and maximum of each axis are given. The data refer to the most significant relative warp scores and describe 13.6% of the variation.

Three canonical discriminant functions were calculated to predict cross type membership; all of which were statistically significant and together explained 100% of the variation ($p < 0.05$; Table 5.5). Together the discriminant functions were correctly able to predict group membership for 64.2% of the samples.

Table 5.5 Tests of canonical discriminant functions to assess suitability of partial warp scores for differentiating between cross types of sticklebacks bred from the Thornton reservoir-stream system.

Function	Canonical correlation	χ^2	df	*p* value
1	0.581	455.9	78	<0.001
2	0.556	249.0	50	<0.001
3	0.343	62.78	24	<0.001

5.3.2. Effect of flow regime during rearing on the morphology of sticklebacks from lakes and rivers

The results indicated that there was a significant effect of rearing environment on depth of the caudal peduncle ($F_{1,360} = 14.1$, $p < 0.001$). Across populations, fish reared under flowing water conditions had a deeper caudal peduncle than those reared under still water conditions (Fig. 5.6b). However, there was also an effect of population of origin ($F_{5,360} = 10.8$, $p < 0.001$; Fig 5.6b). Population-specific analyses showed that the effect of rearing environment on caudal peduncle depth was significant in families reared from Afon Ystwyth ($F_{1,63} = 5.70$, $p = 0.020$) fish and that there was significant interaction between treatment and standard length (SL) in depth of the caudal peduncle was apparent in fish from River Eye ($F_{1,42} = 10.6$, $p = 0.002$).

In terms of body depth, fish showed a significant interaction between rearing environment and SL ($F_{1,349} = 6.54$, $p = 0.011$) and also between population of origin and SL ($F_{5,349} = 2.74$, $p = 0.019$). Further population specific analyses showed the effect of rearing environment on body depth was significant in offspring reared from Llyn

Eiddwen ($F_{1,72}$ = 22.8, p < 0.001), River Welland ($F_{1,73}$ = 9.91, p = 0.002) and Afon Ystwyth ($F_{1,63}$ = 16.4, p < 0.001) fish.

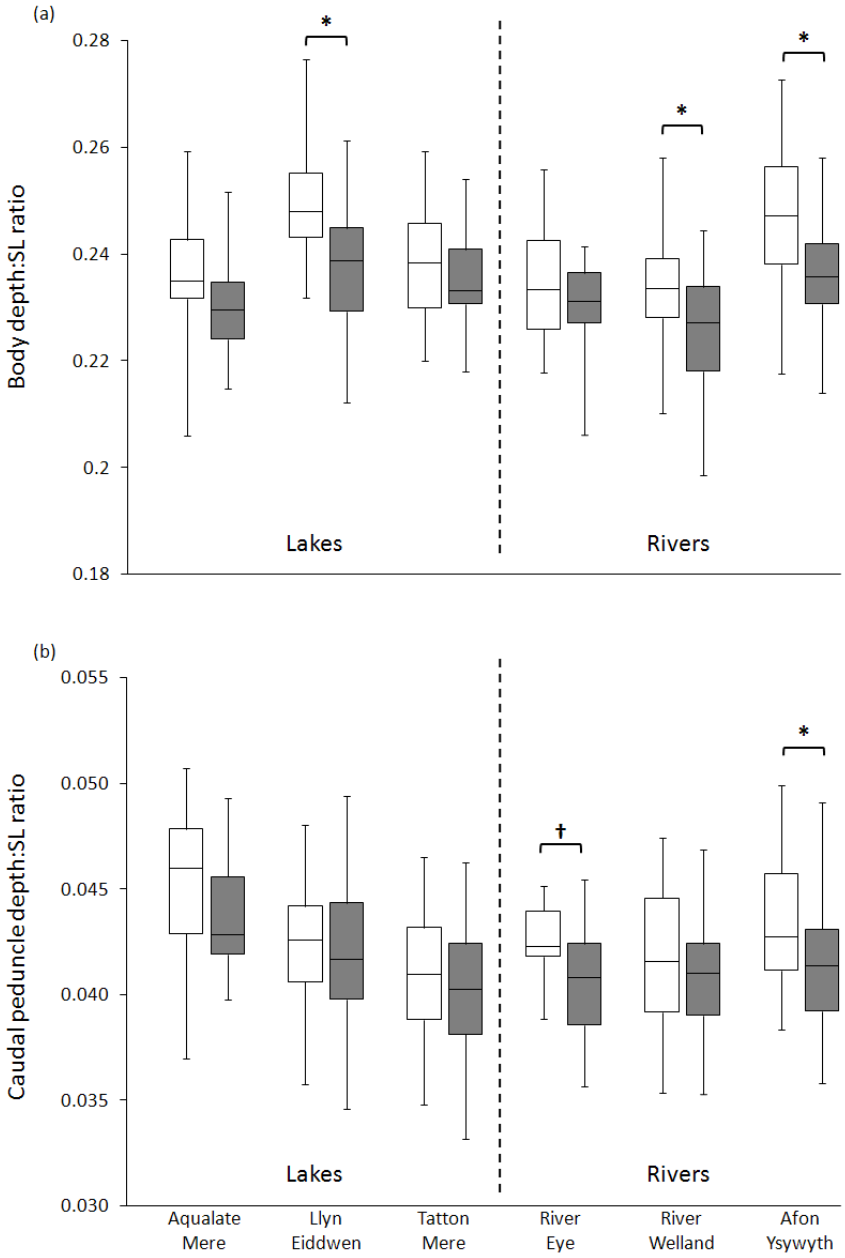


Figure 5.6 Variation in the (a) body depth and (b) caudal peduncle depth of lake and river sticklebacks reared in the laboratory under flowing (open) and still (grey) water conditions. Significant interactions between population of origin and rearing condition were apparent in both traits at the 5% level. * indicates a population-specific difference at the 5% level. † indicates a significant interaction between treatment and SL.

5.4. DISCUSSION

5.4.1. Differences in body shape as a consequence of flow

Several studies have implicated that deep and shallow bodies in fish are an adaptation to life in lotic and lentic environments, respectively (Reimchen *et al.* 1985; Lavin & McPhail 1993; Hendry *et al.* 2002). Deep bodies are considered adaptive in flowing water conditions because of the probable increase in muscle tissue whereas more shallow bodies are considered to be better suited for open-water swimming in still water environments due to the lower hydrodynamic drag (Taylor & McPhail 1986; Walker 1997; Tytell *et al.* 2010). Differences in the body depth in fish from Thornton reservoir and stream were not statistically significant, although followed a pattern similar to those expected based on habitat type (i.e. deeper bodied stream fish and more shallow bodied reservoir fish; Chapter 2). In the current study, the F_1 progeny of crosses between reservoir parents (R♀R♂) and stream parents (S♀S♂) bred from the Thornton reservoir-stream system, after being reared under common still water laboratory conditions, showed differences in body depth that are associated with habitat of origin. This result supports the hypothesis that body depth is at least partially determined by a heritable genetic mechanism. However, the F_1 progeny of lake and river fish reared under still and flowing water conditions did not show the same pattern and population specific analyses showed that some populations responded to changes in flow much more than others. Taken together, these results imply that morphological traits are affected by both genetic and environmental factors but that the extent to which either factor contributes to overall morphology is likely to be population-specific.

The body shape of fish arising from 'hybrid' crosses, i.e. between reservoir males and stream females (R♀S♂), and stream males and reservoir females (S♀R♂), was generally most similar to that of fish in the maternal habitat of origin (Figs. 5.2a

and 5.4). So, for example, S♀R♂ fish had shallower bodies than R♀S♂ fish, and there were no differences in body depth between R♀S♂ and S♀S♂ fish. These results suggest that body shape may be more strongly influenced by maternal genotype (i.e. maternally inherited) in this population. Maternal genetic effects on growth and development are well documented in several species (Heath *et al.* 1999; Keller *et al.* 2001; Perry *et al.* 2005; Raventos & Planes 2008; Brand *et al.* 2010). In addition to a genetic contribution, females govern the cytoplasmic allocation to individual progeny hence female egg size is often correlated with larval size, which in turn is associated with survival in several fish species (Wootton 1998). Maternal effects are usually strongest early in life and decline during an individual's development (e.g. Heath *et al.* 1999) although effects on body weight and length can persist beyond the juvenile stage (Tosh *et al.* 2010). Clutch size is fundamentally constrained by mechanical factors (Roff 1992) and previous work has shown that clutch volume variation correlates strongly with relative body depth (Foster *et al.* 1992). Thus body shape in females affects reproductive success in addition to environment-specific adaptations in swimming performance and could provide an explanation for why body shape may be maternally inherited.

5.4.2. Population differences in the response to changes in flow regime

The results of this study indicated a significant effect of population on body depth and caudal peduncle depth morphology. Overall, across populations, flow regime had a significant effect on caudal peduncle depth morphology. However, the extent of differentiation within each population reared under contrasting environmental conditions differed noticeably, suggesting that population has a substantial effect on

morphology and that responses to changes in the environment differ accordingly. In brown trout *Salmo trutta*, which show an effect of population when reared under common conditions (Pakkasmaa & Piironen 2001), heritability of morphological characters varies greatly within and between populations (Varian & Nichols 2010). Variation in the heritability of a trait can affect a population's ability to respond to changes in the environment and can reflect differences in historical events. For example, population bottlenecks have been found to initially increase heritability (Willis & Orr 1993; Van Buskirk & Willi 2006). However heritability may also increase or decrease over the course of development as a result of changes in the environmental (Wilson & Réale 2006).

Although sites were classified into still or flowing habitat types, various other factors differ in the environment that may also affect morphology. For example, increased predation has been shown to be associated with a plastic response that can increase body depth (Brönmark & Miner 1992; Frommen *et al.* 2011). The response is considered to be adaptive because deeper bodies enhance fast-start escape responses thus minimising the chance of capture (Webb 1984; Walker 1997; Domenici *et al.* 2008) and reduce susceptibility to gape-limited predators (Wahl & Stein 1988); although there is a potential cost in terms of hydrodynamics because deep bodies also produce more drag (Blake *et al.* 2005). Gravid female sticklebacks regularly exposed to predator cues also produce larger eggs and juveniles that exhibit behavioural anti-predator defences (Giesing *et al.* 2011). In these examples, phenotypic plasticity may be considered advantageous. The predation regimes of the populations under investigation were unknown and so it is unclear what effect, if any, this may have had on the population's ability to respond to changes in the environment.

Rearing environment had a significant effect on the morphology of progeny reared from all three river sites but only on one of the lake sites possibly implying that there may be greater plasticity amongst river fish than there is amongst lake fish. Rivers tend to be spatially and temporally variable environments (Hynes 1979) and are therefore likely to favour plasticity (Alpert & Simms 2002). Environmental conditions in lakes however, are less likely to vary over space and time and so are more stable, and long-term success therefore may be reliant on the ability to show specialisations specific to that environment (Schluter 2000).

5.4.3. Polymorphisms in foraging morphology

The current study detected significant differences in the mouth width of fish reared under common conditions that were associated with parental habitat of origin: S♂S♀ fish had wider mouths than did R♂R♀ fish. Furthermore, R♂S♀ and S♂R♀ hybrids showed an intermediate phenotype, but were indistinguishable from one another. Mouth gape can be a limiting factor for determining the maximum sized prey an individual can ingest, particularly for species like stickleback, that consume their prey whole. In most stream habitats, the stickleback diet is dominated by benthic invertebrates (Berner *et al.* 2008) hence a larger mouth gape is expected. Previous work has shown trophic morphology in sticklebacks to both be phenotypically plastic (Day *et al.* 1994) and to have an underlying genetic basis (McPhail 1992) and the current study adds further evidence to suggest an underlying genetic mechanism for maximum gape size.

Foraging efficiency is a key determinant of fitness (e.g. Lemon 1991) and has a strong functional significance (Smith & Skúlason 1996) hence it is perhaps not surprising to see evolutionary adaptations in traits that maximise efficiency

(Waddington 1953; West-Eberhard 1989). Trophic polymorphisms in fish have been detected across a wide range of taxa and almost always include co-existing benthic and pelagic forms (Robinson & Wilson 1994; Skulason & Smith 1995); forms which partition the locally available resources by developing specialised local adaptations. In some cases, microsatellite genetic data has indicated that benthic and pelagic forms are separate and reproductively isolated populations (McPhail 1992; Dynes *et al.* 1999). Studies have shown that the morphological differences in littoral and pelagic brook charr *Salvelinus fontinalis* are heritable but are also regulated by a combination of both genetic and environmental factors (Proulx & Magnan 2004).

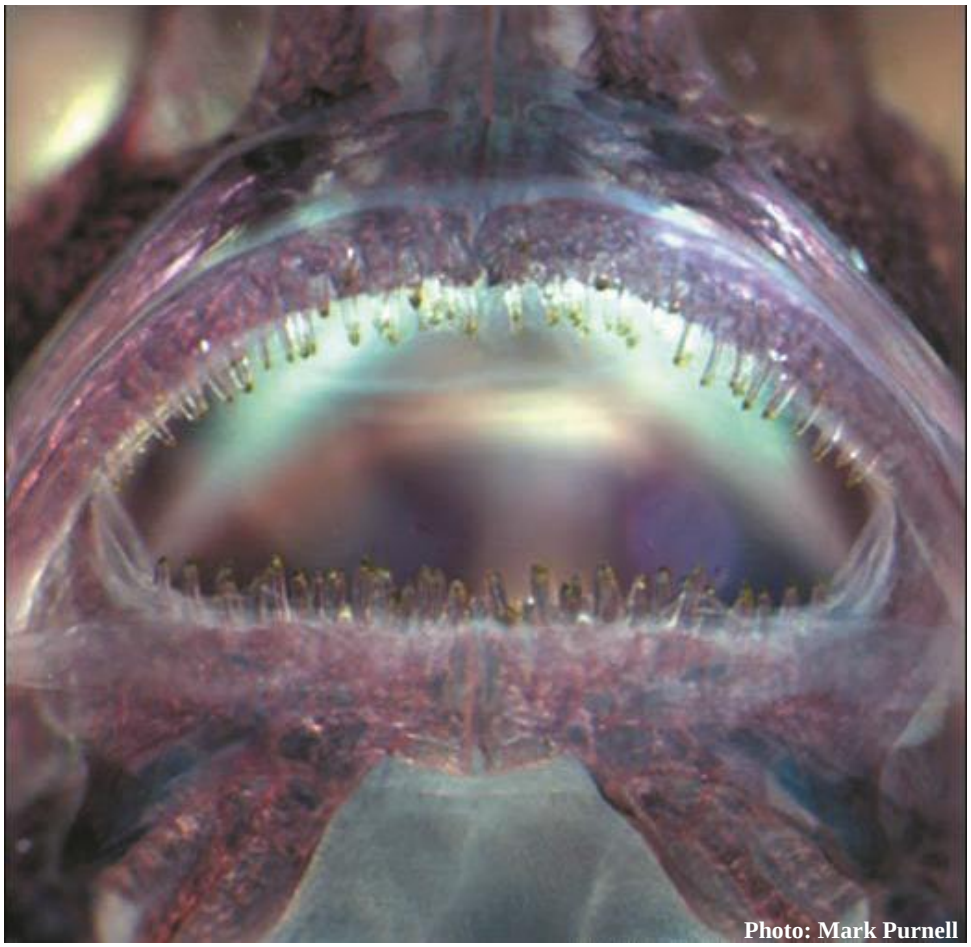
5.4.4. Conclusions

Stickleback morphology is clearly affected by parental habitat of origin (i.e. parental genotype) but is also affected by environmental rearing conditions. The results of this chapter have shown that flow regimes have a consistent and predictable effect on morphology. However, the extent that flow regime affects a population varies and is probably a result of the geographical and genetic isolation of those populations. Plasticity is considered to be more beneficial in environments which are spatially or temporally heterogeneous (reviewed in Alpert & Simms 2002) whereas strong divergent directional selection in isolated populations is more likely to drive evolutionary adaptations for specific traits (Bernatchez *et al.* 1999; Lu & Bernatchez 1999). Plasticity may therefore signify an adaptive response that has evolved in response to environmental heterogeneity (Waddington 1960; Scheiner 1993; Pigliucci 2001) where the degree of plasticity in a population represents the extent of that heterogeneity.

In summary, the extent to which plasticity or fixed genetic effects influence morphology is likely to be mediated by a combination of several factors including the degree of environmental heterogeneity, the strength of selection in each population, the proportion of phenotypic variation of the trait that is genetically determined, the heritability of the trait and migration rates (Donohue 2009).

Chapter 6

Foraging in the three-spined stickleback: does trophic morphology affect prey preference and handling time efficiency?



6.1. INTRODUCTION

Feeding is one of the most critical activities in the daily life of all animals. For small bodied animals with high metabolic rates, for example the common shrew, *Sorex araneus*, forgoing feeding for even a few hours can quickly lead to death by starvation (Vogel 1976). Feeding is associated with a suite of behaviours including searching for food and deciding whether or not to accept it, as well as handling and ingesting it. Strategies for accepting or rejecting food items are based on a compromise between the benefits derived from the food consumed and the costs associated with that strategy, which are expressed in terms of a common currency (Hart 1993).

One such currency commonly employed for explaining foraging behaviours is units of energy and a theory of optimal foraging was developed using fish to explain and predict diet choices (Werner & Hall 1974). One of the predictions that the theory makes is that organisms forage in a way that maximises their net energy intake per unit of time. Under this prediction, certain types of prey may be ignored if the time taken to consume them could be better spent searching for more energetically profitable prey. The profitability of any given prey is therefore influenced by two additional factors: prey abundance, which will affect time spent searching for food, and prey handling time, the period during which a predator consumes its prey and is therefore unable to search for other prey items. Predation risk can also have a profound effect on fish foraging habits and risk-sensitive foragers may shift to different prey types even if by doing so reduces their overall energy intake (Ibrahim & Huntingford 1989c).

Animals can be specialists or generalists in their use of resources. Fundamentally, specialists use a narrow range of resources whereas generalists use a wide range of resources, although individual species typically lie along a continuum between extremes (e.g. Woo *et al.* 2008). Diet specialisation is most likely to occur

when there is competition for resources (Svanback & Persson 2004) and when resources are predictable or homogeneous (Terraube *et al.* 2010). Specialists should therefore, be more efficient feeders on their preferred prey. Generalists, on the other hand, have greater foraging success if the main prey becomes scarce, or if novel profitable prey become available (Terraube *et al.* 2010).

Three-spined sticklebacks, *Gasterosteus aculeatus* are opportunistic feeders; that is, they will eat whatever food is locally available at the time. Studies of stomach content analysis have shown that feeding is largely influenced by prey availability, which itself is strongly influenced by season (for a summary, see Hart & Gill 1994). One of the key predictions that the theory of optimal foraging makes is that, as the preferred prey becomes scarce, individuals should add new prey types to their diet; this is particularly the case if these alternative prey items are more abundant (Visser 1982). Similarly, increases in population density can lead to changes in diet composition due to competition for resources between individuals (Svanback & Bolnick 2007; Sharma & Borgstrøm 2008).

Numerous studies have shown that in polymorphic populations of fish, intra-specific variation in trophic morphology is associated with differences in prey type availability and abundance in different habitats (Gross & Anderson 1984; Lavin & McPhail 1986; Adams *et al.* 2003; Hellig *et al.* 2010). For example, arctic charr, *Salvelinus alpinus*, which in many lakes exist as benthic or pelagic foraging specialists (Jonsson & Skulason 2000), show prey preference that are associated with head morphology (Malmquist 1992; Garduno-Paz & Adams 2010). In laboratory experiments of prey choice, individuals with slender and fusiform bodies and relatively large eyes foraged primarily on pelagic prey whereas those with robust and blunter heads, and bigger mouths were more likely to forage on benthic prey.

Similarly, in a small number of lakes in British Columbia, three-spined sticklebacks coexist as sympatric benthic and limnetic morphs, so called because their morphological divergence, and is related almost completely to their foraging habitat (McPhail 1994). Whereas benthic individuals are specialised for foraging in the littoral zone with deep bodies, wide mouths and a small number of short gill rakers, limnetic sticklebacks are better suited to foraging in the water column, having more slender bodies, numerous and long gill rakers and a narrow, upturned snout (Schluter 1993). In sticklebacks, gill raker length and number has been strongly associated with foraging efficiency (Bentzen & McPhail 1984; Lavin & McPhail 1985; Schluter 1993). Gill rakers are bony protrusions that project from the inner edge of the brachial gill arches (Bell & Foster 1994). They serve as a sieve to prevent the loss of prey from the bucco-pharyngeal chamber during feeding. Whereas a few, short gill rakers are suitable for foraging on large macroinvertebrates, numerous, long gill rakers are required for preventing the loss of smaller zooplankton prey (Gross & Anderson 1984; Schluter 1993).

Differences in trophic morphology are retained in laboratory reared crosses between benthic and limnetic morphs, with F_1 hybrids showing an intermediate phenotype, suggesting an underlying genetic basis to the divergence of the two forms (McPhail 1992). However, diet reversal experiments have shown that there is also a degree of morphological plasticity, particularly for trophic traits and more so in the limnetic morph (Day *et al.* 1994). Greater plasticity in the limnetic morph could evolve because it also forages in the littoral habitat during the breeding season and so is exposed to greater variation in prey type.

Sympatric divergence in the benthic-limnetic stickleback pairs is a special case and only seen in six small lakes in British Columbia. However, similar patterns of trophic morphological divergence are also apparent among different habitats, including lake-stream pairs (McKinnon & Rundle 2002). Research on the diet of lake-dwelling and stream-dwelling stickleback suggests that although both consume a mixture of benthic invertebrates and zooplankton, lake sticklebacks consume more zooplankton and less benthic prey than do stream sticklebacks (Gross & Anderson 1984). As expected from their diet, sticklebacks from lakes consistently show fine, closely spaced and long gill rakers whereas stream sticklebacks typically have relatively few short gill rakers (Moodie 1972a, 1972b; Reimchen *et al.* 1985; Lavin & McPhail 1986; Hendry *et al.* 2002).

Trophic specialisation allows fish to maximise their foraging efficiency on commonly encountered prey, and hence leads to an optimisation of their growth rates. In a reciprocal transfer experiment by Scharsack *et al.* (2007), lake sticklebacks transferred to rivers showed decreased growth when compared to those that remained in lakes and also grew less than native river fish. In contrast, river sticklebacks transferred to lakes grew at the same rate as those that remained in rivers. Furthermore, the decreased growth of river fish in their non-native habitat was less than it was for lake fish in their non native habitat. Together, this strongly suggests specialisation of the lake habitat by lake-resident fish. What is less clear however is whether sticklebacks that show habitat-specific morphological specialisation make foraging decisions that are associated with these specialisations.

6.1.1. Aims

I have previously shown that sticklebacks from Thornton reservoir and its inflowing stream exhibit trophic morphologies that are expected to be suitable for a lake-type and stream habitat, respectively (Chapter 2). Using fish caught from the Thornton reservoir-stream system, the aim of the current study was to test the hypothesis that divergence in morphology has been in tandem with divergence in foraging behaviours, which are associated with habitat of capture.

First, I assessed whether sticklebacks from either habitat showed a preference for typically 'benthic' or 'pelagic' prey items. Due to the mixture of both littoral and pelagic habitats within Thornton reservoir compared to the much more consistent shallow, flowing water conditions of the stream (see section 2.2.1.7 for details); I expected that reservoir-caught sticklebacks would show greater variation in their preferences when compared to stream-caught sticklebacks.

Secondly, I tested the handling efficiency of fish on both prey types. Handling time was measured as the time from initial strike to completion of ingestion, signalled by the cessation of buccal movements (Mackney & Hughes 1995) and is partially dictated by the speed at which prey can be captured and consumed. Previous research has suggested that lake-dwelling sticklebacks show specialisations towards prey resources typically found in lake habitats (Scharsack *et al.* 2007). However, greater habitat variation in the reservoir compared to the stream is likely to be reflected in the prey type available as well. As such, I expected to observe greater specialisation (shorter handling times) by stream-caught sticklebacks towards benthic prey when compared to fish from the reservoir. However, based on the assumption that sticklebacks from the reservoir were exposed to a wider variety of prey types, I

expected that they would show better handling efficiency of zooplankton prey than fish from the stream.

Finally, I tested to see if the individuals in this study showed habitat-specific morphological differences, specifically those which are related to feeding and whether it was possible to relate foraging decisions to these morphological differences.

6.2. METHODS

6.2.1. Fish supply and husbandry

Adult three-spined sticklebacks were caught from Thornton reservoir and its inflowing stream during December 2009 (see section 2.2.1.7 for site details) using unbaited minnow traps (5 x 5mm mesh) left overnight and hand nets (1 x 1mm mesh). On return to the University of Leicester, fish were housed in groups in stock aquaria, which were set up with a sandy substratum, a plastic plant and a bio-filter. They were held for 36h without feeding, prior to testing. The photoperiod was held constant (10L:14D) and temperature set to 8°C throughout.

Prey used in the experiment were chosen for contrasting swimming behaviour and the environment that they are most commonly found in. *Daphnia* sp. are small, planktonic crustaceans found commonly in lakes and pools whereas bloodworm, (*Chironomus* sp. larvae) are primarily benthic dwelling invertebrates.

6.2.2. Experiment 1: Prey preferences

6.2.2.1. Prey choice aquarium set-up

The rear one-third of a 14-L tank was partitioned off using white plastic with a trap-door that could be opened by the researcher using a pulley without disturbing fish. A plastic plant was placed in the partitioned section for cover. Two clear, plastic

containers (approximately 5cm high and 1.5cm wide) were used to hold prey at the front of the tank. A small volume of sand was added to each one and then topped with water. *Daphnia* sp. were added to one and live bloodworm to the other. Each was sealed using Parafilm and placed 5cm from the front of the tank, equidistant from the centre of the trapdoor (Fig. 6.1). The tank was surrounded by sheets of laminated brown paper to minimise external disturbances and an air stone was used to maintain oxygen levels when the tank was not in use. A screen was erected in front of the tank behind which a camcorder (Sony DCR-TRV320E) was set up to record the activity of the fish during the trials.

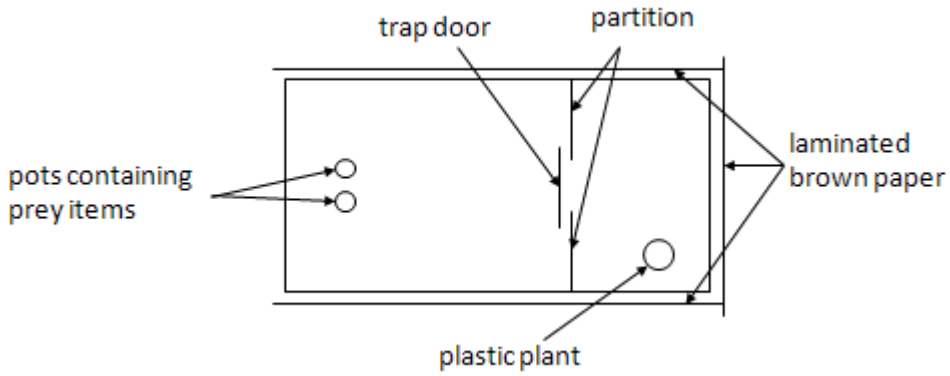


Figure 6.1 Schematic view of the prey choice aquarium viewed from above

6.6.2.2. Experimental procedure

Fish were placed in the partitioned section of the non-feeding aquarium (see Fig. 6.1) with the trap door closed and allowed to settle for 10 mins, after which, recording commenced and the trap door was opened using a pulley.

The first container to be approached and a strike directed towards was recorded as the test fish's initial preference. Hand tally counters were used to count the total number of strikes directed towards the *Daphnia*- and bloodworm-filled containers in 3 minutes, timed from the initial strike. The containers were rotated between trials of successive fish although the contents remained constant. Filming was halted at the end

of each trial. Individuals that did not respond after 10 mins were removed from the study. At the end of the preference trial, fish were removed and housed individually.

6.2.3. Experiment 2: Foraging efficiency

6.2.3.1. Feeding aquarium set-up

A large glass cylindrical divider (diameter 20cm) was placed in the centre of the aquarium, with enough room around the outside to allow fish to move freely around it. The area enclosed by the divider was used as the feeding arena for the test fish.

Additional food and stimulus fish were placed in the tank outside of the central divider to encourage the focal fish to feed and to minimise stress. At the rear of the tank, a mirror was suspended using clamps at a 45° angle (Fig. 6.2), to allow an aerial view of feeding sticklebacks without disturbing the fish. The whole aquarium was placed on top of a sheet of laminated white paper. The water was aerated during periods between trials, but the airstone was removed prior to the start of trials. A screen was erected in front of the tank behind which a camcorder was set up to record the activity of the fish during the trials.

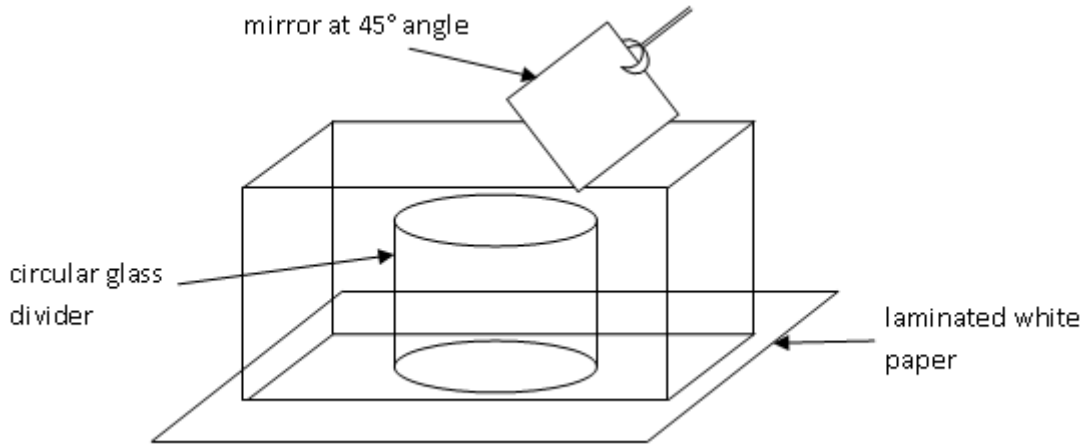


Figure 6.2 Schematic view of the feeding experimental aquarium viewed from the side

6.2.3.2. Experimental procedure

Fish were tested for handling time efficiency the day after prey preferences were tested. Handling time was measured as the time from initial contact with the prey to the cessation of buccal movements or strike at another prey item following ingestion, thus removing the effect of search time. Each fish was randomly assigned bloodworm or *Daphnia* sp. for its initial test. Prey were size-matched across trials. Fish were placed into the central section of the feeding aquarium (Fig. 6.2) containing 20 items of their assigned prey. Filming commenced immediately and continued for 5 mins, after the focal fish had initiated feeding, or had finished all of the prey items. Fish were filmed handling and consuming prey aerially from behind the screen by directing the camera at the angled mirror. On a second handling trial the following day, fish were tested in exactly the same way, but with the alternative prey item.

At the end of the study, all fish were sacrificed using an overdose of Benzocaine anaesthetic and fixed in 10% neutral buffered formalin. They were then processed for morphological investigation using the standard protocol detailed in section 2.2.3.

6.2.4. Data analysis

6.2.4.1. Morphological analysis

Morphological data (both linear measurements and landmark data) were analysed as described in section 2.2.3. However, several additional traits were also measured: jaw length (length between the posterior edge of the angular and anterior tip of the upper jaw), head depth (supraoccipital notch immediately lateral to the dorsal midline to the bottom of the head, perpendicular to the lateral line) and head length (length between the anterior tip of the upper jaw and the posteriodorsal edge of the

operculum). All three of the additional measurements correlated with standard length and so were compared between sites using a standard multivariate ANCOVA.

6.2.4.2. Handling time analysis

Handling time was strongly correlated with fish standard length, so all analyses required fish length to be included as a covariate. Handling times were non-normally distributed, so were first subjected to log transformation. Differences in the handling time of *Daphnia* sp. and bloodworm between fish caught in the reservoir and those caught in the stream were investigated using an ANCOVA. A paired samples t-test was used to investigate if the individuals from either habitat type showed differences in their handling times of bloodworm and *Daphnia* sp.

Handling time data for bloodworm and *Daphnia* sp. were individually regressed onto measures of trophically relevant morphology (SL, body width, body depth, jaw angle, jaw length, head length, mouth width, number of gill rakers and length of the gill rakers) and habitat (reservoir or stream) using stepwise regression to find the best fitting model that could predict handling times. Data were also regressed onto significant relative warp scores to investigate whether overall shape differences could reliably predict the time taken to consume prey items.

A related samples Wilcoxon signed rank test was used to investigate if there were differences within habitat types in the total number of bloodworm and *Daphnia* sp. consumed, and also the number of each prey type rejected (as a proportion of the total number consumed). Mann-Whitney U tests were used to assess whether there were differences between fish from the reservoir and those for the stream in the total number of each prey type consumed and also for the number of each prey type rejected (as a proportion of the total number consumed).

6.2.4.3. Preference data

The prey item attacked first (initial preference) was compared between fish from different habitats using a chi-squared test. Overall preference for prey type was assessed and compared between habitats using the proportion of bites directed towards bloodworm. Proportional data was arcsine transformed and tested using an independent samples t-test. A one sample binomial test was used to test for significant differences within each habitat in the number of fish that preferred bloodworm or *Daphnia* sp.

6.3. RESULTS

6.3.1. Morphological analyses

Consistent with previous findings (Chapter 2) morphological differences between the test fish from the stream and the reservoir were apparent in several of the traits measured. Stream fish were wider ($F_{1,50} = 5.507$, $p = 0.023$) deeper bodied ($F_{1,50} = 9.359$, $p = 0.004$), had wider mouths ($F_{1,50} = 53.065$, $p < 0.001$) and longer heads ($F_{1,50} = 6.795$, $p = 0.012$) than reservoir fish (Figs. 6.3a-d). Stream fish also had a larger jaw angle and hence a more forward pointing snout ($U = 106.0$, $N_R = 30$, $N_S = 24$, $p < 0.001$) although there was no significant difference in jaw length ($F_{1,50} = 0.438$, $p = 0.511$) (Figs. 6.3e-f). There was a significant interaction was between standard length (SL) and habitat in gill raker length ($F_{1,50} = 7.002$, $p = 0.011$). Length of the gill rakers were similar in reservoir and stream fish at a small SL, but with increasing SL, length increased much quicker in reservoir-caught fish than it did in stream-caught fish (Fig. 6.3g). There was also a significant interaction between SL and habitat in spine length so that at smaller sizes stream fish had longer spines than reservoir fish but at larger sizes reservoir fish had longer spines than stream fish ($F[\text{DS1}]_{1,50} = 4.340$, $p = 0.042$; $F[\text{DS2}]_{1,50} = 6.727$, $p = 0.012$; $F[\text{PS}]_{1,50} = 6.759$, $p = 0.012$) (Figs. 6.3h-j).

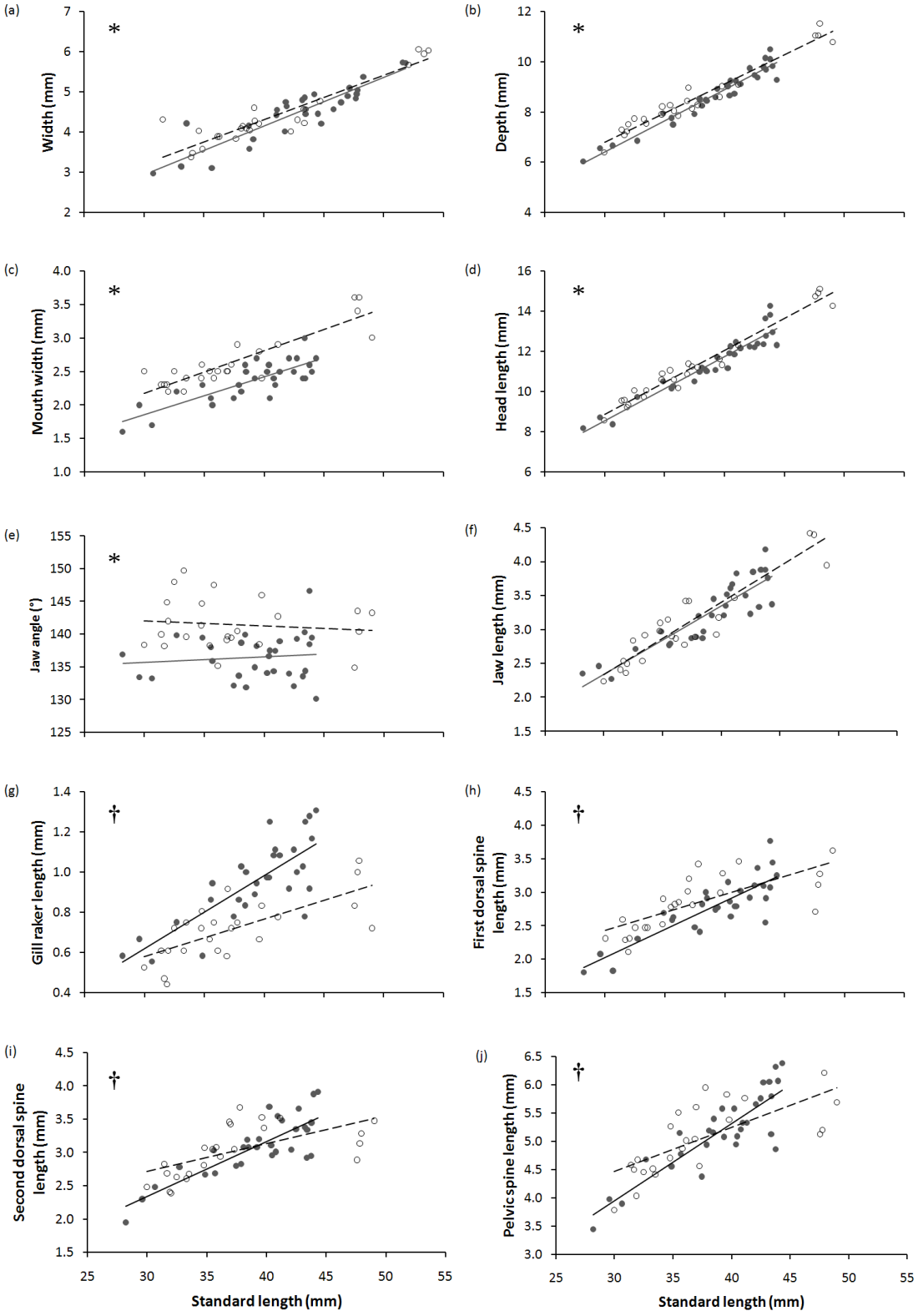


Figure 6.3 The relationship between standard length (SL) and (a) width, (b) body depth, (c) mouth width, (d) head length, (e) jaw angle (f), jaw length, (g) gill raker length, (h) first dorsal spine length, (i) second dorsal spine length and (j) second dorsal spine length in sticklebacks collected from Thornton reservoir (—●—) and the stream (-- ○ --). * indicate a significant difference at the 5% level. † indicates a significant interaction.

There were significant reservoir/stream site differences in 2 of the 26 principal components of shape (relative warps; RW). Although the first relative warp explained 31.15% of the variation, it was not significant ($p = 0.088$) and was related to bending associated to the position of the specimen when it was photographed. RW3 and RW4 however were able to differentiate between fish from the reservoir and those from the stream and accounted for 11.31% ($p < 0.001$) and 11.02% ($p = 0.008$) of shape variation, respectively (Fig. 6.4).

RW3 was related to head length, body depth, ventral fin placement and length and depth of the caudal peduncle. Individuals with increasingly positive scores showed a narrowing of the body, a slight anterior displacement of the ventral fin, a decrease in the overall length of both the dorsal and ventral fins and a shorter, less deep caudal peduncle. In addition, a positive score was associated with a decrease in the angle of the jaw, causing a more upturned snout. These results are consistent with the morphology of reservoir sticklebacks. RW4 was related to length of the lower jaw, ectocoracoid length, length of the ventral fin and head size. Individuals with increasingly positive scores had a smaller head and smaller jaw length, in addition to a longer ectocoracoid and shorter ventral fin.

One canonical discriminant function was calculated to predict habitat membership; which was statistically significant and could explain 100% of the variation (correlation = 0.833, χ^2 (26) = 46.1, $p = 0.009$). The discriminant function was correctly able to predict habitat membership for 96.3% of the samples.

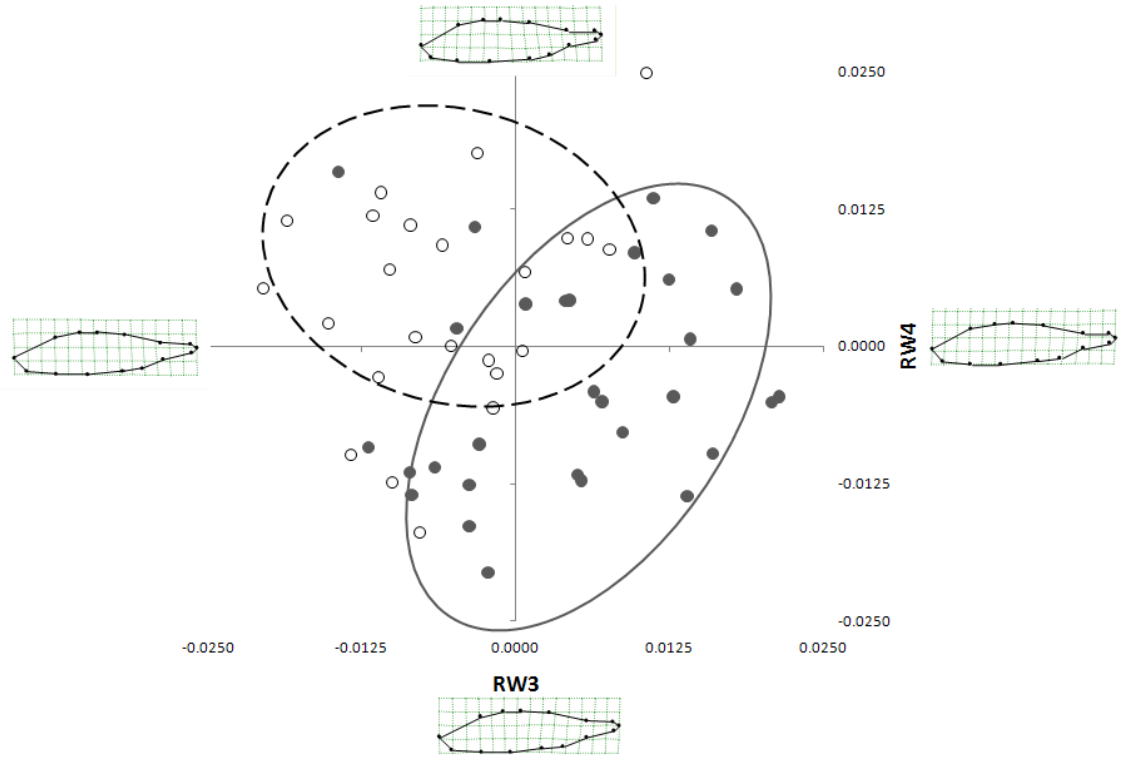


Figure 6.4 Plot of the relative warp (RW) scores significantly differentiating reservoir (filled circles) and stream (open circles) sticklebacks used in prey choice and handling experiments from the **Thornton** system with putative clusters. Deformations associated with the minimum and maximum relative warp scores for each axis are given.

6.3.2. Experiment 1: Prey preferences

Reservoir- and stream-caught fish did not differ in their initial ($\chi^2 = 0.054$, $df = 1$, $p = 0.816$) or overall ($t = 0.381$, $df = 52$, $p = 0.705$) preference towards either bloodworm or *Daphnia* sp. (Fig. 6.5). There was an initial preference for bloodworm over *Daphnia* sp. by fish both from the reservoir (binomial test, $N_B = 28$, $N_D = 2$, $p < 0.001$) and the stream (binomial test, $N_B = 22$, $N_D = 2$, $p < 0.001$). However no differences were detected in the overall preference for either reservoir-caught fish (binomial test, $N_B = 20$, $N_D = 10$, $p > 0.05$) or stream-caught fish (binomial test, $N_B = 17$, $N_D = 7$, $p > 0.05$).

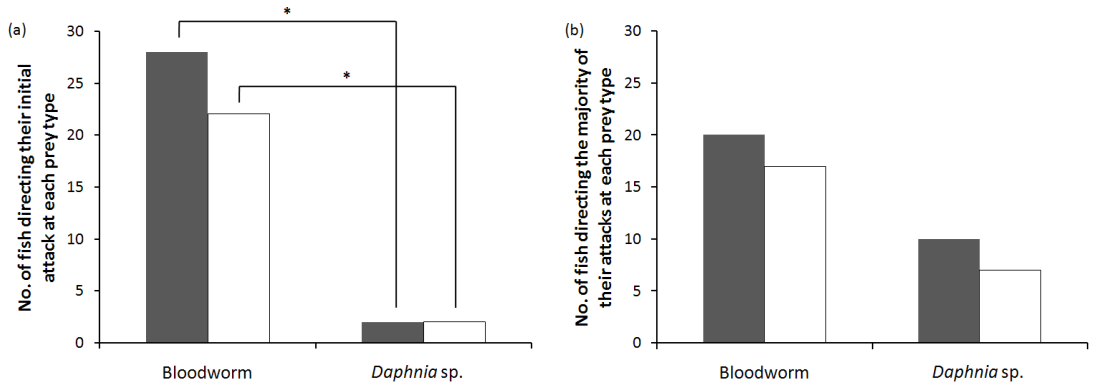


Figure 6.5 The number of stickleback from the reservoir (filled bars) and the stream (open bars) showing a preference towards bloodworm and *Daphnia* sp. (a) gives the initial preference based on the first prey type to be attacked whereas (b) gives the overall preference based on the prey type that was attacked the most. * indicates a significant difference at the 5% level.

6.3.3. Experiment 2: Foraging efficiency

There were no differences between reservoir and stream stickleback in the time it took to handle either bloodworm ($F_{1,35} = 1.12$, $p = 0.298$) or *Daphnia* sp. ($F_{1,35} = 2.55$, $p = 0.120$). However, there was a significant effect of fish standard length (SL) whereby larger individuals were significantly quicker at handling both bloodworm ($F_{1,35} = 68.7$, $p < 0.001$) and *Daphnia* sp. ($F_{1,35} = 37.7$, $p < 0.001$) (Fig 6.6).

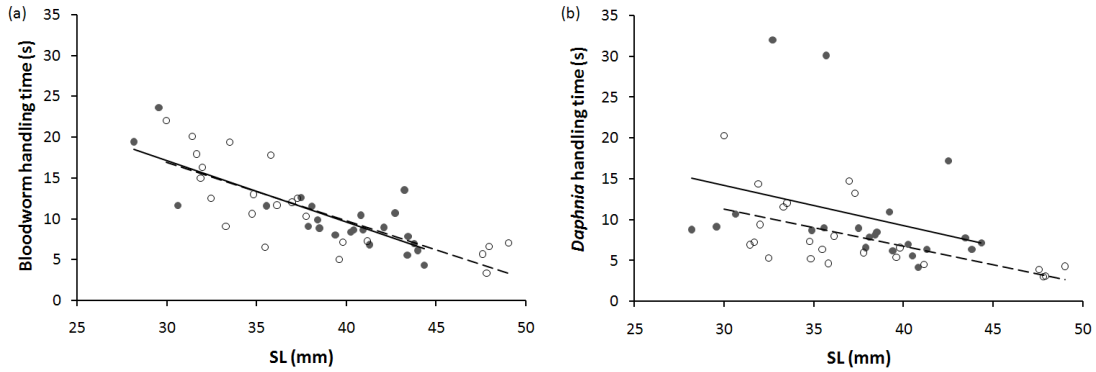


Figure 6.6 The relationship between standard length and the handling times for (a) bloodworm and (b) *Daphnia* sp. for reservoir (—●—) and stream (-- ○--) sticklebacks.

Using a stepwise regression model, the best fitting model to predict bloodworm handling times used only information from an individual's SL ($F_{1,44} = 67.3$, $p < 0.001$, adjusted $R^2 = 0.596$). *Daphnia* sp. handling times were best predicted by an

individual's body depth ($F_{1,43} = 38.7$, $p < 0.001$, $R^2 = 0.461$) although the model was significantly improved by including information about the number on gill rakers and their length (Table 6.1). Neither RW3 nor RW4 were able to reliably predict handling times.

Table 6.1 Model summary of morphological traits significantly able to predict the handling time of sticklebacks feeding on *Daphnia* sp.

Predictor(s)	Adjusted R^2	R^2 change	F Change	df1	df2	Sig. F Change
1. Depth	0.461	0.474	38.7	1	43	0.000
2. Depth, Gill raker number	0.519	0.068	6.20	1	42	0.017
3. Depth, Gill raker number, Gill raker length	0.556	0.045	4.48	1	41	0.040

Sticklebacks from the reservoir consumed more bloodworm during a trial than did fish from the stream ($U = 364.5$, $N_R = 23$, $N_S = 23$, $p = 0.016$) but there was no significant difference in the number of *Daphnia* sp. taken between sites ($U = 189$, $N_R = 22$, $N_S = 23$, $p = 0.134$) (Fig. 6.7a). Stream fish consumed significantly more *Daphnia* sp. than they did bloodworm ($Z = 2.98$, $M_B = 16$, $M_D = 16$, $p = 0.003$), but there was no difference in the number of bloodworm and *Daphnia* sp. consumed by reservoir fish ($Z = -1.27$, $M_B = 20$, $M_D = 18$, $p = 0.206$). Analysis of rejection data showed that the number of prey items rejected (as a proportion of the total number consumed) were greater for *Daphnia* sp. than they were for bloodworm, in both reservoir ($Z = 2.20$, $M_B = 0.05$, $M_D = 0.5$, $p = 0.028$) and stream ($Z = 2.78$, $M_B = 0$, $M_D = 0.15$ $p = 0.005$) sticklebacks (Fig. 6.7b). However, there were no significant differences between fish from different habitats in the proportion of rejected bloodworm ($U = 317.5$, $N_R = 23$, $N_S = 23$, $p = 0.217$) or *Daphnia* sp. ($U = 324.0$, $N_R = 21$, $N_S = 23$, $p = 0.051$).

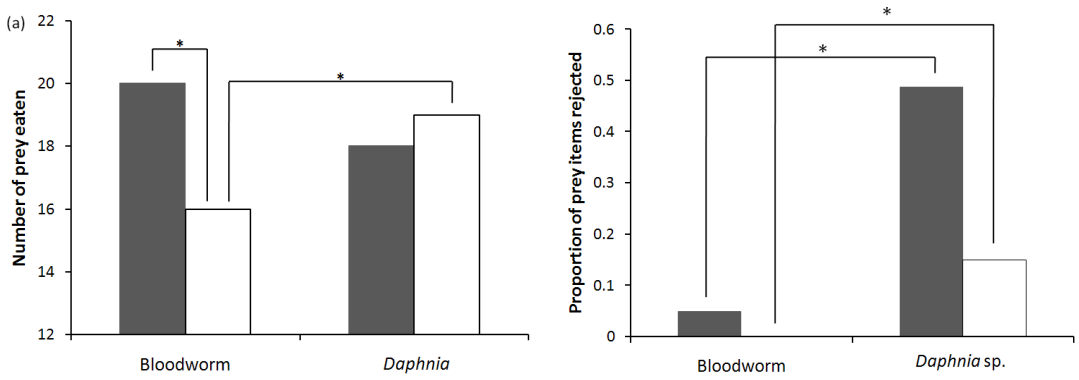


Figure 6.7 (a) Median number of each prey type eaten and (b) median proportion of each prey type rejected during a 5 minute feeding bout by sticklebacks from the reservoir (filled bars) and the stream (open bars). * indicates a significant difference at the 5% level.

6.4. DISCUSSION

6.4.1. Effect of morphology on prey handling times

Individuals in this study showed a pattern of morphological differences that would be expected under adaptive evolution (Reimchen *et al.* 1985; Lavin & McPhail 1986; Hendry *et al.* 2002) with stream fish having deeper bodies, wider mouths, longer heads, a more forward pointing snout and fewer gill rakers when compared to reservoir fish. However, whereas in earlier studies, results have indicated that variation in trophic morphology can produce differences in foraging success (Lavin & McPhail 1986; Berner *et al.* 2008), none of the measures in the current study were able to reliably predict differences in handling efficiency. In general, the time taken to ingest a single prey item (the handling time) of bloodworm was longer than it was for *Daphnia* sp., however handling times did not differ between sites. Instead, they were related closely to the size of the individual, with larger fish ingesting both types of prey quicker.

Gill and Hart (1994) have suggested that the handling times are most likely to differ between individuals and prey types when the probability of capture is low, which corresponds to a situation where the ratio of prey width to predator mouth width is 0.6

or greater. Thus, a predator handling a prey item that has a width 60% or less than its own mouth will nearly always result in successful consumption. If this is the case, it is possible that the size of prey used in this study were too small to reveal a difference between sites, even though mouth width differed between sites.

Associated with this, divergence in trophic morphology may not have been sufficient to cause a noticeable effect on handling time. If juvenile sticklebacks were used instead of fully grown adults, or more widely differing prey (for example, *Gammarus* sp. and *Artemia* sp.), the constraints imposed by trophic morphology may have been more apparent and lead to greater differences in handling times.

6.4.2. Variation in prey handling time

Numerous studies have shown that hungry fish eat quicker and that handling times increase with satiation (Tugendhat 1960; Thomas *et al.* 1985; Croy & Hughes 1991a, 1991b; Gill & Hart 1994; Gill 2003). However, handling times for small prey are relatively constant during a feeding bout whereas those of larger prey increase with prey sequence (Hirvonen & Ranta 1996). Perhaps because of the smaller size of *Daphnia* sp. compared to bloodworm used in this study, handling times remained relatively low so no differences were observed between individuals. The fact that there were no differences between sites in bloodworm handling times may be the result of the observed greater prey variability in the reservoir.

Three-spined sticklebacks can learn to forage more efficiently with experience and are able to retain learning for up to 25 days and is thought to be an adaptive response to prey availability (Mackney & Hughes 1995). Given that greater variability in prey type was observed in the reservoir than in the stream, sticklebacks caught in the reservoir may have learnt to manipulate both prey types used in the current study with

maximum efficiency. Although in general *Daphnia* sp. are less common in streams, they are not always completely absent and stomach content analyses of stream sticklebacks in northwest England show a mixed diet (Hynes 1950). Thus, it is still possible that stream-caught sticklebacks in the current study also had experience of both prey types.

In arctic charr *Salvenius alpinus,* research has shown that foraging anatomy is phenotypically plastic and that diet variability could be the driver for inducing differences in trophic morphology (Adams *et al.* 2003). Similarly, sticklebacks kept on different diets from an earlier age demonstrate greater plasticity in gape width, gill raker length, snout length and head depth (Day *et al.* 1994) but the same pattern is not observed when fish are maintained on different diets at a later age (Day & McPhail 1996). This suggests that for some traits, morphological plasticity and adaptations may be possible early in life but less so as an individual matures. A recent study investigating ontogenetic trends in body shape suggests that sticklebacks in lakes and rivers show differential growth based on habitat, interpreted as developmental plasticity (Spoljaric & Reimchen 2011). It is possible therefore, that fish that have completed ontogenetic development have lost the ability to be plastic once fully matured. Thus, differences in prey consumption early in development may beget morphological differences but learning and behavioural plasticity may negate differences in handling efficiency.

Prey taken into the buccal cavity were frequently rejected several times before being consumed and, on some occasion, rejected all together. Spitting is used to get prey into the desired orientation, which is usually head-first (Croy & Hughes 1991a; Gill & Hart 1994). Whereas the number of *Daphnia* ap. rejected did not differ between reservoir and stream-caught fish, bloodworm were rejected more often by sticklebacks

from the reservoir. However, they also consumed more larvae overall during the 5min feeding bout. The total number of prey consumed were not, in general, limited by the time. Fish were rarely removed from the feeding chamber whilst they were still feeding and variation in prey eaten was probably due to satiation. This suggests that reservoir fish were rejecting prey to manipulate it so it could be eaten with greater ease. Although this is contrary to my initial prediction, it is not completely unexpected. A stream fish manipulating a benthic invertebrate taken from the benthos is liable to lose its prey if it is spat out into the flowing water. A potentially better approach might be to manipulate the prey whilst it remains in the mouth. This might also explain the slightly longer (but non-significant) bloodworm handling time observed for stream fish. A stream fish may also be better experienced at attacking prey head on at the first strike, again requiring fewer spits. Although not statistically significant, stream fish were on average, slightly smaller than their reservoir-dwelling counterparts and may have reach satiation quicker than reservoir fish.

6.4.3. Preferences for different prey types

Sticklebacks from the Thornton reservoir-stream system did not show any differential preference towards either a typically benthic (bloodworm) or pelagic (*Daphnia* sp.*)* prey item; in fact, fish from both habitats appeared to prefer bloodworm. Sticklebacks are predominantly visual feeders (Wootton 1976) and therefore visual cues to expected to play a major role in prey detection. Prey in the current study were presented in clear, plastic pots so that non-visual cues and other factors such as palatability or stomach fullness did not impact on the 'choice' made. Sticklebacks eat their prey whole and so the relative size of prey is likely to be fundamental to understanding prey choices. Most animals are restrained in what they can physically

eat by their morphology. This is either because they must have the strength, speed and agility to catch and kill their prey so it can be consumed piece by piece, or, as a rule of thumb for predators that eat their prey whole, the widest part of their prey should be smaller than their maximum gape size. Given that fish in this experiment were not permitted the opportunity to take any of the prey, they had no way of otherwise knowing for certain whether or not it was physically possible to consume an item. Fish need touch to recognise prey and tactile stimulation is required for acceptance or rejection of prey (Hart & Gill 1992).

Earlier studies have shown that sticklebacks are able to learn to forage more efficiently with increased exposure (Croy & Hughes 1991a; Ibrahim & Huntingford 1992). The fish in this experiment were wild-caught and were maintained on diets which did not include the test prey, hence any learned behaviours were from their site of capture. Hungry fish will feed on large prey items even though they may have a longer handling time, becoming more selective and switching to smaller prey with a greater energetic gain per unit time as they fill up (Hart & Ison 1991).

Under the Basic Prey Model, diet is determined by prey abundance, energetic content and handling time (Stephen & Krebs 1986). In a study of simultaneously presented prey of different size classes on bluegill sunfish *Lepomis macrochirus*, prey of all sizes were eaten without discrimination at low abundances (Werner & Hall 1974). However, as the abundance of prey increased, size classes were sequentially dropped from the diet, which the authors claim was in accordance with the model. Based on this principle, it is perhaps not surprising that when presented simultaneously with an abundance of two prey types, sticklebacks tended towards preferring the larger prey (size here being used as a visual indicator of energetic gain), regardless of whether or not they might have been physically restrained from being able to take it. This is also

under the assumption that bloodworm provide the most energetic gain per unit time (Wissing & Hasler 1971).

Three-spined sticklebacks tend to show a preference for prey which are red, fast moving, straight shaped and larger than the alternative (Ibrahim & Huntingford 1989a, 1989b). If sticklebacks are choosing prey based on the preferred stimulus (colour, shape or movement etc.), then the expected response would be one where bloodworm were preferred over *Daphnia* sp. However, previous experience of morphological constraints from their habitat may have had confounding effects on the results leading to the non significant mixed responses obtained here.

6.4.4. Conclusions

In summary, although three-spined sticklebacks from Thornton reservoir and its inflowing stream show differences in trophic morphology which are consistent with earlier lake-stream studies, they do not affect the behavioural measures of prey choice, acceptance and handling employed in this study. It is possible that if other measures of foraging were also included such as prey search time or if several benthic and pelagic prey types were tested instead of just the two used here, differences may have resulted. Additionally, Thornton reservoir was only 154 years old at the time these fish were caught. Studies looking at trophic and associated behavioural divergence are mostly based on populations that have been separated for many hundreds of years (freshwater populations compared to anadromous ones and ancient lake fish compared to stream fish) or show greater morphological differences due to intense competition (benthic compared to limnetic). Thus, although there are differences in the feeding apparatus of sticklebacks, they may need more time to continue adapting before a noticeable effect can be observed in laboratory-based experiments of behaviour.

Chapter 7

Growth rate of three-spined stickleback in a reservoir-stream system

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7.1. INTRODUCTION

The growth and development of an animal is a complex physiological process that begins the moment an egg is fertilised by a sperm. Growth rate is a measure of the change in size as a function of time and the growth of most fish is indeterminate, that is, they continue growing even when they have reached sexual maturity (Charnov 1993). The rate of growth is important because body size is associated with various measures of fitness including survival (Hutchings 1994) fecundity (Michaletz 1998; Blaxter, 1969, cited in Bone & Moore 2008) and ability to defend nest sites (van den Berghe & Gross 1989). Differences in the rate of individual development within a species may be influenced and modified by the external environment such as quantity and quality of food (Skalski *et al.* 2005; Amundsen *et al.* 2007), temperature (Allen & Wootton 1982a; Xu *et al.* 2010) and population density (Imre *et al.* 2005, 2010). However there are often also species-specific characteristics suggesting that the rate of development is, at least in part, genetically determined (Bone *et al.* 1995; Jobling 2002).

Studies of growth rates between fish in different environments are not consistent, with some researchers reporting that pond fish grow faster than stream fish in their first year (Baker & Foster 2002) whereas others describing how growth in lake fish is slower than stream fish, virtually stopping during the winter months (Allen & Wootton 1982b). This introduction will begin by discussing evolved differences in life history traits that can affect growth. It will then go on to talk about environment factors that can also affect growth rates, many of which differences are applicable to fluvial and still water habitats.

7.1.1. Evolved differences in life history

7.1.1.1. Age at maturity

The age at which an individual reaches maturity (defined as the onset of first reproduction) is a balance between the costs and benefits of early or delayed reproduction, and is particularly relevant to species with indeterminate growth, which includes most fish (Wootton 1998). If natural selection is assumed to act on age-specific expectations of producing future offspring, age at maturity and reproductive effort can be predicted based on the ratio of adult and juvenile survival rates (Gadgil & Bossert 1970; Schaffer 1974). Reproductive effort is defined as the proportion of resource, which could be time or energy, that that are directed towards reproduction rather than growth maintenance (Bell 1980). In pumpkinseed sunfish *Lepomis gibbosus*, populations that experience severe overwinter kills mature earlier and at a smaller size than those that do not suffer poor overwinter survival (Fox & Keast 1991). Increased reproductive effort and earlier maturation in these populations increase the probability of survival to the reproductive age (Gadgil & Bossert 1970).

In wild three-spined stickleback, *Gasterosteus aculeatus*, most populations live for just over one year, dying shortly after spawning, although some show a maximum life span of up to 4 years (Baker 1994). However, even in longer living populations, there is still variation in the age at maturation with some populations maturing after one year but others not maturing until their second summer (Wootton 1976). The reason for this variation remains unclear, though there is evidence for the maintenance of different growth and maturation age in fish kept in the laboratory (Wund, 1928, cited in Wootton 1976; Wright *et al.* 2004) suggesting an evolved genetic adaptation.

7.1.1.2. Costs of early maturation

However, there are also costs associated with early maturation and reproduction. For iteroparous species (those where some adults in the population survive to reproduce more than once), reproduction often incurs a cost of diminished future survival (Bell 1980). Size of an individual is particularly important around breeding time, more so for females than for males as body size determines several reproductive traits (Wootton 1998). In particular, larger females produce larger clutch sizes (Baker & Foster 2002; Poizat *et al.* 2002). In male sticklebacks, although body size does not appear to affect female mate choice (Head *et al.* 2009), larger males tend to dominate larger territories and so are likely to obtain a greater number of matings (Rowland 1988).

If delaying maturity permits additional growth, then earlier maturing individuals have the additional cost of lower fecundity or fewer matings. Delaying maturity may also indirectly benefit offspring survival through higher parental care (Stearns 1992) and increased ability to defend nest sites (van den Berghe & Gross 1989).

7.1.1.3. Effects of predation on growth rates and age of maturation

Predation too can have dramatic effects on life history differences in growth rates between populations of the same species. Sticklebacks are subjected to attack from a variety of predators including fish and avian piscivores, macroinvertebrates and conspecifics (for a summary, see Reimchen 1994). Predation manipulation experiments have shown that growth in sticklebacks is consistently higher when levels of predation by fish and macroinvertebrates are experimentally increased and is inversely related to survival (Rundle *et al.* 2003).

Different predators can also affect maturation by altering age-specific survival through size-specific prey choice in different populations (Reznick *et al.* 1990). In

guppies *Poecilia reticulate*, predation by pike cichlid *Crenicichla alta* is predominantly on large adults whereas predation by killifish *Rivulus hartii* is primarily on smaller individuals (Reznick & Endler 1982). Guppies experimentally transferred from an area of predation by pike cichlid to killifish, showed reproductive phenotypic differences consistent with a theorised response to predation (Reznick & Endler 1982). Within 8-10 generations, guppies in the introduced site were larger, matured later and produced larger offspring; characteristics which were maintained in common garden rearing experiments (Reznick *et al.* 1990). Populations under predation by killifish tended to reproduce over a longer period, exert less reproductive effort per clutch and were longer lived.

Studies have also shown that heavily predated populations also show greater exploratory boldness than those which are less so (Brown *et al.* 2007a; Archard & Braithwaite 2011; Fraser *et al.* 2011) and that this is related to growth because boldness is likely to lead to increased foraging (Brown *et al.* 2007b). Laboratory studies suggest that boldness is a heritable trait that is maintained even under conditions of low predation (Brown *et al.* 2007a), hence populations evolved under high levels of predation may show increased growth rates even if current levels of predation are low.

7.1.1.4. Timing of maturation and length of the reproductive season

In annual populations, many fish produce several clutches over the breeding season so there is scope for individuals maturing earlier in the season to produce greater numbers of clutches. However, the timing of maturation is critical for maximising lifetime production of offspring so that young are born into an environment in which resources are not a limiting factor (Wootton 1998). Fish living in temperate conditions grow primarily during the spring and summer months with little or no growth during the

winter months (Wootton 1998). A larger body size among young-of-the-year at the end of the summer has been associated with an increased likelihood of overwinter survival in lake herring *Coregonus artedi* (Pangle *et al.* 2004), sand smelt *Atherina boyeri* (Henderson *et al.* 1988) and three-spined stickleback (Allen & Wootton 1982b). Fish are poikilotherms and their growth is strongly dependent on environmental water temperatures (Bone *et al.* 1995). However, studies on the Atlantic silverside *Menidia menidia* have highlighted that there may also be a strong genetic component (Conover 1990; Schultz *et al.* 1998). High latitude environments are usually subject to lower temperatures and/or a shorter growing season. In spite of this, high and low latitude populations of the silverside show no difference in mean size at the end of the growing season even though northern populations spawn later and the growth season is much shorter for northern populations, when compared to southern ones. Rearing experiments have shown that the increased growth rates of the northern populations are maintained under controlled laboratory conditions suggesting a strong underling genetic effect (Conover 1990).

7.1.2. Environmental factors affecting growth

7.1.2.1. Temperature

Water temperatures in the natural environment are hardly ever constant, fluctuating on a daily, weekly, monthly and seasonal basis (Hynes 1979). As ectotherms, muscle metabolic capabilities in aquatic species are strongly influenced by abiotic factors such as temperature. Fluctuations in temperature bring about associated changes in physiological rates so that as temperatures rise, so do the maintenance requirements of tissues and metabolic capacities (Guderley *et al.* 1994). As such, feeding rates for fish are higher at higher temperatures (Guderley & Leroy 2001) and

growth rates increase with temperature (Wootton 1998). In several species, including the three-spined stickleback, fertilisation is external and the rate at which the fertilised egg develops depends on the temperature of the water (Wootton 1976). Stickleback eggs kept at 18-19°C usually hatch 7-9 days after fertilisation. Over the following 3 weeks, the larval characteristics are lost and the fish takes on its adult-like form. Fish kept at lower temperature take longer to hatch and the opposite is also true, however very low (0°C) or very high (33°C) temperatures, even for a short time, can lead to abnormalities (Swarup, 1958, cited in Wootton 1976).

7.1.2.2. Food availability

Seasonal fluctuations in food availability are inevitable (Hynes 1950). Cycling through the seasons brings changes to prey availability and there are associated shifts in prey consumption throughout the year, particularly for opportunistic feeders like stickleback (Allen & Wootton 1984). Sticklebacks from Llyn Frongoch in Mid-Wales show a rate of growth slower than expected based on laboratory experiments and is suggested to reflect a poor food supply (Allen & Wootton 1982b). In experimentally fed populations of brown trout *Salmo trutta*, those receiving higher rations of food matured quicker (Bagenal 1969). However, it is not only the quantity of food that is important but he quality too and fish fed on a low quality diet show a reduced growth (Hofer *et al.* 1985). Thus, growth and maturity are also regulated by food quality and availability. However, daily fluctuations in food seem less important than overall availability as sticklebacks are able to make compensatory changes allowing them to maintain growth (Ali & Wootton 2000).

7.1.2.3. Predation

The primary defence against predators is avoidance – sticklebacks actively avoid feeding in patches where there are predatory fish and reduce risk of detection by hiding in vegetation (Fraser & Huntingford 1986; Ibrahim & Huntingford 1989c). However, one of the side effects of doing so is a decrease in time spent foraging and hence slower growth. Although it has been suggested that fish show evolved behavioural adaptations that may mitigate the effects, such as increased exploratory boldness (e.g. Archard & Braithwaite 2011), behavioural plasticity also allows fish to adapt to current situations (Brown *et al.* 2007b). In particular, boldness traits may shift during ontogeny as a result of life experiences including social interaction and habituation (Magnhagen & Staffan 2005; Oosten *et al.* 2010).

In sticklebacks, small and large individuals have the greatest probability of escaping predation by fish piscivores (Reimchen 1991) hence for very young stickleback, it may pay to hide in the weeds. However, as they become intermediate in size, the best strategy may be a risky one that involves feeding even when predation risks are high so that they can achieve a safer, larger size.

7.1.4. Aims

The aim of the current study was to begin investigating the hypothesis that fish showing associated with their habitat. I addressed this question using fish from the Thornton reservoir-stream system. Lakes are thermally stratified with warmer layers at the surface as a result of heat absorption from the atmosphere, which is in contrast to flowing waters which rarely stratify due to the constant flow (Moss 1988). As a result, water temperatures at the surface of lakes and reservoirs can reach over 20°C in temperate zones.

Given that fish in warmer waters tend to grow faster than those in cooler waters, I expected to see a faster growth rate in reservoir-caught fish. Additionally, if maturation in sticklebacks is also affected by temperature (Borg 1982; Borg & Vanveen 1982), then reproduction in the reservoir is also expected to start ahead of that in the stream. Furthermore, Thornton reservoir is host to a wide range of potential predators (section 2.2.1.7) and as such, reservoir-fish may be likely to mature earlier and grow faster.

7.2. METHODS

Sampling began in June 2009 and initially was undertaken every second month. On realising the importance of collecting data at more regular intervals however, sampling intensified and was carried out monthly from October 2009 onwards, and continued until September 2010.

Fish were sampled from Thornton reservoir (N52°40'01" W1°18'34") and its inflowing stream (N52°40'17" W1°19'01") on the same day using unbaited minnow traps left over night (see section 2.2.1.7 for full site details). Ten traps were deployed at each site in the same location each month – five with a 3 x 3 mm mesh and five with a 5 x 5mm mesh. All fish caught were temporarily transferred to a cool box for photographing later. If fewer than 50 fish were collected using the traps, we attempted to increase the number by additionally netting with hand nets (1 x 1mm mesh). Between March and October, hand nets were also used in both habitats to see if any young-of-the-year sticklebacks could be caught, which would otherwise be able to escape the mesh of the traps.

When all the traps had been collected, fish were digitally photographed in dorsal profile in batches of 10-15 in a white bucket. A ruler was included for size

standardising and a pebble was rotated around the bucket to distinguish between batches of fish (Fig. 7.1). Once photographed, all fish were returned back to the water. Standard length measurements were made using ImageTool v3.0.

Figure 7.1 An example of two digitals image taken in the field, which were later used to determine the length-frequency distribution of sticklebacks caught on the same sampling day. The different position of the pebble indicates that these were two different batches of fish.

7.2.1. Statistical analyses

The Kolmogorov-Smirnov test was used to determine whether there were significant differences in the length-frequency distribution between habitats each month. It was also used to test for differences within habitats between months. All multiple tests were corrected using the false discovery rate control (Verhoeven *et al.* 2005).

To assess the growth rate of only one year class, individuals were assigned as young-of-the-year (YOY) or adults based on how they clustered. The 2009 cohort was used to assess the overall difference in growth rate measured by the increase in SL. Monthly specific growth rates (SGR) were compared between habitats using a one-way ANOVA. SGR was calculated as:

$$\mathbf{SGR = [\ln(SL_2 - SL_1)]}\boldsymbol{T}$$

where SL_1 is the SL in the first month, SL_2 is the SL in the second month and T is the number of days between sampling visits.

7.3. RESULTS

7.3.1. General patterns of growth in reservoirs and streams

Changes in the length-frequency distribution of sticklebacks caught from Thornton reservoir and its inflowing stream between June 2009 and October 2010 are shown in Fig. 7.2 and summarised in Table 7.1. The significance of the differences between successive months for each habitat type is given in Table 7.2.

Table 7.1 Modal ranges and sample sizes for sticklebacks caught from the reservoir and the stream on a single day in a given month

Month	Reservoir modal range(s) (mm)	Reservoir sample size (N_R)	Stream modal range(s) (mm)	Stream sample size (N_S)
June '09	20-22 ; 54-56	45	42-44	157
August '09	38-40	49	16-18 ; 44-46	103
October '09	44-46	67	44-46	76
November '09	46-48	70	46-48	108
December '09	48-50	253	44-46	62
January '10	44-46	191	44-46	62
February '10	48-50	218	46-48	59
March '10	50-52	96	46-48	39
April '10	48-50	347	48-52	49
May '10	50-52	117	42-44	86
June '10	8-10 ; 52-54	100	40-42	433
July '10	28-30 ; 52-54	498	42-46	149
August '10	20-22 ; 40-42	17	8-10 ; 42-44	90
September '10	40-42	53	42-44	43

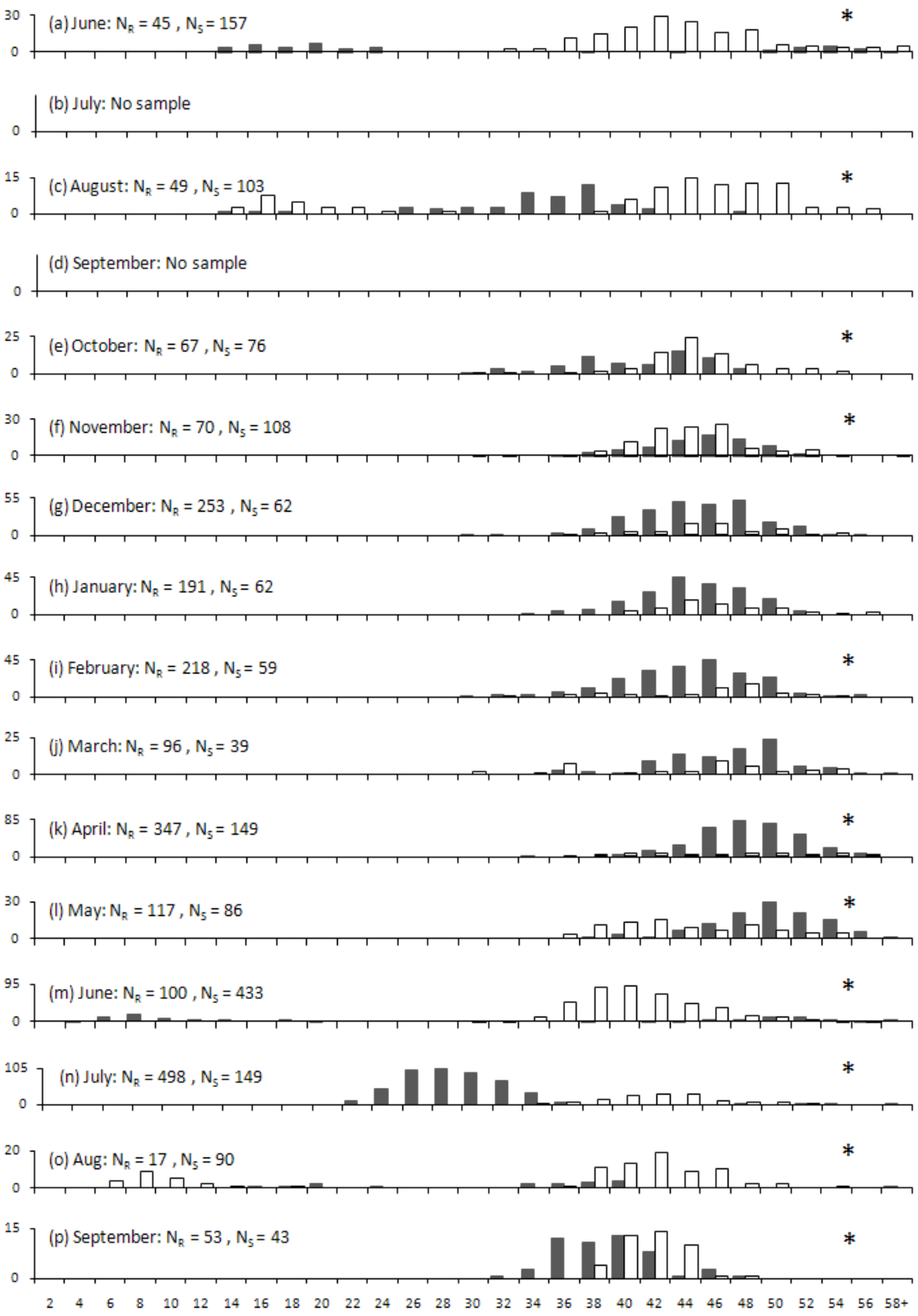

Figure 7.2 Length-frequency distribution of sticklebacks caught on a single day in a given month from June 2009 to September 2010 from Thornton reservoir (filled bars) and its inflowing stream (empty bars). No sampling was undertaken in July and September 2009. * indicates a significance difference between distributions at the 5% level.

Table 7.2 Results from the Kolmogorov-Smirnov test comparing the length-frequency distribution between successive months of sticklebacks caught from the reservoir and those caught from the stream on a single day in a given month.

	Reservoir		Stream	
Months compared	Kolmogorov-Smirnov *D*‡ statistic	p	Kolmogorov-Smirnov *D** statistic	p
June '09/Aug '09	0.56	< 0.001*	0.23	0.002*
Aug '09/Oct '09	0.52	< 0.001*	0.23	0.014*
Oct '09/Nov '09	0.39	< 0.001*	0.13	0.388
Nov '09/Dec '09	0.10	0.657	0.24	0.020*
Dec '09/Jan '10	0.07	0.724	0.81	0.984
Jan '10/Feb '10	0.11	0.162	0.22	0.091
Feb '10/Mar '10	0.31	< 0.001*	0.17	0.456
Mar '10/Apr '10	0.19	0.006*	0.24	0.153
Apr '10/May '10	0.20	0.001*	0.23	0.054
May '10/Jun '10	0.52	< 0.001*	0.31	< 0.001*
Jun '10/Jul '10	0.51	< 0.001*	0.19	< 0.001*
Jul '10/Aug '10	0.62	< 0.001*	0.24	0.002*
Aug '10/Sep '10	0.40	0.025*	0.28	0.014*

‡The *D* statistic is the maximum difference between cumulative distributions
* indicates a significance difference at the 5% level.

7.4.2. Comparing the growth rates of reservoir and stream fish

Results from the Kolmogorov-Smirnov test comparing the length-frequency distribution of reservoir-caught and stream-caught fish over the year are given in Table 7.3. There were significant differences in the length-frequency distribution between fish from the reservoir and those from the stream throughout the majority of the year ($p < 0.05$; Fig. 7.2). The only months where this was not the case were December 2009, January 2010 and March 2010.

In the reservoir, length-frequency distributions showed significant differences ($p < 0.05$) on a month to month basis. The exception to this was through the winter months from November 2009 to February 2010, when the distribution remained constant, reflecting no growth.

The stream however, showed more consistency in size throughout the year. Statistically significant differences between successive sampling efforts were only apparent from June 2009 until October 2009, November 2009 to December 2009 and from May through until September 2010 ($p < 0.05$). For the remaining part of the year (October 2009 to November 2009 and December 2009 until June 2010), the distribution remained constant.

Of note, is that in the 2 months after young were detected in the reservoir (July and August), the number of adults (45mm+) fell to almost none. In the stream however, large adults (50mm+) were observed throughout the year, apart from September 2010.

Table 7.3 Results from the Kolmogorov-Smirnov test comparing the length-frequency distribution between sticklebacks caught from the reservoir with that of those caught from the stream on a single day in a given month.

Month	Kolmogorov-Smirnov *D*‡ statistic	*p*
June '09	0.62	< 0.001*
August '09	0.67	< 0.001*
October '09	0.37	< 0.001*
November '09	0.22	0.032*
December '09	0.12	0.418
January '10	0.11	0.633
February '10	0.21	0.033*
March '10	0.23	0.103
April '10	0.29	0.001*
May '10	0.50	< 0.001*
June '10	0.51	< 0.001*
July '10	0.92	< 0.001*
August '10	0.44	0.005*
September '10	0.48	< 0.001*

‡The *D* statistic is the maximum difference between cumulative distributions.
* indicates a significant difference at the 5% level.

There was a significant interaction between month and standard length (SL) ($F_{1,2697} = 251$, $p < 0.001$). SL increased much quicker in stream fish than it did in reservoir fish (Fig. 7.3). Although specific growth rate (SGR) varied month to month

(Fig. 7.4), there was no significant difference in the overall SGR between reservoir and stream stickleback ($F_{1,30} = 8.01$, $p = 0.093$).

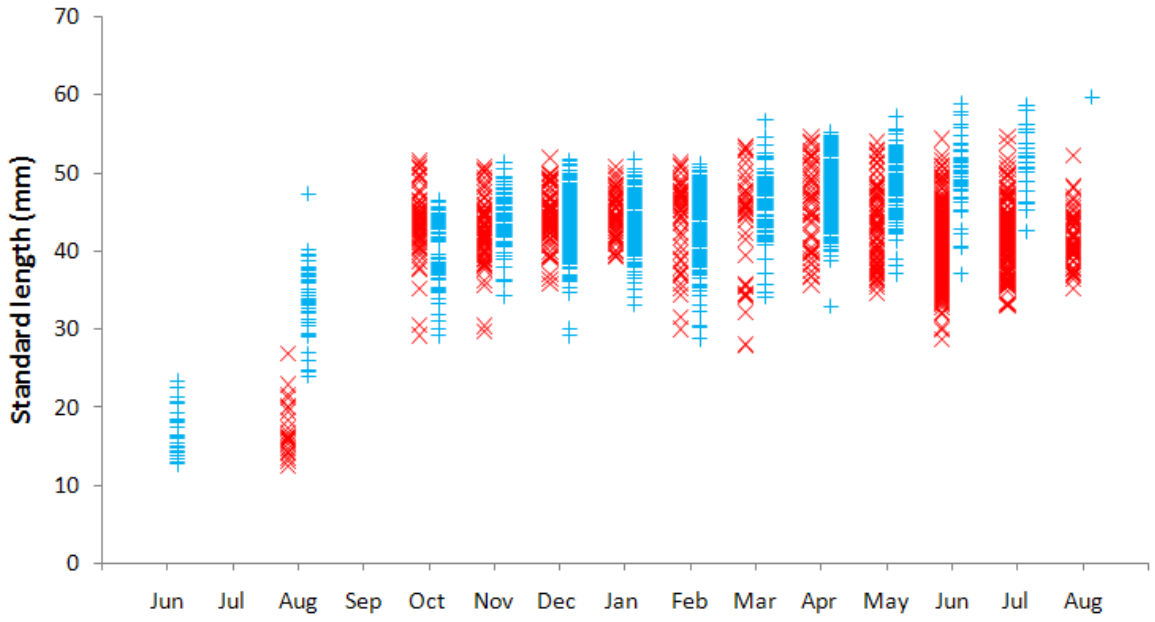

Figure 7.3 Standard length of sticklebacks caught from the Thornton reservoir (blue) and its inflowing stream (red) from June 2009 to August 2010. Data plotted are for the 2009 cohort only.

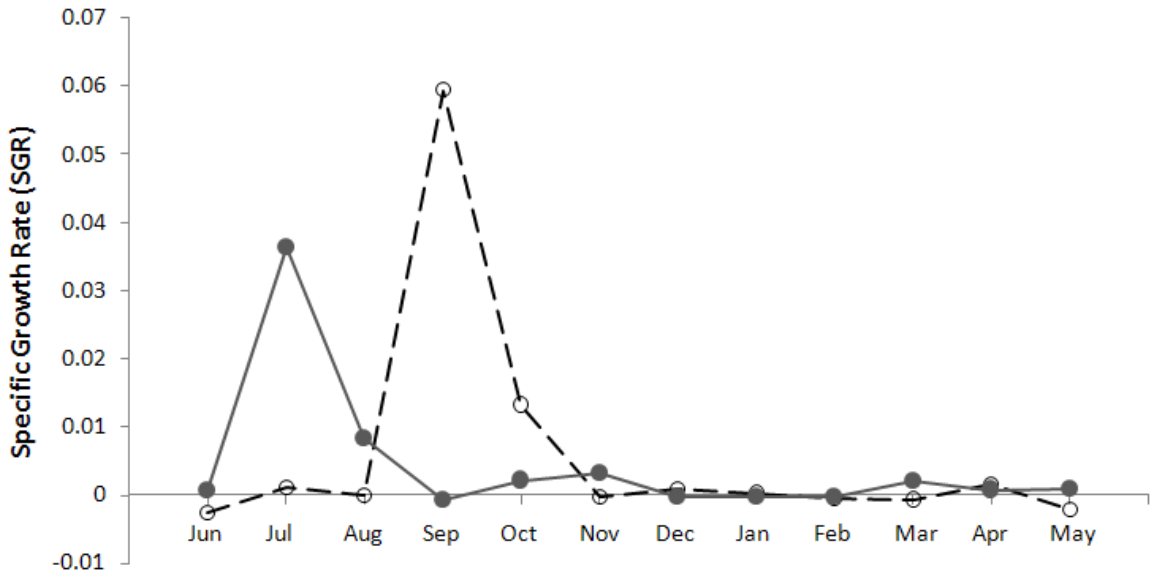

Figure 7.4 Changes in the specific growth rate (SGR) of stickleback caught from the reservoir (—●—) and the stream (--○--) collected from October 2009 to September 2010. Data collected from 2010 are plotted ahead of that collected from 2009 to assist with the interpretation of the results as June appears to marks the start of the main growing season.

7.4. DISCUSSION

Patterns of growth in three-spined sticklebacks collected from Thornton reservoir and its inflowing stream differed and seemed to be related to the onset of the breeding season at different times. Importantly, young-of-the-year (YOY) (< 25mm in length) were only caught from the stream during August whereas from the reservoir, they were caught in June, July and August. The length of the growing season varied between habitats so that sticklebacks from the reservoir continued growing throughout the year, except during the coldest winter months (November to February; Met Office regional statistic for 2010), whereas the growing season in the stream was effectively halted for much longer, from October through until May. Although fish from the stream seemed to be growing for less of the year than their reservoir-dwelling counterparts, there were no differences in the length-frequency distribution between December and March. The small size of sticklebacks means that they are less tolerant of starvation because they exhaust their energy stores earlier; hence overwinter survival is likely to be size-dependent (Shuter & Post 1990). The results of this study suggest that although sticklebacks in the stream reproduce up to two months later than those in the reservoir, they appear to show an early accelerated growth so that by December, they show no signs of their later start in life. There were also no differences in the annual specific growth rate (SGR), further suggesting that the observed accelerated growth is compensatory.

Alternatively, it is possible that differences in the size of individuals caught in the two habitats throughout the year could reflect movement between the habitats and differential use of the reservoir and stream by different sized fish. However, the physical structure of Thornton reservoir makes this a highly unlikely possibility; there are two streams that feed the reservoir, and weirs provide unidirectional obstruction to

movement in both arms. For the majority of the year, the water level was 20cm below the weir, which is where fish were sampled from. Although at times of extremely high rainfall, water levels in the reservoir rose so that the water in the reservoir was just a few cm below the weir. It therefore seems unlikely that the results were the result of fish migration.

7.4.1. The onset of reproduction and the breeding season

The timing of sexual maturation and hence breeding in sticklebacks is prompted both by day length and water temperature (Borg 1982; Borg & Vanveen 1982). Thornton reservoir is surrounded by a relatively open landscape hence a permanent stratification is unlikely to form and surface temperatures in the littoral zone ranged from freezing to over 20°C during the course of sampling. The characteristics of the stream flowing into Thornton reservoir where stickleback were captured (shallow, slow flowing and shaded; see section 2.2.1.7 for further details) meant that the water was less likely to vary in the short term (Hynes 1979) and that it was generally cooler than in the reservoir (Null *et al.* 2010). Given that the surface temperature of waters are strongly affected by sunshine (Moss 1988) but that the stream was primarily shaded, earlier and greater temperature rises in the reservoir are likely could therefore be a reason for the earlier onset of breeding observed.

During the breeding season, feeding becomes a secondary activity for males, particularly those who are in close proximity to a gravid female or another male showing breeding colouration (Noakes 1986). Energy reserves are depleted through vigorous courtship of females (Chellappa & Huntingford 1989) and presumably by maintenance of intense nuptial colouration (Von Hippel 2000). Similarly for females, who although feed voraciously during the breeding season, much of the energy

consumed is directed towards producing clutches of eggs (Wootton 1976). Furthermore, there are also higher maintenance requirements of tissues and metabolic capacities associated with higher temperatures (Guderley 2004).

7.4.2. Life span

In annual species like the stickleback, post-breeding mortality is high (Chellappa *et al.* 1989) and could explain why in the reservoir, few adults from the previous year were caught 2 months after YOY were first detected. In the stream however, large adults (50mm+) were detected 2 months after the first appearance of YOY. Water temperatures are cooled by increasing flow rates (Hutchinson 2005) hence it is possible that stream sticklebacks have lower maintenance requirements and as a consequence, survive longer. The fact that larger sticklebacks are detected throughout the year in the stream, may point towards a longer lived population that survives two breeding seasons. Unfortunately, it is difficult to confirm this from the length-frequency information collected here. These data would benefit from additional otolith data (growth rings in the calcified tissue of bony fishes; Wright & Huntingford 1993) which would be able to age fish more accurately.

7.4.3. Compensatory growth and length of the growing season

Different patterns of growth have also been observed in the Atlantic silverside, *Menidia menidia* and are considered adaptive to the environment. Here, the length of the growing season varies between populations so that it is shorter at higher latitudes, although like the stickleback in the current study, size at the end of the growing season does not differ (Conover 1990). The increased growth rate of northern silverside populations is considered a compensatory response to the shorter growing season.

However, many organisms grow at a slower rate than they are physically capable of because accelerated growth is considered costly (Arendt 1997). Increased feeding is associated with a greater risk of mortality because of the associated increase in activity (Anholt & Werner 1998). Accelerated growth has also been linked to a decrease in lifespan in laboratory-kept rodents which is independent of calorific intake (Rollo 2002). In perch *Perca fluviatilis*, the average growth rate of a year-class is the strongest predictor of its subsequent adult mortality (Metcalfe & Monaghan 2003). However, it is unclear whether this is due to the earlier onset of reproduction and the associated reproductive costs.

In the current study, growth in both habitats slowed and appeared to halt during the coldest and darkest winter months (November to February) when often fish cannot swim fast enough to capture moving food items and intervals between feeding bouts are longer because of a reduced speed of digestion (Wootton 1998). The results suggest that in the reservoir, the growth season begins after February whereas in the stream, it does not re-commence until May.

7.4.4. Earlier onset of the growing season in the reservoir

Earlier growth in the reservoir may be an adaptation to increased predation through the spring months (Arendt 1997). Thornton reservoir is a stocked trout fishery which closes during the winter spawning months, and its re-opening in February coincides with the ongoing growth seen in reservoir sticklebacks. In addition, Thornton reservoir is also a nesting site for several bird species such as the great-crested grebe and tufted duck, which are known to predate on sticklebacks (Reimchen 1994). These too make an appearance around the same time. Heavily predated populations show

higher growth rates (Rundle *et al.* 2003) presumably because in general, larger fish are stronger swimmers hence better able to escape than smaller ones (Garenc *et al.* 1999).

Predation in the stream however, is less intense. There were no sightings of piscivorous fish and the only predatory bird seen was the kingfisher *Alcedo atthis*. Furthermore, given that stream fish do not start breeding until later in the year and that accelerated growth is costly (Metcalfe & Monaghan 2003), it may be more beneficial for stream fish to slow their growth trajectory for longer, thus giving them more time to replenish their energy reserves. In sticklebacks, a decelerating growth trajectory improves swimming performance against a strong current of water (Lee *et al.* 2010), which is likely to be more important in a lotic environment than in a lentic one.

7.4.5. Variation in growth rates as a result of plasticity or evolved differences?

Standard length is only one of many life history traits that can affect an individual's fitness. This study has demonstrated that sticklebacks living in flowing streams and still water reservoirs, even when they are connected and less than 1km apart, show differences in their growth and breeding patterns. However, it remains unclear whether these are responses *to* the environment or constraints imposed *by* the environment.

Sticklebacks have been shown to demonstrate plasticity for reproductive life span (Baker & Foster 2002); hence whether these fish are showing evolutionary adaptations or phenotypic plasticity is difficult to ascertain using field-based sampling methods. Continued sampling over several years may go a little way to shedding some light on this matter as reciprocal transfer experiments would be difficult to undertake in the Thornton system. Common garden experiments have yielded that there is genetic

component for growth in other species (Conover 1990; Reznick *et al.* 1990; Henryon *et al.* 2002; Kause *et al.* 2006). Monitoring growth and the timing of reproduction in reservoir and stream lab-bred fish reared in both flowing and still water would be the next logical step in determining whether there is an underlying genetic component to differences in growth rates observed here. Setting conditions (flow rate, temperature, photoperiod etc.) to match those in the natural environment may also help with understanding the complex interactions between growth and development and the environment.

Chapter 8

Synopsis

8.1. SUMMARY OF MAIN FINDINGS

8.1.1. Changes in the morphology and behaviour of impounded three-spined stickleback

Previous research has shown that sticklebacks from streams and lakes show morphological differences that are consistent with functional morphologies (e.g. Moodie 1972b; Lavin & McPhail 1993). The purpose of this thesis was to investigate if those differences are also apparent between streams and man-made lakes, which share many of the same characteristics as natural lakes, but have been in existence for a much shorter evolutionary timeframe.

8.1.1.1. Body shape and swimming performance

Comparing the morphology of sticklebacks from several streams with the reservoirs they flow into, has shown that there are a number of traits that respond in a way which is consistent with hypotheses based on lake-stream divergence. Variation in body depth is associated with differences in swimming modality so that shallow bodies are considered better suited to sustained, open water swimming whereas deep bodies are considered more beneficial for burst swimming and for providing greater inertia in flowing conditions (Webb 1984; Walker 1997; Blake 2004).

Results from the current research have shown that sticklebacks from streams are consistently deeper bodied and wider than their reservoir-dwelling counterparts (Chapter 2) and that differences in body depth are, at least in part, determined by a heritable genetic mechanism (Chapter 5). Additionally, the body depth of reservoir fish is, on average, intermediate between that of sticklebacks from streams and those from natural, ancient lakes (Chapter 4). Evidence for a genetic basis underpinning body depth differences and an overall intermediate phenotype amongst reservoir fish suggests

that they represent the interim stage of divergence from a stream-typical form to a lake-typical one. There is also an indication that body depth may be maternally inherited. The progeny of laboratory-bred reservoir and stream fish reared under common-garden conditions showed body depth morphology that was most similar to the maternal habitat of origin (Chapter 5).

The role of the caudal peduncle is to provide thrust and propulsion, particularly during fast-start manoeuvres, and as such, is strongly affected by depth (Bone & Moore 2008). Significantly deeper caudal peduncles were detected in the stream fish from four of the seven reservoir-stream systems with a non-significant pattern in the same direction detected in a further two (Chapter 2). Although caudal peduncle morphology in reservoir fish was intermediate between stream fish and lake fish (Chapter 4), laboratory-bred reservoir and stream fish reared under common laboratory conditions showed no differences in depth (Chapter 5). Furthermore, lake and river fish bred in the laboratory and reared under still and flowing conditions showed morphologies that were associated with those expected under the different rearing conditions (Chapter 5).

8.1.1.2. Trophic morphology and feeding

Numerous studies have shown that variation in fish trophic morphology is associated prey type availability and abundance in different habitats (Gross & Anderson 1984; Lavin & McPhail 1986; Adams *et al.* 2003). Stomach content analyses have shown that sticklebacks in lakes have a diet that comprises primarily of pelagic zooplankton prey items whereas those in streams consume a great deal more benthic prey (Berner *et al.* 2009). Consistent with the expected prey consumption of stream- and reservoir-dwelling fish, a greater mouth width was observed in stream fish (Chapter 2 and Chapter 6). Differences in gape were maintained in laboratory-bred reservoir and

stream fish reared under common conditions, with hybrids showing an intermediate phenotype (Chapter 5), suggesting a potentially large genetic contribution to the differences observed. Furthermore, the gape of reservoir fish was indistinguishable from that of lake fish (Chapter 4) suggesting that divergence has been the result of strong selective pressures on this trait.

Differences in gill raker morphology between reservoir- and stream-caught fish were less consistent. In three of the seven systems, stream sticklebacks showed significantly shorter gill rakers than did those caught in the reservoir (Chapter 2). Of the remaining four systems, two showed patterns of differences in the same direction whereas two showed the opposite, of which one was statistically significant (Chapter 2).

Despite differences in trophic morphology, reservoir and stream fish did not differ in the time taken to ingest, or show a preference for typically pelagic (*Daphnia* sp.) or benthic (*Chironomus* sp. larvae – bloodworm) prey (Chapter 6). In fact, stream fish were slower at handing bloodworm than they were at handling *Daphnia* sp. Handling times for bloodworm were best predicted by the standard length of individual fish, whereas the handling time for *Daphnia* sp. were best predicted by body depth, together with the number of gill rakers and their length (Chapter 6).

8.1.1.3. Length of the dorsal and pelvic spines

The dorsal and pelvic spines of three-spined stickleback are considered an adaptation against gape-limited predators (Hoogland *et al.* 1957). Patterns of armour morphology divergence differed considerably between the seven reservoir-stream systems under investigation (Chapter 2). Three of the systems showed little differentiation in spine length based on habitat of capture. Of the four that did show significant variation in spine length, reservoir fish had longer spines in two of the

systems, whereas stream fish had longer spines in the remaining two systems. Although the direction of differences were not the same across systems, there was consistency within systems between the dorsal and ventral spines, i.e. the dorsal and pelvic spines in a single system were all longer or all shorter in a given habitat type.

Although there is strong evidence for the genetic control of both dorsal and pelvic spine lengths (Peichel *et al.* 2001), only length of the pelvic spine appeared to have a genetic basis in the current research (Chapter 5). Similarly, there were no clear pattern of differences or similarities between stream, reservoir and lake fish (Chapter 4).

8.1.2. Neutral genetic differentiation between populations

Genome-wide neutral divergence and genetic structure of stickleback populations are associated with habitat types (Reusch *et al.* 2001a; Leinonen *et al.* 2006; Makinen *et al.* 2006), suggesting that separate populations have evolved independently in response to a specific habitat, but that genetic responses are, to some degree, similar for similar habitats. Using data on genetic variation among UK samples, this thesis has shown that geographically separate systems can be differentiated based on microsatellite variation, and that divergence is not due to isolation-by-distance (Chapter 3). However, fish living in parapatry were more genetically similar than fish that were geographically separated but living in similar habitat types, suggesting that at a macro-geographical scale, habitat has little or no effect on genetic differentiation. Individual analyses showed that only fish in three of the seven reservoir-stream systems could be genetically differentiated into stream and reservoir sticklebacks

8.1.3. Differences in the growth rates of sticklebacks sampled from a reservoir and its inflowing stream

The growth rate of individuals within a species are dictated by a number of environmental factors, including temperature (Wootton 1976), food availability (Bagenal 1969; Hofer *et al.* 1985) and predation (Reimchen 1991). In addition, there are also evolved differences in growth rates such as age and timing of maturity (Conover 1990; Fox & Keast 1991). Monthly sampling and assessment of length-frequency distributions from a single reservoir-stream system showed that the growth of sticklebacks differed between habitat types, and that this was caused by a later onset of breeding in stream fish (Chapter 7). Although stream fish appeared to begin breeding around 2 months after those from the reservoir, there were no differences in size prior to winter, due to an overall accelerated growth rate amongst stream fish.

8.2. DISCUSSION AND DIRECTION FOR FUTURE WORK

One of the key assumptions maintained throughout this thesis has been that sticklebacks in reservoirs were ancestrally river-dwelling and that there is limited movement from the reservoir back into the inflowing stream. In general, movement from the reservoir back into the upstream reaches of the inflowing stream is unlikely, predominantly due to the landscape surrounding the area where the stream joins the reservoir. Population structure analyses confirm that sticklebacks within a reservoir-stream system are more closely related to one another than they are to sticklebacks in a separate location, further supporting the assumption. However, reservoir function is also likely to have an effect on the results. For example, Carsington Water is fed not only by the streams that flow into it, but also by water pumped directly from the River Derwent via a tunnels and aqueducts. It is thus feasible that sticklebacks sampled from

Carsington Water were translocated there unintentionally from over 10km away. As such, interpreting results based on multiple field studies should keep in mind that there are likely to be unaccountable differences due to external and uncontrollable factors.

One of the reoccurring results was the apparent lack of consistency in the direction of morphological differences between habitat types across systems (e.g. for armour and trophic morphology). However, as discussed in Chapter 1, there are a several aspects of the ecosystem that are altered when a river is impounded, in addition to the most obvious changes to the flow regime.

Sticklebacks are predated upon by a wide variety of birds, piscivorous fish and invertebrates (for a summary, see Reimchen 1994) and variation in armour morphology is associated with differences in the type and number of predators (Hagen & Gilbertson 1972; Moodie 1972a; Moodie & Reimchen 1976; Gross 1978). Thus, is seems likely that differences in stickleback armour morphology are indirectly affected by river impoundments, via the effects on their predators. For example, many of the reservoirs sampled were fisheries stocked with trout, a gape-limited predator that preferentially feeds on fish with small or absent spines (Hoogland *et al.* 1957). Of note, is that of the four systems where spine length was longer in the stream, young brown trout *Salmo trutta* were spotted in three of them. In general, the point along the streams where sticklebacks were sampled from was often too shallow to support larger fish.

Although spine length is considered to be under genetic control (Peichel *et al.* 2001), only an effect of family was detected using a common-garden laboratory rearing approach. The observed family effect may have arisen as a true effect of family, but could also be a tank artefact. The densities of fish in each tank were not kept constant across families hence differences in morphology could actually be the result of differences in density (e.g. Lorenzen & Enberg 2002). However, spine length in the

parental population used for the common-garden rearing experiment (Thornton) also showed a significant interaction with standard length (SL), so that spines were longer in reservoir fish, but only when fish SL was 37mm or more. Complexity in the matter is further intensified as the genetic control of spines has been mapped to four separate linkage groups (Peichel *et al.* 2001) and so may not be inherited together.

Similarly inconsistent results were also detected for gill raker morphology but not for mouth width, both of which appear to be under genetic control (Gross & Anderson 1984 and Chapter 6, respectively). Prey type and availability in each of the habitat types were not formally assessed and were assumed to be primarily benthic and zooplanktonic in streams and reservoirs respectively (Berner *et al.* 2009). Formal assessment of prey consumption using stable isotope or stomach content analyses would aid the interpretation of the results and may go some way to clarify the unexpected and conflicting findings (e.g. Harrod *et al.* 2010). Doing so would also potentially assist with the interpretation of the results on prey handling times, which did not show an association with habitat of capture.

If reservoir fish represent an intermediate form between streams and lakes, then one would expect that given time, they would eventually be indistinguishable from sticklebacks from natural lakes. Although the morphology of some traits supports this hypothesis, many do not. Furthermore, although it is clear that differences are not purely the result of morphological plasticity, neither genetic nor morphological differentiation between habitat types increased with reservoir age, which may have been expected under a theory of evolved adaptive divergence.

For evolved responses, the rate of adaptation and diversification is often related to the strength of divergent selection (Bernatchez *et al.* 1999; Lu & Bernatchez 1999); ergo, strong selective pressures over relatively short evolutionary timescales can induce

phenotypic changes of large effect (e.g. Kristjánsson *et al.* 2002; Bell *et al.* 2004; Kristjánsson 2005). Equally however, weak divergent selection over a considerably longer timeframe may result in statistically imperceptible phenotypic differences. Although all of the systems investigated were an on-line reservoir and an inflowing stream sampled, it is clear that each one is very individual (see section 2.2.1 for details).

This is further exemplified by the population-specific degree of plasticity observed in lake and stream fish reared under still and flowing conditions, and the extent of neutral genetic differentiation within each system. Although phenotypic divergence may be due to genetic differentiation, adaptive traits may evolve more rapidly than neutral DNA (Ballentine & Greenberg 2010) and may explain why genetic and morphological differentiation did not appear to be correlated.

An interesting way to investigate this further, without genotyping individuals at every known quantitative trait locus, would be to breed and rear fish from all seven reservoir-stream systems under both still and flowing water conditions. This would help to shed light on which systems were more amenable to environmentally-induced changes, and could give an indication of temporal heterogeneity and how fixed each system has become for particular phenotypes.

However, if phenotypic differences are evolved, then it is not sufficient to consider only the current ecological situation. Studying evolved differences must also consider the likely ecological state of the environment during the period of divergence (Schluter 2001). For example, whereas sticklebacks in a particular reservoir may have historically been subject to low levels of piscivorous predation, conversion to a stocked trout fishery imparts a major change to the predatory regime and thus selection pressures. Unfortunately, detailed and accurate information about predation regimes was not collected and is notoriously challenging, either current or historical, and thus

interpretation of differences thought to be under divergent selection should be approached with caution. This applies to most studies of this kind.

8.3. CONCLUDING COMMENTS

This thesis has shown that reservoirs can function as a valuable proxy for studying divergence in lake-stream stickleback pairs but with additional insight into the timescales involved. I have demonstrated that adaptive changes in some morphological traits can occur in less than 20 years; but only given the right conditions. By comparing environments that do and do not induce changes, a better understanding of the mechanisms involved may be gained.

Crucially, I have highlighted that the process of river impoundments can have very different effects on the biology of one particular species and this highlights the importance of studying several systems before making generalisations based on only one or two. Although a number of studies use the degree of neutral divergence to infer how differentiated populations are, I have shown that it does not necessarily correlate with phenotypic divergence. Populations that are similar may have reached the same end-point via adaptive divergence or plasticity and only detailed laboratory studies can tease the detail between these two theories apart.

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Appendix

CHAPTER 2

Alaw

Body width: with interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	43.130[a]	3	14.377	169.487	.000
Intercept	.098	1	.098	1.159	.286
Site	.315	1	.315	3.714	.059
SL_img_a	10.797	1	10.797	127.288	.000
Site * SL_img_a	1.171	1	1.171	13.811	.000
Error	4.750	56	.085		
Total	1166.673	60			
Corrected Total	47.880	59			

a. R Squared = .901 (Adjusted R Squared = .895)

Gill raker length: with interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.272[a]	3	.091	8.679	.000
Intercept	.005	1	.005	.506	.482
Site	.109	1	.109	10.449	.003
SL_img_l	.081	1	.081	7.750	.009
Site * SL_img_l	.123	1	.123	11.805	.002
Error	.366	35	.010		
Total	22.556	39			
Corrected Total	.638	38			

a. R Squared = .427 (Adjusted R Squared = .377)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	87.211[a]	2	43.606	491.417	.000
	Length of first dorsal spine (mm)	3.104[b]	2	1.552	20.482	.000
	Length of second dorsal spine (mm)	2.788[c]	2	1.394	23.933	.000
	Angle of jaw	10.601[d]	2	5.301	.591	.557
	Mouth width	7.574[e]	2	3.787	72.960	.000
	Length of pelvic spine	9.506[f]	2	4.753	32.860	.000
	Length of pelvic girdle	68.643[g]	2	34.321	151.594	.000
	Depth of caudal peduncle	2.261[h]	2	1.131	88.391	.000

	Length of caudal peduncle	22.195[i]	2	11.097	39.577	.000
Intercept	Depth (mm)	.101	1	.101	1.142	.290
	Length of first dorsal spine (mm)	.234	1	.234	3.085	.084
	Length of second dorsal spine (mm)	.717	1	.717	12.305	.001
	Angle of jaw	12585.739	1	12585.739	1402.723	.000
	Mouth width	.208	1	.208	4.003	.050
	Length of pelvic spine	.690	1	.690	4.770	.033
	Length of pelvic girdle	.441	1	.441	1.947	.168
	Depth of caudal peduncle	.001	1	.001	.047	.829
	Length of caudal peduncle	.119	1	.119	.425	.517
SL_img_l	Depth (mm)	47.410	1	47.410	534.294	.000
	Length of first dorsal spine (mm)	2.440	1	2.440	32.200	.000
	Length of second dorsal spine (mm)	1.980	1	1.980	33.988	.000
	Angle of jaw	1.254	1	1.254	.140	.710
	Mouth width	6.011	1	6.011	115.815	.000
	Length of pelvic spine	8.595	1	8.595	59.428	.000
	Length of pelvic girdle	56.385	1	56.385	249.046	.000
	Depth of caudal peduncle	1.635	1	1.635	127.800	.000
	Length of caudal peduncle	21.779	1	21.779	77.671	.000
Site	Depth (mm)	19.835	1	19.835	223.537	.000
	Length of first dorsal spine (mm)	1.367	1	1.367	18.032	.000
	Length of second dorsal spine (mm)	1.472	1	1.472	25.263	.000
	Angle of jaw	7.264	1	7.264	.810	.372
	Mouth width	.383	1	.383	7.385	.009
	Length of pelvic spine	2.675	1	2.675	18.495	.000
	Length of pelvic girdle	2.492	1	2.492	11.007	.002
	Depth of caudal peduncle	.210	1	.210	16.426	.000
	Length of caudal peduncle	3.082	1	3.082	10.993	.002
Error	Depth (mm)	4.969	56	.089		
	Length of first dorsal spine (mm)	4.244	56	.076		
	Length of second dorsal spine (mm)	3.262	56	.058		
	Angle of jaw	502.452	56	8.972		
	Mouth width	2.906	56	.052		
	Length of pelvic spine	8.100	56	.145		

	Length of pelvic girdle	12.679	56	.226		
	Depth of caudal peduncle	.716	56	.013		
	Length of caudal peduncle	15.702	56	.280		
Total	Depth (mm)	4529.053	59			
	Length of first dorsal spine (mm)	367.598	59			
	Length of second dorsal spine (mm)	444.026	59			
	Angle of jaw	1113722.449	59			
	Mouth width	350.320	59			
	Length of pelvic spine	1235.415	59			
	Length of pelvic girdle	4086.126	59			
	Depth of caudal peduncle	148.281	59			
	Length of caudal peduncle	1638.253	59			
Corrected Total	Depth (mm)	92.180	58			
	Length of first dorsal spine (mm)	7.348	58			
	Length of second dorsal spine (mm)	6.050	58			
	Angle of jaw	513.054	58			
	Mouth width	10.480	58			
	Length of pelvic spine	17.605	58			
	Length of pelvic girdle	81.321	58			
	Depth of caudal peduncle	2.978	58			
	Length of caudal peduncle	37.897	58			

a. R Squared = .946 (Adjusted R Squared = .944)
b. R Squared = .422 (Adjusted R Squared = .402)
c. R Squared = .461 (Adjusted R Squared = .442)
d. R Squared = .021 (Adjusted R Squared = -.014)
e. R Squared = .723 (Adjusted R Squared = .713)
f. R Squared = .540 (Adjusted R Squared = .523)
g. R Squared = .844 (Adjusted R Squared = .839)
h. R Squared = .759 (Adjusted R Squared = .751)
i. R Squared = .586 (Adjusted R Squared = .571)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	87.425[a]	3	29.142	337.070	.000
	Length of first dorsal spine (mm)	3.172[b]	3	1.057	13.922	.000
	Length of second dorsal spine (mm)	3.291[c]	3	1.097	21.861	.000

	Angle of jaw	11.163[d]	3	3.721	.408	.748
	Mouth width	7.902[e]	3	2.634	56.183	.000
	Length of pelvic spine	9.624[f]	3	3.208	22.109	.000
	Length of pelvic girdle	69.461[g]	3	23.154	107.369	.000
	Depth of caudal peduncle	2.273[h]	3	.758	59.157	.000
	Length of caudal peduncle	22.196[i]	3	7.399	25.918	.000
Intercept	Depth (mm)	.077	1	.077	.896	.348
	Length of first dorsal spine (mm)	.254	1	.254	3.339	.073
	Length of second dorsal spine (mm)	.816	1	.816	16.255	.000
	Angle of jaw	12483.103	1	12483.103	1367.969	.000
	Mouth width	.165	1	.165	3.518	.066
	Length of pelvic spine	.734	1	.734	5.057	.029
	Length of pelvic girdle	.544	1	.544	2.522	.118
	Depth of caudal peduncle	.001	1	.001	.088	.767
	Length of caudal peduncle	.121	1	.121	.423	.518
Site	Depth (mm)	.001	1	.001	.011	.915
	Length of first dorsal spine (mm)	.017	1	.017	.219	.642
	Length of second dorsal spine (mm)	.326	1	.326	6.491	.014
	Angle of jaw	.200	1	.200	.022	.883
	Mouth width	.407	1	.407	8.672	.005
	Length of pelvic spine	.026	1	.026	.181	.672
	Length of pelvic girdle	.525	1	.525	2.436	.124
	Depth of caudal peduncle	.025	1	.025	1.967	.166
	Length of caudal peduncle	.056	1	.056	.195	.660
SL_img_l	Depth (mm)	47.620	1	47.620	550.800	.000
	Length of first dorsal spine (mm)	2.386	1	2.386	31.418	.000
	Length of second dorsal spine (mm)	1.860	1	1.860	37.075	.000
	Angle of jaw	1.348	1	1.348	.148	.702
	Mouth width	5.831	1	5.831	124.376	.000
	Length of pelvic spine	8.451	1	8.451	58.241	.000
	Length of pelvic girdle	56.982	1	56.982	264.236	.000
	Depth of caudal peduncle	1.613	1	1.613	125.959	.000
	Length of caudal peduncle	21.729	1	21.729	76.120	.000
Site * SL_img_l	Depth (mm)	.214	1	.214	2.476	.121
	Length of first dorsal spine (mm)	.067	1	.067	.886	.351

	Length of second dorsal spine (mm)	.502	1	.502	10.013	.003
	Angle of jaw	.562	1	.562	.062	.805
	Mouth width	.328	1	.328	6.999	.011
	Length of pelvic spine	.119	1	.119	.818	.370
	Length of pelvic girdle	.818	1	.818	3.794	.057
	Depth of caudal peduncle	.012	1	.012	.925	.340
	Length of caudal peduncle	.002	1	.002	.006	.937
Error	Depth (mm)	4.755	55	.086		
	Length of first dorsal spine (mm)	4.177	55	.076		
	Length of second dorsal spine (mm)	2.760	55	.050		
	Angle of jaw	501.891	55	9.125		
	Mouth width	2.578	55	.047		
	Length of pelvic spine	7.981	55	.145		
	Length of pelvic girdle	11.861	55	.216		
	Depth of caudal peduncle	.705	55	.013		
	Length of caudal peduncle	15.701	55	.285		
Total	Depth (mm)	4529.053	59			
	Length of first dorsal spine (mm)	367.598	59			
	Length of second dorsal spine (mm)	444.026	59			
	Angle of jaw	1113722.449	59			
	Mouth width	350.320	59			
	Length of pelvic spine	1235.415	59			
	Length of pelvic girdle	4086.126	59			
	Depth of caudal peduncle	148.281	59			
	Length of caudal peduncle	1638.253	59			
Corrected Total	Depth (mm)	92.180	58			
	Length of first dorsal spine (mm)	7.348	58			
	Length of second dorsal spine (mm)	6.050	58			
	Angle of jaw	513.054	58			
	Mouth width	10.480	58			
	Length of pelvic spine	17.605	58			
	Length of pelvic girdle	81.321	58			
	Depth of caudal peduncle	2.978	58			
	Length of caudal peduncle	37.897	58			

a. R Squared = .948 (Adjusted R Squared = .946)
b. R Squared = .432 (Adjusted R Squared = .401)

c. R Squared = .544 (Adjusted R Squared = .519)
d. R Squared = .022 (Adjusted R Squared = -.032)
e. R Squared = .754 (Adjusted R Squared = .741)
f. R Squared = .547 (Adjusted R Squared = .522)
g. R Squared = .854 (Adjusted R Squared = .846)
h. R Squared = .763 (Adjusted R Squared = .751)
i. R Squared = .586 (Adjusted R Squared = .563)

Blackbrook

Width: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.648[a]	2	7.824	98.820	.000
Intercept	.056	1	.056	.704	.405
SL_img_a	13.995	1	13.995	176.762	.000
Site	2.216	1	2.216	27.983	.000
Error	4.988	63	.079		
Total	1014.692	66			
Corrected Total	20.636	65			

a. R Squared = .758 (Adjusted R Squared = .751)

Gill raker length: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.080[a]	2	.040	3.671	.035
Intercept	.017	1	.017	1.524	.225
SL_img_I	.024	1	.024	2.203	.146
Site	.055	1	.055	5.054	.031
Error	.405	37	.011		
Total	25.375	40			
Corrected Total	.485	39			

a. R Squared = .166 (Adjusted R Squared = .120)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	38.090[a]	2	19.045	252.215	.000
	Caudal peduncle length	11.773[b]	2	5.886	54.458	.000
	Caudal peduncle depth	1.179[c]	2	.589	151.024	.000
	Length of first dorsal spine (mm)	4.320[d]	2	2.160	44.915	.000
	Length of second dorsal spine (mm)	5.309[e]	2	2.654	61.731	.000

	Length of pelvic spine (mm)	13.803[f]	2	6.902	63.146	.000
	Length of pelvic girdle (mm)	31.597[g]	2	15.799	133.884	.000
	Angle of jaw	32.459[h]	2	16.230	3.842	.027
	Mouth width (mm)	3.196[i]	2	1.598	53.018	.000
Intercept	Depth (mm)	.097	1	.097	1.291	.260
	Caudal peduncle length	.363	1	.363	3.360	.072
	Caudal peduncle depth	.000	1	.000	.046	.831
	Length of first dorsal spine (mm)	8.992E-5	1	8.992E-5	.002	.966
	Length of second dorsal spine (mm)	.001	1	.001	.033	.855
	Length of pelvic spine (mm)	.094	1	.094	.863	.357
	Length of pelvic girdle (mm)	.032	1	.032	.270	.605
	Angle of jaw	8798.630	1	8798.630	2082.769	.000
	Mouth width (mm)	.012	1	.012	.392	.533
SL_img_I	Depth (mm)	38.070	1	38.070	504.164	.000
	Caudal peduncle length	11.720	1	11.720	108.427	.000
	Caudal peduncle depth	1.163	1	1.163	298.011	.000
	Length of first dorsal spine (mm)	4.285	1	4.285	89.102	.000
	Length of second dorsal spine (mm)	5.302	1	5.302	123.311	.000
	Length of pelvic spine (mm)	12.674	1	12.674	115.961	.000
	Length of pelvic girdle (mm)	31.427	1	31.427	266.328	.000
	Angle of jaw	31.453	1	31.453	7.445	.008
	Mouth width (mm)	3.029	1	3.029	100.515	.000
Site	Depth (mm)	1.283	1	1.283	16.992	.000
	Caudal peduncle length	.105	1	.105	.971	.328
	Caudal peduncle depth	.002	1	.002	.632	.430
	Length of first dorsal spine (mm)	.268	1	.268	5.575	.021
	Length of second dorsal spine (mm)	.085	1	.085	1.970	.165
	Length of pelvic spine (mm)	.226	1	.226	2.071	.155
	Length of pelvic girdle (mm)	.245	1	.245	2.079	.154
	Angle of jaw	3.582	1	3.582	.848	.361
	Mouth width (mm)	.466	1	.466	15.466	.000
Error	Depth (mm)	4.682	62	.076		

	Caudal peduncle length	6.702	62	.108		
	Caudal peduncle depth	.242	62	.004		
	Length of first dorsal spine (mm)	2.981	62	.048		
	Length of second dorsal spine (mm)	2.666	62	.043		
	Length of pelvic spine (mm)	6.776	62	.109		
	Length of pelvic girdle (mm)	7.316	62	.118		
	Angle of jaw	261.918	62	4.224		
	Mouth width (mm)	1.868	62	.030		
Total	Depth (mm)	4356.649	65			
	Caudal peduncle length	2052.732	65			
	Caudal peduncle depth	150.474	65			
	Length of first dorsal spine (mm)	542.757	65			
	Length of second dorsal spine (mm)	651.363	65			
	Length of pelvic spine (mm)	1330.807	65			
	Length of pelvic girdle (mm)	3727.334	65			
	Angle of jaw	1252320.356	65			
	Mouth width (mm)	342.960	65			
Corrected Total	Depth (mm)	42.771	64			
	Caudal peduncle length	18.474	64			
	Caudal peduncle depth	1.421	64			
	Length of first dorsal spine (mm)	7.301	64			
	Length of second dorsal spine (mm)	7.975	64			
	Length of pelvic spine (mm)	20.580	64			
	Length of pelvic girdle (mm)	38.914	64			
	Angle of jaw	294.377	64			
	Mouth width (mm)	5.064	64			

a. R Squared = .891 (Adjusted R Squared = .887)
b. R Squared = .637 (Adjusted R Squared = .626)
c. R Squared = .830 (Adjusted R Squared = .824)
d. R Squared = .592 (Adjusted R Squared = .578)
e. R Squared = .666 (Adjusted R Squared = .655)
f. R Squared = .671 (Adjusted R Squared = .660)
g. R Squared = .812 (Adjusted R Squared = .806)
h. R Squared = .110 (Adjusted R Squared = .082)

i. R Squared = .631 (Adjusted R Squared = .619)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	38.156[a]	3	12.719	168.113	.000
	Caudal peduncle length	11.780[b]	3	3.927	35.783	.000
	Caudal peduncle depth	1.187[c]	3	.396	103.423	.000
	Length of first dorsal spine (mm)	4.355[d]	3	1.452	30.059	.000
	Length of second dorsal spine (mm)	5.315[e]	3	1.772	40.633	.000
	Length of pelvic spine (mm)	14.025[f]	3	4.675	43.504	.000
	Length of pelvic girdle (mm)	31.704[g]	3	10.568	89.423	.000
	Angle of jaw	32.722[h]	3	10.907	2.543	.064
	Mouth width (mm)	3.197[i]	3	1.066	34.819	.000
Intercept	Depth (mm)	.102	1	.102	1.345	.251
	Caudal peduncle length	.360	1	.360	3.282	.075
	Caudal peduncle depth	.000	1	.000	.031	.861
	Length of first dorsal spine (mm)	.000	1	.000	.004	.947
	Length of second dorsal spine (mm)	.002	1	.002	.037	.849
	Length of pelvic spine (mm)	.102	1	.102	.950	.333
	Length of pelvic girdle (mm)	.029	1	.029	.243	.624
	Angle of jaw	8789.700	1	8789.700	2049.157	.000
	Mouth width (mm)	.012	1	.012	.379	.541
Site	Depth (mm)	.131	1	.131	1.730	.193
	Caudal peduncle length	.013	1	.013	.123	.727
	Caudal peduncle depth	.009	1	.009	2.437	.124
	Length of first dorsal spine (mm)	.055	1	.055	1.146	.289
	Length of second dorsal spine (mm)	.011	1	.011	.256	.615
	Length of pelvic spine (mm)	.180	1	.180	1.677	.200
	Length of pelvic girdle (mm)	.078	1	.078	.664	.418
	Angle of jaw	.471	1	.471	.110	.742
	Mouth width (mm)	.001	1	.001	.020	.888

SL_img_I	Depth (mm)	38.103	1	38.103	503.626	.000
	Caudal peduncle length	11.725	1	11.725	106.847	.000
	Caudal peduncle depth	1.165	1	1.165	304.505	.000
	Length of first dorsal spine (mm)	4.293	1	4.293	88.899	.000
	Length of second dorsal spine (mm)	5.306	1	5.306	121.691	.000
	Length of pelvic spine (mm)	12.713	1	12.713	118.302	.000
	Length of pelvic girdle (mm)	31.379	1	31.379	265.514	.000
	Angle of jaw	31.518	1	31.518	7.348	.009
	Mouth width (mm)	3.027	1	3.027	98.905	.000
Site * SL_img_I	Depth (mm)	.067	1	.067	.881	.352
	Caudal peduncle length	.007	1	.007	.068	.795
	Caudal peduncle depth	.009	1	.009	2.230	.141
	Length of first dorsal spine (mm)	.035	1	.035	.733	.395
	Length of second dorsal spine (mm)	.006	1	.006	.143	.706
	Length of pelvic spine (mm)	.221	1	.221	2.060	.156
	Length of pelvic girdle (mm)	.107	1	.107	.906	.345
	Angle of jaw	.263	1	.263	.061	.805
	Mouth width (mm)	.001	1	.001	.048	.826
Error	Depth (mm)	4.615	61	.076		
	Caudal peduncle length	6.694	61	.110		
	Caudal peduncle depth	.233	61	.004		
	Length of first dorsal spine (mm)	2.946	61	.048		
	Length of second dorsal spine (mm)	2.660	61	.044		
	Length of pelvic spine (mm)	6.555	61	.107		
	Length of pelvic girdle (mm)	7.209	61	.118		
	Angle of jaw	261.655	61	4.289		
	Mouth width (mm)	1.867	61	.031		
Total	Depth (mm)	4356.649	65			
	Caudal peduncle length	2052.732	65			
	Caudal peduncle depth	150.474	65			
	Length of first dorsal spine (mm)	542.757	65			

	Length of second dorsal spine (mm)	651.363	65			
	Length of pelvic spine (mm)	1330.807	65			
	Length of pelvic girdle (mm)	3727.334	65			
	Angle of jaw	1252320.356	65			
	Mouth width (mm)	342.960	65			
Corrected Total	Depth (mm)	42.771	64			
	Caudal peduncle length	18.474	64			
	Caudal peduncle depth	1.421	64			
	Length of first dorsal spine (mm)	7.301	64			
	Length of second dorsal spine (mm)	7.975	64			
	Length of pelvic spine (mm)	20.580	64			
	Length of pelvic girdle (mm)	38.914	64			
	Angle of jaw	294.377	64			
	Mouth width (mm)	5.064	64			

a. R Squared = .892 (Adjusted R Squared = .887)
b. R Squared = .638 (Adjusted R Squared = .620)
c. R Squared = .836 (Adjusted R Squared = .828)
d. R Squared = .596 (Adjusted R Squared = .577)
e. R Squared = .666 (Adjusted R Squared = .650)
f. R Squared = .681 (Adjusted R Squared = .666)
g. R Squared = .815 (Adjusted R Squared = .806)
h. R Squared = .111 (Adjusted R Squared = .067)
i. R Squared = .631 (Adjusted R Squared = .613)

Cefni

Width: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	59.585[a]	2	29.793	173.056	.000
Intercept	1.380	1	1.380	8.015	.006
SL_img_a	55.303	1	55.303	321.238	.000
Site	2.015	1	2.015	11.702	.001
Error	14.805	86	.172		
Total	2175.757	89			
Corrected Total	74.391	88			

a. R Squared = .801 (Adjusted R Squared = .796)

Gill raker number and length: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Number of gill rakers	17.295[a]	2	8.647	2.667	.082
	Gill raker length	.154[b]	2	.077	4.442	.018
Intercept	Number of gill rakers	141.197	1	141.197	43.554	.000
	Gill raker length	.006	1	.006	.339	.564
SL_img_l	Number of gill rakers	5.795	1	5.795	1.788	.189
	Gill raker length	.153	1	.153	8.821	.005
Site	Number of gill rakers	11.578	1	11.578	3.571	.066
	Gill raker length	.001	1	.001	.056	.814
Error	Number of gill rakers	123.193	38	3.242		
	Gill raker length	.658	38	.017		
Total	Number of gill rakers	11585.000	41			
	Gill raker length	28.463	41			
Corrected Total	Number of gill rakers	140.488	40			
	Gill raker length	.812	40			

a. R Squared = .123 (Adjusted R Squared = .077)
b. R Squared = .189 (Adjusted R Squared = .147)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	CPL	54.671[a]	2	27.336	89.186	.000
	CPD	4.127[b]	2	2.063	125.632	.000
	Length of first dorsal spine (mm)	2.680[c]	2	1.340	8.114	.001
	Length of second dorsal spine (mm)	5.393[d]	2	2.696	29.114	.000
	PS	8.269[e]	2	4.135	14.035	.000
	PG	176.135[f]	2	88.067	379.482	.000
	Jaw	28.930[g]	2	14.465	1.642	.200
	Mouth	12.672[h]	2	6.336	69.151	.000
Intercept	CPL	.037	1	.037	.121	.729
	CPD	.013	1	.013	.810	.371
	Length of first dorsal spine (mm)	2.581	1	2.581	15.626	.000
	Length of second dorsal spine (mm)	1.590	1	1.590	17.173	.000
	PS	7.334	1	7.334	24.896	.000
	PG	3.937	1	3.937	16.965	.000
	Jaw	25539.116	1	25539.116	2899.927	.000

	Mouth	.003	1	.003	.029	.866
SL_img_l	CPL	48.514	1	48.514	158.284	.000
	CPD	3.675	1	3.675	223.764	.000
	Length of first dorsal spine (mm)	2.398	1	2.398	14.521	.000
	Length of second dorsal spine (mm)	5.188	1	5.188	56.017	.000
	PS	8.136	1	8.136	27.618	.000
	PG	165.378	1	165.378	712.611	.000
	Jaw	28.695	1	28.695	3.258	.075
	Mouth	12.641	1	12.641	137.966	.000
Site	CPL	.802	1	.802	2.617	.109
	CPD	.066	1	.066	3.999	.049
	Length of first dorsal spine (mm)	.047	1	.047	.287	.594
	Length of second dorsal spine (mm)	.377	1	.377	4.069	.047
	PS	2.567	1	2.567	8.712	.004
	PG	7.941	1	7.941	34.216	.000
	Jaw	3.848	1	3.848	.437	.510
	Mouth	2.054	1	2.054	22.421	.000
Error	CPL	25.746	84	.306		
	CPD	1.380	84	.016		
	Length of first dorsal spine (mm)	13.872	84	.165		
	Length of second dorsal spine (mm)	7.779	84	.093		
	PS	24.746	84	.295		
	PG	19.494	84	.232		
	Jaw	739.772	84	8.807		
	Mouth	7.697	84	.092		
Total	CPL	3118.807	87			
	CPD	250.585	87			
	Length of first dorsal spine (mm)	610.772	87			
	Length of second dorsal spine (mm)	758.594	87			
	PS	1874.303	87			
	PG	7183.354	87			
	Jaw	1639449.985	87			
	Mouth	746.250	87			
Corrected Total	CPL	80.417	86			
	CPD	5.506	86			
	Length of first dorsal spine (mm)	16.552	86			
	Length of second dorsal spine (mm)	13.172	86			
	PS	33.015	86			
	PG	195.629	86			
	Jaw	768.702	86			
	Mouth	20.369	86			

a. R Squared = .680 (Adjusted R Squared = .672)
b. R Squared = .749 (Adjusted R Squared = .743)
c. R Squared = .162 (Adjusted R Squared = .142)
d. R Squared = .409 (Adjusted R Squared = .395)
e. R Squared = .250 (Adjusted R Squared = .233)
f. R Squared = .900 (Adjusted R Squared = .898)
g. R Squared = .038 (Adjusted R Squared = .015)
h. R Squared = .622 (Adjusted R Squared = .613)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	CPL	54.672[a]	3	18.224	58.754	.000
	CPD	4.284[b]	3	1.428	96.938	.000
	Length of first dorsal spine (mm)	3.253[c]	3	1.084	6.767	.000
	Length of second dorsal spine (mm)	5.565[d]	3	1.855	20.241	.000
	PS	10.172[e]	3	3.391	12.319	.000
	PG	176.256[f]	3	58.752	251.718	.000
	Jaw	44.118[g]	3	14.706	1.685	.177
	Mouth	13.125[h]	3	4.375	50.131	.000
Intercept	CPL	.036	1	.036	.115	.735
	CPD	.007	1	.007	.501	.481
	Length of first dorsal spine (mm)	2.391	1	2.391	14.924	.000
	Length of second dorsal spine (mm)	1.506	1	1.506	16.431	.000
	PS	6.757	1	6.757	24.550	.000
	PG	4.018	1	4.018	17.214	.000
	Jaw	25492.375	1	25492.375	2920.112	.000
	Mouth	.010	1	.010	.116	.734
Site	CPL	.009	1	.009	.029	.864
	CPD	.126	1	.126	8.567	.004
	Length of first dorsal spine (mm)	.515	1	.515	3.213	.077
	Length of second dorsal spine (mm)	.104	1	.104	1.136	.290
	PS	1.287	1	1.287	4.676	.033
	PG	.004	1	.004	.016	.901
	Jaw	17.131	1	17.131	1.962	.165
	Mouth	.211	1	.211	2.423	.123
SL_img_l	CPL	47.644	1	47.644	153.603	.000
	CPD	3.816	1	3.816	259.067	.000
	Length of first dorsal spine (mm)	2.685	1	2.685	16.757	.000
	Length of second dorsal spine (mm)	5.351	1	5.351	58.389	.000
	PS	9.097	1	9.097	33.054	.000
	PG	163.429	1	163.429	700.196	.000

	Jaw	22.697	1	22.697	2.600	.111
	Mouth	13.064	1	13.064	149.698	.000
Site * SL_img_l	CPL	.001	1	.001	.004	.952
	CPD	.157	1	.157	10.659	.002
	Length of first dorsal spine (mm)	.573	1	.573	3.574	.062
	Length of second dorsal spine (mm)	.172	1	.172	1.882	.174
	PS	1.903	1	1.903	6.913	.010
	PG	.122	1	.122	.521	.472
	Jaw	15.188	1	15.188	1.740	.191
	Mouth	.453	1	.453	5.191	.025
Error	CPL	25.745	83	.310		
	CPD	1.223	83	.015		
	Length of first dorsal spine (mm)	13.299	83	.160		
	Length of second dorsal spine (mm)	7.607	83	.092		
	PS	22.844	83	.275		
	PG	19.373	83	.233		
	Jaw	724.584	83	8.730		
	Mouth	7.243	83	.087		
Total	CPL	3118.807	87			
	CPD	250.585	87			
	Length of first dorsal spine (mm)	610.772	87			
	Length of second dorsal spine (mm)	758.594	87			
	PS	1874.303	87			
	PG	7183.354	87			
	Jaw	1639449.985	87			
	Mouth	746.250	87			
Corrected Total	CPL	80.417	86			
	CPD	5.506	86			
	Length of first dorsal spine (mm)	16.552	86			
	Length of second dorsal spine (mm)	13.172	86			
	PS	33.015	86			
	PG	195.629	86			
	Jaw	768.702	86			
	Mouth	20.369	86			

a. R Squared = .680 (Adjusted R Squared = .668)
b. R Squared = .778 (Adjusted R Squared = .770)
c. R Squared = .197 (Adjusted R Squared = .167)
d. R Squared = .422 (Adjusted R Squared = .402)
e. R Squared = .308 (Adjusted R Squared = .283)
f. R Squared = .901 (Adjusted R Squared = .897)
g. R Squared = .057 (Adjusted R Squared = .023)
h. R Squared = .644 (Adjusted R Squared = .632)

Carsington

Gill raker number and length: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Number of gill rakers	14.684[a]	2	7.342	5.821	.007
	Length of gill rakers	.246[b]	2	.123	6.317	.005
Intercept	Number of gill rakers	118.092	1	118.092	93.630	.000
	Length of gill rakers	.009	1	.009	.460	.503
SL_img_I	Number of gill rakers	4.917	1	4.917	3.899	.057
	Length of gill rakers	.245	1	.245	12.570	.001
Site	Number of gill rakers	13.337	1	13.337	10.574	.003
	Length of gill rakers	.034	1	.034	1.741	.196
Error	Number of gill rakers	41.621	33	1.261		
	Length of gill rakers	.642	33	.019		
Total	Number of gill rakers	9113.000	36			
	Length of gill rakers	19.062	36			
Corrected Total	Number of gill rakers	56.306	35			
	Length of gill rakers	.888	35			

a. R Squared = .261 (Adjusted R Squared = .216)
b. R Squared = .277 (Adjusted R Squared = .233)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	105.419[a]	2	52.709	270.200	.000
	Caudal peduncle length	20.169[b]	2	10.084	27.472	.000
	Caudal peduncle depth	2.275[c]	2	1.138	72.539	.000
	Length of first dorsal spine (mm)	2.868[d]	2	1.434	16.681	.000
	Length of second dorsal spine (mm)	2.756[e]	2	1.378	8.983	.001

	Length of pelvic spine (mm)	7.337[f]	2	3.668	10.043	.000
	Length of pelvic girdle (mm)	68.165[g]	2	34.083	85.818	.000
	Angle of jaw	163.544[h]	2	81.772	7.183	.002
	Mouth width (mm)	12.855[i]	2	6.428	82.796	.000
Intercept	Depth (mm)	.351	1	.351	1.798	.188
	Caudal peduncle length	1.504	1	1.504	4.098	.050
	Caudal peduncle depth	.003	1	.003	.206	.652
	Length of first dorsal spine (mm)	.526	1	.526	6.120	.018
	Length of second dorsal spine (mm)	.708	1	.708	4.612	.038
	Length of pelvic spine (mm)	2.754	1	2.754	7.540	.009
	Length of pelvic girdle (mm)	.001	1	.001	.002	.968
	Angle of jaw	13066.742	1	13066.742	1147.813	.000
	Mouth width (mm)	.108	1	.108	1.386	.247
SL_img_I	Depth (mm)	82.063	1	82.063	420.672	.000
	Caudal peduncle length	20.145	1	20.145	54.878	.000
	Caudal peduncle depth	1.793	1	1.793	114.333	.000
	Length of first dorsal spine (mm)	2.303	1	2.303	26.788	.000
	Length of second dorsal spine (mm)	2.743	1	2.743	17.883	.000
	Length of pelvic spine (mm)	6.990	1	6.990	19.136	.000
	Length of pelvic girdle (mm)	54.281	1	54.281	136.676	.000
	Angle of jaw	61.603	1	61.603	5.411	.026
	Mouth width (mm)	8.597	1	8.597	110.739	.000
Site	Depth (mm)	4.171	1	4.171	21.384	.000
	Caudal peduncle length	1.286	1	1.286	3.504	.069
	Caudal peduncle depth	.080	1	.080	5.098	.030
	Length of first dorsal spine (mm)	.082	1	.082	.956	.334
	Length of second dorsal spine (mm)	.133	1	.133	.867	.358
	Length of pelvic spine (mm)	.036	1	.036	.100	.754
	Length of pelvic girdle (mm)	2.147	1	2.147	5.406	.026
	Angle of jaw	55.242	1	55.242	4.853	.034
	Mouth width (mm)	1.299	1	1.299	16.729	.000
Error	Depth (mm)	7.218	37	.195		

	Caudal peduncle length	13.582	37	.367		
	Caudal peduncle depth	.580	37	.016		
	Length of first dorsal spine (mm)	3.181	37	.086		
	Length of second dorsal spine (mm)	5.676	37	.153		
	Length of pelvic spine (mm)	13.514	37	.365		
	Length of pelvic girdle (mm)	14.694	37	.397		
	Angle of jaw	421.209	37	11.384		
	Mouth width (mm)	2.872	37	.078		
Total	Depth (mm)	3604.676	40			
	Caudal peduncle length	1646.396	40			
	Caudal peduncle depth	98.089	40			
	Length of first dorsal spine (mm)	253.903	40			
	Length of second dorsal spine (mm)	317.124	40			
	Length of pelvic spine (mm)	936.987	40			
	Length of pelvic girdle (mm)	2751.835	40			
	Angle of jaw	747658.509	40			
	Mouth width (mm)	344.630	40			
Corrected Total	Depth (mm)	112.636	39			
	Caudal peduncle length	33.750	39			
	Caudal peduncle depth	2.855	39			
	Length of first dorsal spine (mm)	6.049	39			
	Length of second dorsal spine (mm)	8.433	39			
	Length of pelvic spine (mm)	20.851	39			
	Length of pelvic girdle (mm)	82.860	39			
	Angle of jaw	584.753	39			
	Mouth width (mm)	15.728	39			

a. R Squared = .936 (Adjusted R Squared = .932)
b. R Squared = .598 (Adjusted R Squared = .576)
c. R Squared = .797 (Adjusted R Squared = .786)
d. R Squared = .474 (Adjusted R Squared = .446)
e. R Squared = .327 (Adjusted R Squared = .290)
f. R Squared = .352 (Adjusted R Squared = .317)
g. R Squared = .823 (Adjusted R Squared = .813)
h. R Squared = .280 (Adjusted R Squared = .241)

i. R Squared = .817 (Adjusted R Squared = .807)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	105.508[a]	3	35.169	177.614	.000
	Caudal peduncle length	20.354[b]	3	6.785	18.232	.000
	Caudal peduncle depth	2.347[c]	3	.782	55.470	.000
	Length of first dorsal spine (mm)	2.942[d]	3	.981	11.362	.000
	Length of second dorsal spine (mm)	2.756[e]	3	.919	5.827	.002
	Length of pelvic spine (mm)	7.697[f]	3	2.566	7.022	.001
	Length of pelvic girdle (mm)	68.206[g]	3	22.735	55.855	.000
	Angle of jaw	176.679[h]	3	58.893	5.196	.004
	Mouth width (mm)	13.036[i]	3	4.345	58.109	.000
Intercept	Depth (mm)	.392	1	.392	1.978	.168
	Caudal peduncle length	1.347	1	1.347	3.621	.065
	Caudal peduncle depth	.008	1	.008	.588	.448
	Length of first dorsal spine (mm)	.468	1	.468	5.425	.026
	Length of second dorsal spine (mm)	.694	1	.694	4.402	.043
	Length of pelvic spine (mm)	2.969	1	2.969	8.125	.007
	Length of pelvic girdle (mm)	9.707E-7	1	9.707E-7	.000	.999
	Angle of jaw	12743.765	1	12743.765	1124.245	.000
	Mouth width (mm)	.073	1	.073	.979	.329
Site	Depth (mm)	8.243E-6	1	8.243E-6	.000	.995
	Caudal peduncle length	.350	1	.350	.941	.338
	Caudal peduncle depth	.095	1	.095	6.703	.014
	Length of first dorsal spine (mm)	.051	1	.051	.596	.445
	Length of second dorsal spine (mm)	.004	1	.004	.024	.878
	Length of pelvic spine (mm)	.320	1	.320	.876	.355
	Length of pelvic girdle (mm)	.000	1	.000	.000	.982
	Angle of jaw	6.241	1	6.241	.551	.463
	Mouth width (mm)	.344	1	.344	4.605	.039

SL_img_I	Depth (mm)	81.901	1	81.901	413.619	.000
	Caudal peduncle length	20.329	1	20.329	54.630	.000
	Caudal peduncle depth	1.716	1	1.716	121.659	.000
	Length of first dorsal spine (mm)	2.358	1	2.358	27.322	.000
	Length of second dorsal spine (mm)	2.724	1	2.724	17.279	.000
	Length of pelvic spine (mm)	6.659	1	6.659	18.224	.000
	Length of pelvic girdle (mm)	54.120	1	54.120	132.960	.000
	Angle of jaw	66.227	1	66.227	5.843	.021
	Mouth width (mm)	8.312	1	8.312	111.161	.000
Site * SL_img_I	Depth (mm)	.089	1	.089	.452	.506
	Caudal peduncle length	.185	1	.185	.498	.485
	Caudal peduncle depth	.072	1	.072	5.131	.030
	Length of first dorsal spine (mm)	.074	1	.074	.855	.361
	Length of second dorsal spine (mm)	6.610E-5	1	6.610E-5	.000	.984
	Length of pelvic spine (mm)	.360	1	.360	.986	.327
	Length of pelvic girdle (mm)	.041	1	.041	.101	.753
	Angle of jaw	13.135	1	13.135	1.159	.289
	Mouth width (mm)	.180	1	.180	2.412	.129
Error	Depth (mm)	7.128	36	.198		
	Caudal peduncle length	13.396	36	.372		
	Caudal peduncle depth	.508	36	.014		
	Length of first dorsal spine (mm)	3.107	36	.086		
	Length of second dorsal spine (mm)	5.676	36	.158		
	Length of pelvic spine (mm)	13.154	36	.365		
	Length of pelvic girdle (mm)	14.653	36	.407		
	Angle of jaw	408.074	36	11.335		
	Mouth width (mm)	2.692	36	.075		
Total	Depth (mm)	3604.676	40			
	Caudal peduncle length	1646.396	40			
	Caudal peduncle depth	98.089	40			
	Length of first dorsal spine (mm)	253.903	40			

	Length of second dorsal spine (mm)	317.124	40			
	Length of pelvic spine (mm)	936.987	40			
	Length of pelvic girdle (mm)	2751.835	40			
	Angle of jaw	747658.509	40			
	Mouth width (mm)	344.630	40			
Corrected Total	Depth (mm)	112.636	39			
	Caudal peduncle length	33.750	39			
	Caudal peduncle depth	2.855	39			
	Length of first dorsal spine (mm)	6.049	39			
	Length of second dorsal spine (mm)	8.433	39			
	Length of pelvic spine (mm)	20.851	39			
	Length of pelvic girdle (mm)	82.860	39			
	Angle of jaw	584.753	39			
	Mouth width (mm)	15.728	39			

a. R Squared = .937 (Adjusted R Squared = .931)
b. R Squared = .603 (Adjusted R Squared = .570)
c. R Squared = .822 (Adjusted R Squared = .807)
d. R Squared = .486 (Adjusted R Squared = .444)
e. R Squared = .327 (Adjusted R Squared = .271)
f. R Squared = .369 (Adjusted R Squared = .317)
g. R Squared = .823 (Adjusted R Squared = .808)
h. R Squared = .302 (Adjusted R Squared = .244)
i. R Squared = .829 (Adjusted R Squared = .815)

Kendoon

Width: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17.132[a]	2	8.566	40.749	.000
Intercept	.715	1	.715	3.400	.071
SL_img_a	16.463	1	16.463	78.314	.000
Site	3.022	1	3.022	14.378	.000
Error	10.301	49	.210		
Total	1566.191	52			
Corrected Total	27.433	51			

a. R Squared = .625 (Adjusted R Squared = .609)

Gill raker length: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.267[a]	2	.133	9.338	.001
Intercept	.006	1	.006	.427	.519
SL_img_l	.103	1	.103	7.213	.012
Site	.170	1	.170	11.875	.002
Error	.414	29	.014		
Total	27.202	32			
Corrected Total	.681	31			

a. R Squared = .392 (Adjusted R Squared = .350)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	38.965[a]	2	19.483	127.489	.000
	CPL	8.765[b]	2	4.382	17.950	.000
	CPD	.963[c]	2	.482	33.811	.000
	Length of first dorsal spine (mm)	1.963[d]	2	.982	10.686	.000
	Length of pelvic spine (mm)	3.948[e]	2	1.974	7.814	.001
	Length of pelvic girdle (mm)	34.605[f]	2	17.303	54.333	.000
	Angle of jaw	68.465[g]	2	34.232	3.519	.037
	Mouth	5.113[h]	2	2.557	36.654	.000
	Average number of lateral plates	1.906[i]	2	.953	1.655	.202
Intercept	Depth (mm)	.428	1	.428	2.801	.101
	CPL	.829	1	.829	3.396	.072
	CPD	1.438E-5	1	1.438E-5	.001	.975
	Length of first dorsal spine (mm)	.019	1	.019	.208	.650
	Length of pelvic spine (mm)	.523	1	.523	2.070	.157
	Length of pelvic girdle (mm)	.066	1	.066	.209	.650
	Angle of jaw	4778.993	1	4778.993	491.285	.000
	Mouth	.131	1	.131	1.878	.177
	Average number of lateral plates	1.782	1	1.782	3.095	.085
SL_img_l	Depth (mm)	38.803	1	38.803	253.916	.000
	CPL	8.532	1	8.532	34.946	.000
	CPD	.903	1	.903	63.422	.000
	Length of first dorsal spine (mm)	1.962	1	1.962	21.360	.000
	Length of pelvic spine (mm)	3.606	1	3.606	14.272	.000

	Length of pelvic girdle (mm)	30.270	1	30.270	95.052	.000
	Angle of jaw	66.160	1	66.160	6.801	.012
	Mouth	4.379	1	4.379	62.782	.000
	Average number of lateral plates	1.822	1	1.822	3.165	.082
Site	Depth (mm)	1.368	1	1.368	8.952	.004
	CPL	.705	1	.705	2.889	.096
	CPD	.130	1	.130	9.109	.004
	Length of first dorsal spine (mm)	.020	1	.020	.213	.647
	Length of pelvic spine (mm)	.665	1	.665	2.632	.111
	Length of pelvic girdle (mm)	1.921	1	1.921	6.034	.018
	Angle of jaw	6.310	1	6.310	.649	.425
	Mouth	1.230	1	1.230	17.631	.000
	Average number of lateral plates	.014	1	.014	.025	.875
Error	Depth (mm)	7.335	48	.153		
	CPL	11.719	48	.244		
	CPD	.684	48	.014		
	Length of first dorsal spine (mm)	4.410	48	.092		
	Length of pelvic spine (mm)	12.126	48	.253		
	Length of pelvic girdle (mm)	15.286	48	.318		
	Angle of jaw	466.922	48	9.728		
	Mouth	3.348	48	.070		
	Average number of lateral plates	27.633	48	.576		
Total	Depth (mm)	5158.508	51			
	CPL	2432.199	51			
	CPD	147.240	51			
	Length of first dorsal spine (mm)	399.188	51			
	Length of pelvic spine (mm)	1138.063	51			
	Length of pelvic girdle (mm)	4726.273	51			
	Angle of jaw	993387.613	51			
	Mouth	486.250	51			
	Average number of lateral plates	1235.500	51			
Corrected Total	Depth (mm)	46.301	50			
	CPL	20.483	50			
	CPD	1.647	50			
	Length of first dorsal spine (mm)	6.373	50			

	Length of pelvic spine (mm)	16.074	50			
	Length of pelvic girdle (mm)	49.891	50			
	Angle of jaw	535.386	50			
	Mouth	8.462	50			
	Average number of lateral plates	29.539	50			

a. R Squared = .842 (Adjusted R Squared = .835)
b. R Squared = .428 (Adjusted R Squared = .404)
c. R Squared = .585 (Adjusted R Squared = .568)
d. R Squared = .308 (Adjusted R Squared = .279)
e. R Squared = .246 (Adjusted R Squared = .214)
f. R Squared = .694 (Adjusted R Squared = .681)
g. R Squared = .128 (Adjusted R Squared = .092)
h. R Squared = .604 (Adjusted R Squared = .588)
i. R Squared = .065 (Adjusted R Squared = .026)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	39.066[a]	3	13.022	84.593	.000
	CPL	9.207[b]	3	3.069	12.791	.000
	CPD	.964[c]	3	.321	22.117	.000
	Length of first dorsal spine (mm)	1.969[d]	3	.656	7.004	.001
	Length of pelvic spine (mm)	4.004[e]	3	1.335	5.198	.003
	Length of pelvic girdle (mm)	35.358[f]	3	11.786	38.115	.000
	Angle of jaw	75.752[g]	3	25.251	2.582	.064
	Mouth	5.132[h]	3	1.711	24.144	.000
	Average number of lateral plates	1.911[i]	3	.637	1.084	.365
Intercept	Depth (mm)	.525	1	.525	3.412	.071
	CPL	1.188	1	1.188	4.953	.031
	CPD	.000	1	.000	.014	.908
	Length of first dorsal spine (mm)	.010	1	.010	.110	.741
	Length of pelvic spine (mm)	.345	1	.345	1.342	.253
	Length of pelvic girdle (mm)	.006	1	.006	.019	.892
	Angle of jaw	4273.259	1	4273.259	436.963	.000
	Mouth	.149	1	.149	2.107	.153
	Average number of lateral plates	1.481	1	1.481	2.519	.119
Site	Depth (mm)	.049	1	.049	.321	.574
	CPL	.533	1	.533	2.221	.143
	CPD	3.870E-10	1	3.870E-10	.000	1.000

	Length of first dorsal spine (mm)	.004	1	.004	.042	.838
	Length of pelvic spine (mm)	.029	1	.029	.115	.736
	Length of pelvic girdle (mm)	.569	1	.569	1.839	.182
	Angle of jaw	8.360	1	8.360	.855	.360
	Mouth	.002	1	.002	.030	.863
	Average number of lateral plates	.007	1	.007	.011	.916
SL_img_l	Depth (mm)	34.749	1	34.749	225.737	.000
	CPL	6.049	1	6.049	25.213	.000
	CPD	.796	1	.796	54.780	.000
	Length of first dorsal spine (mm)	1.760	1	1.760	18.787	.000
	Length of pelvic spine (mm)	3.421	1	3.421	13.322	.001
	Length of pelvic girdle (mm)	22.820	1	22.820	73.798	.000
	Angle of jaw	42.665	1	42.665	4.363	.042
	Mouth	3.964	1	3.964	55.956	.000
	Average number of lateral plates	1.636	1	1.636	2.782	.102
Site * SL_img_l	Depth (mm)	.100	1	.100	.652	.424
	CPL	.442	1	.442	1.844	.181
	CPD	.001	1	.001	.057	.812
	Length of first dorsal spine (mm)	.006	1	.006	.059	.809
	Length of pelvic spine (mm)	.056	1	.056	.219	.642
	Length of pelvic girdle (mm)	.753	1	.753	2.434	.125
	Angle of jaw	7.287	1	7.287	.745	.392
	Mouth	.018	1	.018	.258	.614
	Average number of lateral plates	.005	1	.005	.009	.925
Error	Depth (mm)	7.235	47	.154		
	CPL	11.276	47	.240		
	CPD	.683	47	.015		
	Length of first dorsal spine (mm)	4.404	47	.094		
	Length of pelvic spine (mm)	12.070	47	.257		
	Length of pelvic girdle (mm)	14.533	47	.309		
	Angle of jaw	459.634	47	9.779		
	Mouth	3.330	47	.071		
	Average number of lateral plates	27.628	47	.588		
Total	Depth (mm)	5158.508	51			
	CPL	2432.199	51			
	CPD	147.240	51			

	Length of first dorsal spine (mm)	399.188	51			
	Length of pelvic spine (mm)	1138.063	51			
	Length of pelvic girdle (mm)	4726.273	51			
	Angle of jaw	993387.613	51			
	Mouth	486.250	51			
	Average number of lateral plates	1235.500	51			
Corrected Total	Depth (mm)	46.301	50			
	CPL	20.483	50			
	CPD	1.647	50			
	Length of first dorsal spine (mm)	6.373	50			
	Length of pelvic spine (mm)	16.074	50			
	Length of pelvic girdle (mm)	49.891	50			
	Angle of jaw	535.386	50			
	Mouth	8.462	50			
	Average number of lateral plates	29.539	50			

a. R Squared = .844 (Adjusted R Squared = .834)
b. R Squared = .449 (Adjusted R Squared = .414)
c. R Squared = .585 (Adjusted R Squared = .559)
d. R Squared = .309 (Adjusted R Squared = .265)
e. R Squared = .249 (Adjusted R Squared = .201)
f. R Squared = .709 (Adjusted R Squared = .690)
g. R Squared = .141 (Adjusted R Squared = .087)
h. R Squared = .606 (Adjusted R Squared = .581)
i. R Squared = .065 (Adjusted R Squared = .005)

Stithians

Width: with interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47.354[a]	3	15.785	97.016	.000
Intercept	1.053	1	1.053	6.474	.013
Site	1.582	1	1.582	9.720	.003
SL_img_a	42.201	1	42.201	259.378	.000
Site * SL_img_a	.884	1	.884	5.434	.023
Error	10.250	63	.163		
Total	2418.993	67			
Corrected Total	57.604	66			

a. R Squared = .822 (Adjusted R Squared = .814)

Gill raker length: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.143[a]	2	.071	3.306	.048
Intercept	.003	1	.003	.141	.710
SL_img_l	.084	1	.084	3.913	.055
Site	.056	1	.056	2.612	.115
Error	.798	37	.022		
Total	32.869	40			
Corrected Total	.940	39			

a. R Squared = .152 (Adjusted R Squared = .106)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	126.585[a]	2	63.292	209.840	.000
	CPL	34.026[b]	2	17.013	51.150	.000
	CPD	2.254[c]	2	1.127	89.296	.000
	Length of first dorsal spine (mm)	4.670[d]	2	2.335	19.273	.000
	Length of second dorsal spine (mm)	7.913[e]	2	3.956	25.610	.000
	Length of pelvic spine (mm)	19.786[f]	2	9.893	20.652	.000
	Length of pelvic girdle (mm)	121.243[g]	2	60.621	123.496	.000
	Angle of jaw	64.579[h]	2	32.290	4.738	.012
	Mouth	7.784[i]	2	3.892	57.363	.000
	Average number of lateral plates	1.405[j]	2	.703	.889	.416
Intercept	Depth (mm)	.778	1	.778	2.579	.113
	CPL	.458	1	.458	1.377	.245
	CPD	.084	1	.084	6.678	.012
	Length of first dorsal spine (mm)	.750	1	.750	6.188	.015
	Length of second dorsal spine (mm)	.268	1	.268	1.734	.193
	Length of pelvic spine (mm)	.701	1	.701	1.464	.231
	Length of pelvic girdle (mm)	1.521	1	1.521	3.100	.083
	Angle of jaw	15363.677	1	15363.677	2254.399	.000
	Mouth	.065	1	.065	.961	.331
	Average number of lateral plates	37.677	1	37.677	47.677	.000
SL_img_l	Depth (mm)	126.565	1	126.565	419.614	.000
	CPL	29.052	1	29.052	87.345	.000
	CPD	2.235	1	2.235	177.088	.000

	Length of first dorsal spine (mm)	4.654	1	4.654	38.417	.000
	Length of second dorsal spine (mm)	7.897	1	7.897	51.117	.000
	Length of pelvic spine (mm)	19.651	1	19.651	41.023	.000
	Length of pelvic girdle (mm)	117.073	1	117.073	238.496	.000
	Angle of jaw	24.751	1	24.751	3.632	.061
	Mouth	7.298	1	7.298	107.557	.000
	Average number of lateral plates	1.295	1	1.295	1.639	.205
Site	Depth (mm)	5.423	1	5.423	17.978	.000
	CPL	.989	1	.989	2.972	.090
	CPD	.214	1	.214	16.927	.000
	Length of first dorsal spine (mm)	.123	1	.123	1.018	.317
	Length of second dorsal spine (mm)	.243	1	.243	1.575	.214
	Length of pelvic spine (mm)	1.768	1	1.768	3.691	.059
	Length of pelvic girdle (mm)	.144	1	.144	.294	.590
	Angle of jaw	52.537	1	52.537	7.709	.007
	Mouth	1.620	1	1.620	23.875	.000
	Average number of lateral plates	.328	1	.328	.415	.522
Error	Depth (mm)	19.304	64	.302		
	CPL	21.287	64	.333		
	CPD	.808	64	.013		
	Length of first dorsal spine (mm)	7.753	64	.121		
	Length of second dorsal spine (mm)	9.887	64	.154		
	Length of pelvic spine (mm)	30.658	64	.479		
	Length of pelvic girdle (mm)	31.416	64	.491		
	Angle of jaw	436.158	64	6.815		
	Mouth	4.342	64	.068		
	Average number of lateral plates	50.576	64	.790		
Total	Depth (mm)	8223.216	67			
	CPL	2851.847	67			
	CPD	242.808	67			
	Length of first dorsal spine (mm)	703.055	67			
	Length of second dorsal spine (mm)	853.033	67			
	Length of pelvic spine (mm)	2140.267	67			

	Length of pelvic girdle (mm)	7087.481	67			
	Angle of jaw	1265127.602	67			
	Mouth	666.580	67			
	Average number of lateral plates	1966.052	67			
Corrected Total	Depth (mm)	145.888	66			
	CPL	55.313	66			
	CPD	3.062	66			
	Length of first dorsal spine (mm)	12.423	66			
	Length of second dorsal spine (mm)	17.800	66			
	Length of pelvic spine (mm)	50.443	66			
	Length of pelvic girdle (mm)	152.659	66			
	Angle of jaw	500.738	66			
	Mouth	12.127	66			
	Average number of lateral plates	51.981	66			

a. R Squared = .868 (Adjusted R Squared = .864)
b. R Squared = .615 (Adjusted R Squared = .603)
c. R Squared = .736 (Adjusted R Squared = .728)
d. R Squared = .376 (Adjusted R Squared = .356)
e. R Squared = .445 (Adjusted R Squared = .427)
f. R Squared = .392 (Adjusted R Squared = .373)
g. R Squared = .794 (Adjusted R Squared = .788)
h. R Squared = .129 (Adjusted R Squared = .102)
i. R Squared = .642 (Adjusted R Squared = .631)
j. R Squared = .027 (Adjusted R Squared = -.003)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	127.264[a]	3	42.421	143.493	.000
	CPL	34.124[b]	3	11.375	33.820	.000
	CPD	2.260[c]	3	.753	59.253	.000
	Length of first dorsal spine (mm)	4.672[d]	3	1.557	12.657	.000
	Length of second dorsal spine (mm)	8.014[e]	3	2.671	17.199	.000
	Length of pelvic spine (mm)	19.798[f]	3	6.599	13.567	.000
	Length of pelvic girdle (mm)	121.269[g]	3	40.423	81.130	.000
	Angle of jaw	66.039[h]	3	22.013	3.190	.030
	Mouth	7.882[i]	3	2.627	38.997	.000
	Average number of lateral plates	3.363[j]	3	1.121	1.453	.236

Intercept	Depth (mm)	.602	1	.602	2.038	.158
	CPL	.503	1	.503	1.497	.226
	CPD	.078	1	.078	6.096	.016
	Length of first dorsal spine (mm)	.729	1	.729	5.929	.018
	Length of second dorsal spine (mm)	.226	1	.226	1.455	.232
	Length of pelvic spine (mm)	.714	1	.714	1.467	.230
	Length of pelvic girdle (mm)	1.548	1	1.548	3.106	.083
	Angle of jaw	15176.080	1	15176.080	2199.436	.000
	Mouth	.085	1	.085	1.258	.266
	Average number of lateral plates	39.214	1	39.214	50.815	.000
Site	Depth (mm)	1.191	1	1.191	4.027	.049
	CPL	.038	1	.038	.112	.739
	CPD	.001	1	.001	.054	.816
	Length of first dorsal spine (mm)	1.244E-5	1	1.244E-5	.000	.992
	Length of second dorsal spine (mm)	.067	1	.067	.430	.514
	Length of pelvic spine (mm)	.071	1	.071	.145	.704
	Length of pelvic girdle (mm)	.014	1	.014	.027	.869
	Angle of jaw	4.199	1	4.199	.609	.438
	Mouth	.211	1	.211	3.139	.081
	Average number of lateral plates	1.749	1	1.749	2.267	.137
SL_img_l	Depth (mm)	121.147	1	121.147	409.788	.000
	CPL	27.935	1	27.935	83.057	.000
	CPD	2.222	1	2.222	174.733	.000
	Length of first dorsal spine (mm)	4.582	1	4.582	37.239	.000
	Length of second dorsal spine (mm)	7.989	1	7.989	51.432	.000
	Length of pelvic spine (mm)	19.084	1	19.084	39.232	.000
	Length of pelvic girdle (mm)	115.053	1	115.053	230.914	.000
	Angle of jaw	22.499	1	22.499	3.261	.076
	Mouth	6.896	1	6.896	102.359	.000
	Average number of lateral plates	1.773	1	1.773	2.298	.135
Site * SL_img_l	Depth (mm)	.679	1	.679	2.296	.135
	CPL	.098	1	.098	.291	.591
	CPD	.007	1	.007	.516	.475
	Length of first dorsal spine (mm)	.002	1	.002	.016	.898
	Length of second dorsal spine (mm)	.101	1	.101	.653	.422

	Length of pelvic spine (mm)	.012	1	.012	.025	.874
	Length of pelvic girdle (mm)	.026	1	.026	.053	.819
	Angle of jaw	1.459	1	1.459	.211	.647
	Mouth	.098	1	.098	1.453	.233
	Average number of lateral plates	1.958	1	1.958	2.538	.116
Error	Depth (mm)	18.625	63	.296		
	CPL	21.189	63	.336		
	CPD	.801	63	.013		
	Length of first dorsal spine (mm)	7.751	63	.123		
	Length of second dorsal spine (mm)	9.786	63	.155		
	Length of pelvic spine (mm)	30.645	63	.486		
	Length of pelvic girdle (mm)	31.390	63	.498		
	Angle of jaw	434.699	63	6.900		
	Mouth	4.245	63	.067		
	Average number of lateral plates	48.618	63	.772		
Total	Depth (mm)	8223.216	67			
	CPL	2851.847	67			
	CPD	242.808	67			
	Length of first dorsal spine (mm)	703.055	67			
	Length of second dorsal spine (mm)	853.033	67			
	Length of pelvic spine (mm)	2140.267	67			
	Length of pelvic girdle (mm)	7087.481	67			
	Angle of jaw	1265127.602	67			
	Mouth	666.580	67			
	Average number of lateral plates	1966.052	67			
Corrected Total	Depth (mm)	145.888	66			
	CPL	55.313	66			
	CPD	3.062	66			
	Length of first dorsal spine (mm)	12.423	66			
	Length of second dorsal spine (mm)	17.800	66			
	Length of pelvic spine (mm)	50.443	66			
	Length of pelvic girdle (mm)	152.659	66			
	Angle of jaw	500.738	66			
	Mouth	12.127	66			

	Average number of lateral plates	51.981	66			

a. R Squared = .872 (Adjusted R Squared = .866)
b. R Squared = .617 (Adjusted R Squared = .599)
c. R Squared = .738 (Adjusted R Squared = .726)
d. R Squared = .376 (Adjusted R Squared = .346)
e. R Squared = .450 (Adjusted R Squared = .424)
f. R Squared = .392 (Adjusted R Squared = .364)
g. R Squared = .794 (Adjusted R Squared = .785)
h. R Squared = .132 (Adjusted R Squared = .091)
i. R Squared = .650 (Adjusted R Squared = .633)
j. R Squared = .065 (Adjusted R Squared = .020)

Thornton

Width: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.951[a]	2	13.975	101.798	.000
Intercept	.079	1	.079	.575	.453
SL_img_a	26.998	1	26.998	196.658	.000
Site	.829	1	.829	6.035	.018
Error	5.766	42	.137		
Total	962.874	45			
Corrected Total	33.717	44			

a. R Squared = .829 (Adjusted R Squared = .821)

Gill raker length: no interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.636[a]	2	.818	47.008	.000
Intercept	.109	1	.109	6.264	.016
SL_img_l	.332	1	.332	19.092	.000
Site	1.252	1	1.252	71.937	.000
Error	.713	41	.017		
Total	39.145	44			
Corrected Total	2.349	43			

a. R Squared = .696 (Adjusted R Squared = .682)

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	100.539[a]	2	50.270	292.353	.000
	CPL	38.129[b]	2	19.064	86.765	.000
	CPD	4.542[c]	2	2.271	112.578	.000
	Length of first dorsal spine (mm)	5.902[d]	2	2.951	30.135	.000
	Length of second dorsal spine (mm)	8.102[e]	2	4.051	41.321	.000

	Length of pelvic spine (mm)	21.209[f]	2	10.605	38.511	.000
	Length of pelvic girdle (mm)	94.592[g]	2	47.296	125.408	.000
	Angle of jaw	64.313[h]	2	32.157	2.823	.071
	Mouth	12.508[i]	2	6.254	79.654	.000
Intercept	Depth (mm)	.005	1	.005	.029	.866
	CPL	1.550	1	1.550	7.056	.011
	CPD	.066	1	.066	3.278	.077
	Length of first dorsal spine (mm)	.495	1	.495	5.053	.030
	Length of second dorsal spine (mm)	.470	1	.470	4.796	.034
	Length of pelvic spine (mm)	.736	1	.736	2.674	.110
	Length of pelvic girdle (mm)	.161	1	.161	.428	.517
	Angle of jaw	19013.702	1	19013.702	1668.928	.000
	Mouth	.286	1	.286	3.641	.063
SL_img_l	Depth (mm)	100.017	1	100.017	581.667	.000
	CPL	36.619	1	36.619	166.660	.000
	CPD	4.275	1	4.275	211.939	.000
	Length of first dorsal spine (mm)	5.472	1	5.472	55.876	.000
	Length of second dorsal spine (mm)	6.891	1	6.891	70.294	.000
	Length of pelvic spine (mm)	20.132	1	20.132	73.110	.000
	Length of pelvic girdle (mm)	93.598	1	93.598	248.182	.000
	Angle of jaw	26.493	1	26.493	2.325	.135
	Mouth	11.532	1	11.532	146.875	.000
Site	Depth (mm)	.580	1	.580	3.375	.073
	CPL	1.452	1	1.452	6.608	.014
	CPD	.258	1	.258	12.809	.001
	Length of first dorsal spine (mm)	.418	1	.418	4.273	.045
	Length of second dorsal spine (mm)	1.188	1	1.188	12.120	.001
	Length of pelvic spine (mm)	1.041	1	1.041	3.781	.059
	Length of pelvic girdle (mm)	1.070	1	1.070	2.836	.100
	Angle of jaw	38.066	1	38.066	3.341	.075
	Mouth	1.002	1	1.002	12.768	.001
Error	Depth (mm)	7.222	42	.172		
	CPL	9.228	42	.220		
	CPD	.847	42	.020		
	Length of first dorsal spine (mm)	4.113	42	.098		

	Length of second dorsal spine (mm)	4.117	42	.098		
	Length of pelvic spine (mm)	11.566	42	.275		
	Length of pelvic girdle (mm)	15.840	42	.377		
	Angle of jaw	478.496	42	11.393		
	Mouth	3.298	42	.079		
Total	Depth (mm)	4211.492	45			
	CPL	2283.405	45			
	CPD	142.363	45			
	Length of first dorsal spine (mm)	399.801	45			
	Length of second dorsal spine (mm)	474.556	45			
	Length of pelvic spine (mm)	1232.992	45			
	Length of pelvic girdle (mm)	3683.074	45			
	Angle of jaw	872419.227	45			
	Mouth	353.100	45			
Corrected Total	Depth (mm)	107.761	44			
	CPL	47.357	44			
	CPD	5.389	44			
	Length of first dorsal spine (mm)	10.016	44			
	Length of second dorsal spine (mm)	12.219	44			
	Length of pelvic spine (mm)	32.775	44			
	Length of pelvic girdle (mm)	110.431	44			
	Angle of jaw	542.809	44			
	Mouth	15.806	44			

a. R Squared = .933 (Adjusted R Squared = .930)
b. R Squared = .805 (Adjusted R Squared = .796)
c. R Squared = .843 (Adjusted R Squared = .835)
d. R Squared = .589 (Adjusted R Squared = .570)
e. R Squared = .663 (Adjusted R Squared = .647)
f. R Squared = .647 (Adjusted R Squared = .630)
g. R Squared = .857 (Adjusted R Squared = .850)
h. R Squared = .118 (Adjusted R Squared = .077)
i. R Squared = .791 (Adjusted R Squared = .781)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth (mm)	100.670[a]	3	33.557	194.028	.000
	CPL	38.867[b]	3	12.956	62.566	.000
	CPD	4.609[c]	3	1.536	80.805	.000

	Length of first dorsal spine (mm)	6.548[d]	3	2.183	25.811	.000
	Length of second dorsal spine (mm)	8.574[e]	3	2.858	32.154	.000
	Length of pelvic spine (mm)	24.125[f]	3	8.042	38.114	.000
	Length of pelvic girdle (mm)	94.645[g]	3	31.548	81.939	.000
	Angle of jaw	72.775[h]	3	24.258	2.116	.113
	Mouth	12.581[i]	3	4.194	53.324	.000
Intercept	Depth (mm)	.046	1	.046	.267	.608
	CPL	.599	1	.599	2.893	.097
	CPD	.117	1	.117	6.155	.017
	Length of first dorsal spine (mm)	.093	1	.093	1.098	.301
	Length of second dorsal spine (mm)	.114	1	.114	1.277	.265
	Length of pelvic spine (mm)	.005	1	.005	.022	.882
	Length of pelvic girdle (mm)	.213	1	.213	.554	.461
	Angle of jaw	15411.451	1	15411.451	1344.305	.000
	Mouth	.139	1	.139	1.773	.190
Site	Depth (mm)	.216	1	.216	1.248	.270
	CPL	.466	1	.466	2.252	.141
	CPD	.035	1	.035	1.831	.183
	Length of first dorsal spine (mm)	.498	1	.498	5.888	.020
	Length of second dorsal spine (mm)	.280	1	.280	3.147	.083
	Length of pelvic spine (mm)	2.398	1	2.398	11.363	.002
	Length of pelvic girdle (mm)	.140	1	.140	.363	.550
	Angle of jaw	13.994	1	13.994	1.221	.276
	Mouth	.016	1	.016	.209	.650
SL_img_l	Depth (mm)	85.139	1	85.139	492.283	.000
	CPL	34.249	1	34.249	165.398	.000
	CPD	3.942	1	3.942	207.329	.000
	Length of first dorsal spine (mm)	6.054	1	6.054	71.584	.000
	Length of second dorsal spine (mm)	7.134	1	7.134	80.261	.000
	Length of pelvic spine (mm)	22.934	1	22.934	108.701	.000
	Length of pelvic girdle (mm)	78.790	1	78.790	204.636	.000
	Angle of jaw	34.727	1	34.727	3.029	.089
	Mouth	8.808	1	8.808	111.997	.000
Site * SL_img_l	Depth (mm)	.131	1	.131	.757	.389
	CPL	.738	1	.738	3.566	.066

	CPD	.068	1	.068	3.556	.066
	Length of first dorsal spine (mm)	.646	1	.646	7.638	.009
	Length of second dorsal spine (mm)	.473	1	.473	5.319	.026
	Length of pelvic spine (mm)	2.915	1	2.915	13.817	.001
	Length of pelvic girdle (mm)	.054	1	.054	.139	.711
	Angle of jaw	8.462	1	8.462	.738	.395
	Mouth	.073	1	.073	.930	.341
Error	Depth (mm)	7.091	41	.173		
	CPL	8.490	41	.207		
	CPD	.780	41	.019		
	Length of first dorsal spine (mm)	3.467	41	.085		
	Length of second dorsal spine (mm)	3.645	41	.089		
	Length of pelvic spine (mm)	8.650	41	.211		
	Length of pelvic girdle (mm)	15.786	41	.385		
	Angle of jaw	470.034	41	11.464		
	Mouth	3.225	41	.079		
Total	Depth (mm)	4211.492	45			
	CPL	2283.405	45			
	CPD	142.363	45			
	Length of first dorsal spine (mm)	399.801	45			
	Length of second dorsal spine (mm)	474.556	45			
	Length of pelvic spine (mm)	1232.992	45			
	Length of pelvic girdle (mm)	3683.074	45			
	Angle of jaw	872419.227	45			
	Mouth	353.100	45			
Corrected Total	Depth (mm)	107.761	44			
	CPL	47.357	44			
	CPD	5.389	44			
	Length of first dorsal spine (mm)	10.016	44			
	Length of second dorsal spine (mm)	12.219	44			
	Length of pelvic spine (mm)	32.775	44			
	Length of pelvic girdle (mm)	110.431	44			
	Angle of jaw	542.809	44			
	Mouth	15.806	44			

a. R Squared = .934 (Adjusted R Squared = .929)

b. R Squared = .821 (Adjusted R Squared = .808)
c. R Squared = .855 (Adjusted R Squared = .845)
d. R Squared = .654 (Adjusted R Squared = .628)
e. R Squared = .702 (Adjusted R Squared = .680)
f. R Squared = .736 (Adjusted R Squared = .717)
g. R Squared = .857 (Adjusted R Squared = .847)
h. R Squared = .134 (Adjusted R Squared = .071)
i. R Squared = .796 (Adjusted R Squared = .781)

CHAPTER 4

Width

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	9.157	1	9.157	.945	.343
	Error	194.820	20.107	9.689[a]		
Site	Hypothesis	139.708	2	69.854	7.234	.004
	Error	194.243	20.116	9.656[b]		
Population(Site)	Hypothesis	202.632	20	10.132	18.091	.000
	Error	411.078	734	.560[c]		

a. .954 MS(Population(Site)) + .046 MS(Error)
b. .950 MS(Population(Site)) + .050 MS(Error)
c. MS(Error)

Depth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.000	1	.000	.000	.995
	Error	182.422	21.531	8.473[a]		
Site	Hypothesis	132.923	2	66.461	7.696	.003
	Error	185.494	21.480	8.636[b]		
Population(Site)	Hypothesis	222.905	21	10.615	21.377	.000
	Error	370.911	747	.497[c]		

a. .788 MS(Population(Site)) + .212 MS(Error)
b. .804 MS(Population(Site)) + .196 MS(Error)
c. MS(Error)

First dorsal spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	2.506	1	2.506	.252	.620
	Error	214.356	21.590	9.928[a]		
Site	Hypothesis	6.751	2	3.375	.334	.720
	Error	217.570	21.538	10.102[b]		
Population(Site)	Hypothesis	259.404	21	12.353	18.735	.000
	Error	485.924	737	.659[c]		

a. .793 MS(Population(Site)) + .207 MS(Error)
b. .808 MS(Population(Site)) + .192 MS(Error)
c. MS(Error)

Second dorsal spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4.716	1	4.716	.428	.520
	Error	236.570	21.490	11.008[a]		
Site	Hypothesis	11.855	2	5.928	.529	.597
	Error	240.372	21.444	11.209[b]		
Population(Site)	Hypothesis	287.014	21	13.667	22.058	.000
	Error	456.041	736	.620[c]		

a. .796 MS(Population(Site)) + .204 MS(Error)
b. .812 MS(Population(Site)) + .188 MS(Error)
c. MS(Error)

Pelvic spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.075	1	.075	.007	.932
	Error	218.152	21.604	10.098[a]		
Site	Hypothesis	3.027	2	1.514	.147	.864
	Error	221.694	21.547	10.289[b]		
Population(Site)	Hypothesis	264.927	21	12.616	18.697	.000
	Error	503.361	746	.675[c]		

a. .789 MS(Population(Site)) + .211 MS(Error)
b. .805 MS(Population(Site)) + .195 MS(Error)
c. MS(Error)

Pelvic girdle length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	9.987	1	9.987	1.115	.303
	Error	193.784	21.641	8.955[a]		
Site	Hypothesis	30.776	2	15.388	1.687	.209
	Error	196.883	21.580	9.123[b]		
Population(Site)	Hypothesis	234.744	21	11.178	17.649	.000
	Error	472.498	746	.633[c]		

a. .789 MS(Population(Site)) + .211 MS(Error)
b. .805 MS(Population(Site)) + .195 MS(Error)
c. MS(Error)

Caudal peduncle depth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	2.130	1	2.130	.289	.596
	Error	160.928	21.831	7.371[a]		
Site	Hypothesis	48.721	2	24.360	3.244	.058
	Error	163.322	21.752	7.508[b]		
Population(Site)	Hypothesis	192.594	21	9.171	13.695	.000
	Error	500.260	747	.670[c]		

a. .788 MS(Population(Site)) + .212 MS(Error)
b. .804 MS(Population(Site)) + .196 MS(Error)
c. MS(Error)

Caudal peduncle length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	9.436	1	9.436	1.247	.276
	Error	165.791	21.912	7.566[a]		
Site	Hypothesis	15.771	2	7.885	1.023	.376
	Error	168.170	21.825	7.705[b]		
Population(Site)	Hypothesis	197.321	21	9.396	12.497	.000
	Error	561.637	747	.752[c]		

a. .788 MS(Population(Site)) + .212 MS(Error)
b. .804 MS(Population(Site)) + .196 MS(Error)
c. MS(Error)

Mouth width

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	2.539	1	2.539	.754	.394
	Error	77.852	23.126	3.366[a]		
Site	Hypothesis	120.697	2	60.349	17.647	.000
	Error	78.386	22.921	3.420[b]		
Population(Site)	Hypothesis	85.453	21	4.069	5.431	.000
	Error	559.664	747	.749[c]		

a. .788 MS(Population(Site)) + .212 MS(Error)
b. .804 MS(Population(Site)) + .196 MS(Error)
c. MS(Error)

Jaw angle

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	9699214.327	1	9699214.327	117158.116	.000
	Error	1818.046	21.960	82.787[a]		
Site	Hypothesis	15.341	2	7.670	.091	.913
	Error	1843.573	21.869	84.302[b]		
Population(Site)	Hypothesis	2156.639	21	102.697	11.873	.000
	Error	6461.354	747	8.650[c]		

a. .788 MS(Population(Site)) + .212 MS(Error)
b. .804 MS(Population(Site)) + .196 MS(Error)
c. MS(Error)

Average number of lateral plates

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	15054.237	1	15054.237	378.334	.000
	Error	854.092	21.465	39.791[a]		
Site	Hypothesis	22.762	2	11.381	.281	.758
	Error	868.688	21.420	40.554[b]		
Population(Site)	Hypothesis	1046.156	21	49.817	24.155	.000
	Error	1536.488	745	2.062[c]		

a. .790 MS(Population(Site)) + .210 MS(Error)
b. .806 MS(Population(Site)) + .194 MS(Error)
c. MS(Error)

CHAPTER 5

Common garden

Width

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	16.862	1	16.862	2.150	.159
	Error	148.378	18.922	7.842[a]		
Genotype	Hypothesis	6.603	3	2.201	.275	.843
	Error	150.950	18.863	8.002[b]		
Family(Genotype)	Hypothesis	209.349	18	11.630	20.581	.000
	Error	288.204	510	.565[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

Depth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	5.754	1	5.754	1.795	.195
	Error	67.985	21.205	3.206[a]		
Genotype	Hypothesis	51.196	3	17.065	5.234	.007
	Error	68.457	20.995	3.261[b]		
Family(Genotype)	Hypothesis	80.845	18	4.491	6.089	.000
	Error	376.210	510	.738[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

First dorsal spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.020	1	.020	.005	.946
	Error	87.022	20.598	4.225[a]		
Genotype	Hypothesis	12.302	3	4.101	.954	.433
	Error	87.852	20.429	4.300[b]		
Family(Genotype)	Hypothesis	108.092	18	6.005	7.456	.000
	Error	410.770	510	.805[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

Second dorsal spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4.515	1	4.515	.942	.343
	Error	96.855	20.200	4.795[a]		
Genotype	Hypothesis	6.866	3	2.289	.469	.707
	Error	97.953	20.056	4.884[b]		
Family(Genotype)	Hypothesis	123.670	18	6.871	8.719	.000

	Error	401.095	509	.788[c]		

a. .659 MS(Family(Genotype)) + .341 MS(Error)
b. .673 MS(Family(Genotype)) + .327 MS(Error)
c. MS(Error)

Pelvic spine length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	5.474	1	5.474	1.209	.285
	Error	85.485	18.887	4.526[a]		
Genotype	Hypothesis	48.724	3	16.241	3.467	.037
	Error	87.468	18.670	4.685[b]		
Family(Genotype)	Hypothesis	112.344	17	6.608	9.964	.000
	Error	314.381	474	.663[c]		

a. .650 MS(Family(Genotype)) + .350 MS(Error)
b. .676 MS(Family(Genotype)) + .324 MS(Error)
c. MS(Error)

Pelvic girdle length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	26.093	1	26.093	8.540	.008
	Error	64.137	20.990	3.056[a]		
Genotype	Hypothesis	4.952	3	1.651	.525	.670
	Error	64.541	20.520	3.145[b]		
Family(Genotype)	Hypothesis	71.932	17	4.231	4.838	.000
	Error	414.556	474	.875[c]		

a. .650 MS(Family(Genotype)) + .350 MS(Error)
b. .676 MS(Family(Genotype)) + .324 MS(Error)
c. MS(Error)

Caudal peduncle depth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	5.787	1	5.787	1.055	.317
	Error	108.113	19.716	5.484[a]		
Genotype	Hypothesis	29.278	3	9.759	1.746	.191
	Error	109.587	19.604	5.590[b]		
Family(Genotype)	Hypothesis	143.384	18	7.966	11.163	.000
	Error	363.215	509	.714[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

Caudal peduncle length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	11.024	1	11.024	1.458	.242
	Error	143.998	19.049	7.559[a]		
Genotype	Hypothesis	5.210	3	1.737	.225	.878
	Error	146.426	18.981	7.714[b]		

Family(Genotype)	Hypothesis	201.100	18	11.172	18.110	.000
	Error	314.002	509	.617[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

Mouth width

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3.117	1	3.117	.663	.425
	Error	93.226	19.831	4.701[a]		
Genotype	Hypothesis	57.884	3	19.295	4.028	.022
	Error	94.436	19.713	4.791[b]		
Family(Genotype)	Hypothesis	122.591	18	6.811	10.483	.000
	Error	331.337	510	.650[c]		

a. .658 MS(Family(Genotype)) + .342 MS(Error)
b. .672 MS(Family(Genotype)) + .328 MS(Error)
c. MS(Error)

Jaw angle

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3167708.853	1	3167708.853	49876.897	.000
	Error	553.124	8.709	63.511[a]		
Genotype	Hypothesis	19.897	1	19.897	.312	.591
	Error	554.755	8.694	63.811[b]		
Family(Genotype)	Hypothesis	659.739	8	82.467	8.174	.000
	Error	2592.854	257	10.089[c]		

a. .738 MS(Family(Genotype)) + .262 MS(Error)
b. .742 MS(Family(Genotype)) + .258 MS(Error)
c. MS(Error)

Average number of lateral plates

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	6165.446	1	6165.446	1610.575	.000
	Error	33.015	8.624	3.828[a]		
Genotype	Hypothesis	2.811	1	2.811	.731	.416
	Error	33.123	8.611	3.847[b]		
Family(Genotype)	Hypothesis	39.961	8	4.995	9.261	.000
	Error	138.619	257	.539[c]		

a. .738 MS(Family(Genotype)) + .262 MS(Error)
b. .742 MS(Family(Genotype)) + .258 MS(Error)
c. MS(Error)

Rearing environment

Depth and Caudal peduncle depth: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth	276.989[a]	12	23.082	138.668	.000
	CPD	9.938[b]	12	.828	57.117	.000
Intercept	Depth	.042	1	.042	.252	.616
	CPD	.009	1	.009	.595	.441
SL	Depth	230.210	1	230.210	1382.989	.000
	CPD	6.615	1	6.615	456.213	.000
Treatment	Depth	5.736	1	5.736	34.457	.000
	CPD	.204	1	.204	14.066	.000
Site	Depth	12.140	5	2.428	14.586	.000
	CPD	.781	5	.156	10.775	.000
Treatment * Site	Depth	1.027	5	.205	1.234	.292
	CPD	.067	5	.013	.926	.464
Error	Depth	59.925	360	.166		
	CPD	5.220	360	.014		
Total	Depth	34377.606	373			
	CPD	1087.288	373			
Corrected Total	Depth	336.914	372			
	CPD	15.158	372			

a. R Squared = .822 (Adjusted R Squared = .816)
b. R Squared = .656 (Adjusted R Squared = .644)

Depth and caudal peduncle depth: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Depth	281.116[a]	23	12.222	76.447	.000
	CPD	10.119[b]	23	.440	30.472	.000
Intercept	Depth	.504	1	.504	3.151	.077
	CPD	.006	1	.006	.389	.533
Treatment	Depth	1.351	1	1.351	8.449	.004
	CPD	.028	1	.028	1.962	.162
Site	Depth	2.195	5	.439	2.746	.019
	CPD	.072	5	.014	1.000	.417
SL	Depth	107.670	1	107.670	673.442	.000
	CPD	3.589	1	3.589	248.607	.000
Treatment * Site	Depth	.490	5	.098	.613	.690
	CPD	.094	5	.019	1.301	.263
Treatment * SL	Depth	1.046	1	1.046	6.542	.011
	CPD	.017	1	.017	1.187	.277
Site * SL	Depth	2.190	5	.438	2.740	.019
	CPD	.075	5	.015	1.043	.392
Treatment * Site * SL	Depth	.541	5	.108	.676	.642
	CPD	.093	5	.019	1.282	.271
Error	Depth	55.798	349	.160		

	CPD	5.039	349	.014		
Total	Depth	34377.606	373			
	CPD	1087.288	373			
Corrected Total	Depth	336.914	372			
	CPD	15.158	372			

a. R Squared = .834 (Adjusted R Squared = .823)
b. R Squared = .668 (Adjusted R Squared = .646)

CHAPTER 6

Morphology

All morphology: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Width (mm)	24.888[a]	2	12.444	155.606	.000
	Depth (mm)	75.457[b]	2	37.729	443.566	.000
	Length of first dorsal spine (mm)	5.819[c]	2	2.909	41.147	.000
	Length of second dorsal spine (mm)	4.677[d]	2	2.338	26.446	.000
	Length of jaw (mm)	14.451[e]	2	7.226	135.565	.000
	Width of mouth (mm)	5.726[f]	2	2.863	83.953	.000
	Length of head (mm)	135.177[g]	2	67.588	387.552	.000
	head depth	57.470[h]	2	28.735	352.308	.000
	Length of pelvic girdle (mm)	54.554[i]	2	27.277	82.319	.000
	Length of pelvic spine (mm)	14.309[j]	2	7.155	39.241	.000
	Length of 4th gillraker on first gill arch	1.643[k]	2	.822	51.548	.000
Intercept	Width (mm)	.601	1	.601	7.517	.008
	Depth (mm)	.259	1	.259	3.050	.087
	Length of first dorsal spine (mm)	.063	1	.063	.897	.348
	Length of second dorsal spine (mm)	.560	1	.560	6.333	.015
	Length of jaw (mm)	.539	1	.539	10.104	.003
	Width of mouth (mm)	.034	1	.034	1.003	.321
	Length of head (mm)	.683	1	.683	3.919	.053
	head depth	.234	1	.234	2.866	.097
	Length of pelvic girdle (mm)	.224	1	.224	.676	.415
	Length of pelvic spine (mm)	1.216	1	1.216	6.671	.013

	Length of 4th gillraker on first gill arch	.026	1	.026	1.646	.205
SL_img_i	Width (mm)	24.829	1	24.829	310.475	.000
	Depth (mm)	74.985	1	74.985	881.577	.000
	Length of first dorsal spine (mm)	5.806	1	5.806	82.108	.000
	Length of second dorsal spine (mm)	4.605	1	4.605	52.079	.000
	Length of jaw (mm)	14.162	1	14.162	265.707	.000
	Width of mouth (mm)	4.796	1	4.796	140.623	.000
	Length of head (mm)	134.124	1	134.124	769.071	.000
	head depth	56.808	1	56.808	696.498	.000
	Length of pelvic girdle (mm)	53.981	1	53.981	162.909	.000
	Length of pelvic spine (mm)	13.978	1	13.978	76.667	.000
	Length of 4th gillraker on first gill arch	.908	1	.908	56.993	.000
Site	Width (mm)	.440	1	.440	5.507	.023
	Depth (mm)	.796	1	.796	9.359	.004
	Length of first dorsal spine (mm)	.302	1	.302	4.266	.044
	Length of second dorsal spine (mm)	.016	1	.016	.177	.676
	Length of jaw (mm)	.023	1	.023	.438	.511
	Width of mouth (mm)	1.810	1	1.810	53.065	.000
	Length of head (mm)	1.185	1	1.185	6.795	.012
	head depth	.319	1	.319	3.911	.053
	Length of pelvic girdle (mm)	.344	1	.344	1.038	.313
	Length of pelvic spine (mm)	.012	1	.012	.068	.796
	Length of 4th gillraker on first gill arch	.449	1	.449	28.200	.000
Error	Width (mm)	4.079	51	.080		
	Depth (mm)	4.338	51	.085		
	Length of first dorsal spine (mm)	3.606	51	.071		
	Length of second dorsal spine (mm)	4.509	51	.088		
	Length of jaw (mm)	2.718	51	.053		
	Width of mouth (mm)	1.739	51	.034		
	Length of head (mm)	8.894	51	.174		
	head depth	4.160	51	.082		
	Length of pelvic girdle (mm)	16.899	51	.331		

	Length of pelvic spine (mm)	9.299	51	.182		
	Length of 4th gillraker on first gill arch	.813	51	.016		
Total	Width (mm)	1090.041	54			
	Depth (mm)	4072.087	54			
	Length of first dorsal spine (mm)	434.691	54			
	Length of second dorsal spine (mm)	515.494	54			
	Length of jaw (mm)	568.595	54			
	Width of mouth (mm)	342.470	54			
	Length of head (mm)	7096.174	54			
	head depth	3054.445	54			
	Length of pelvic girdle (mm)	3756.239	54			
	Length of pelvic spine (mm)	1440.825	54			
	Length of 4th gillraker on first gill arch	41.074	54			
Corrected Total	Width (mm)	28.967	53			
	Depth (mm)	79.795	53			
	Length of first dorsal spine (mm)	9.425	53			
	Length of second dorsal spine (mm)	9.186	53			
	Length of jaw (mm)	17.170	53			
	Width of mouth (mm)	7.465	53			
	Length of head (mm)	144.071	53			
	head depth	61.630	53			
	Length of pelvic girdle (mm)	71.453	53			
	Length of pelvic spine (mm)	23.608	53			
	Length of 4th gillraker on first gill arch	2.456	53			

a. R Squared = .859 (Adjusted R Squared = .854)
b. R Squared = .946 (Adjusted R Squared = .944)
c. R Squared = .617 (Adjusted R Squared = .602)
d. R Squared = .509 (Adjusted R Squared = .490)
e. R Squared = .842 (Adjusted R Squared = .835)
f. R Squared = .767 (Adjusted R Squared = .758)
g. R Squared = .938 (Adjusted R Squared = .936)
h. R Squared = .933 (Adjusted R Squared = .930)
i. R Squared = .763 (Adjusted R Squared = .754)
j. R Squared = .606 (Adjusted R Squared = .591)
k. R Squared = .669 (Adjusted R Squared = .656)

All morphology: with interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Width (mm)	25.028[a]	3	8.343	105.908	.000
	Depth (mm)	75.554[b]	3	25.185	296.901	.000
	Length of first dorsal spine (mm)	6.107[c]	3	2.036	30.675	.000
	Length of second dorsal spine (mm)	5.212[d]	3	1.737	21.853	.000
	Length of jaw (mm)	14.462[e]	3	4.821	89.021	.000
	Width of mouth (mm)	5.742[f]	3	1.914	55.545	.000
	Length of head (mm)	135.178[g]	3	45.059	253.333	.000
	head depth	57.471[h]	3	19.157	230.284	.000
	Length of pelvic girdle (mm)	54.605[i]	3	18.202	54.017	.000
	Length of pelvic spine (mm)	15.417[j]	3	5.139	31.368	.000
	Length of 4th gillraker on first gill arch	1.743[k]	3	.581	40.744	.000
Intercept	Width (mm)	.679	1	.679	8.616	.005
	Depth (mm)	.304	1	.304	3.583	.064
	Length of first dorsal spine (mm)	.028	1	.028	.415	.522
	Length of second dorsal spine (mm)	.392	1	.392	4.935	.031
	Length of jaw (mm)	.503	1	.503	9.284	.004
	Width of mouth (mm)	.041	1	.041	1.189	.281
	Length of head (mm)	.675	1	.675	3.796	.057
	head depth	.226	1	.226	2.712	.106
	Length of pelvic girdle (mm)	.187	1	.187	.555	.460
	Length of pelvic spine (mm)	.859	1	.859	5.246	.026
	Length of 4th gillraker on first gill arch	.044	1	.044	3.058	.086
Site	Width (mm)	.209	1	.209	2.649	.110
	Depth (mm)	.180	1	.180	2.117	.152
	Length of first dorsal spine (mm)	.364	1	.364	5.483	.023
	Length of second dorsal spine (mm)	.550	1	.550	6.913	.011
	Length of jaw (mm)	.007	1	.007	.127	.724
	Width of mouth (mm)	.002	1	.002	.066	.798
	Length of head (mm)	.030	1	.030	.167	.684

	head depth	.003	1	.003	.038	.847
	Length of pelvic girdle (mm)	.090	1	.090	.267	.607
	Length of pelvic spine (mm)	1.119	1	1.119	6.831	.012
	Length of 4th gillraker on first gill arch	.051	1	.051	3.601	.064
SL_img_i	Width (mm)	24.886	1	24.886	315.916	.000
	Depth (mm)	74.378	1	74.378	876.846	.000
	Length of first dorsal spine (mm)	6.048	1	6.048	91.143	.000
	Length of second dorsal spine (mm)	4.945	1	4.945	62.212	.000
	Length of jaw (mm)	13.812	1	13.812	255.057	.000
	Width of mouth (mm)	4.639	1	4.639	134.608	.000
	Length of head (mm)	131.870	1	131.870	741.402	.000
	head depth	55.778	1	55.778	670.506	.000
	Length of pelvic girdle (mm)	53.472	1	53.472	158.689	.000
	Length of pelvic spine (mm)	14.785	1	14.785	90.251	.000
	Length of 4th gillraker on first gill arch	.973	1	.973	68.250	.000
Site * SL_img_i	Width (mm)	.140	1	.140	1.776	.189
	Depth (mm)	.097	1	.097	1.140	.291
	Length of first dorsal spine (mm)	.288	1	.288	4.340	.042
	Length of second dorsal spine (mm)	.535	1	.535	6.727	.012
	Length of jaw (mm)	.011	1	.011	.197	.659
	Width of mouth (mm)	.016	1	.016	.471	.496
	Length of head (mm)	.001	1	.001	.006	.940
	head depth	.000	1	.000	.004	.952
	Length of pelvic girdle (mm)	.051	1	.051	.152	.698
	Length of pelvic spine (mm)	1.107	1	1.107	6.759	.012
	Length of 4th gillraker on first gill arch	.100	1	.100	7.002	.011
Error	Width (mm)	3.939	50	.079		
	Depth (mm)	4.241	50	.085		
	Length of first dorsal spine (mm)	3.318	50	.066		
	Length of second dorsal spine (mm)	3.975	50	.079		
	Length of jaw (mm)	2.708	50	.054		

	Width of mouth (mm)	1.723	50	.034		
	Length of head (mm)	8.893	50	.178		
	head depth	4.159	50	.083		
	Length of pelvic girdle (mm)	16.848	50	.337		
	Length of pelvic spine (mm)	8.191	50	.164		
	Length of 4th gillraker on first gill arch	.713	50	.014		
Total	Width (mm)	1090.041	54			
	Depth (mm)	4072.087	54			
	Length of first dorsal spine (mm)	434.691	54			
	Length of second dorsal spine (mm)	515.494	54			
	Length of jaw (mm)	568.595	54			
	Width of mouth (mm)	342.470	54			
	Length of head (mm)	7096.174	54			
	head depth	3054.445	54			
	Length of pelvic girdle (mm)	3756.239	54			
	Length of pelvic spine (mm)	1440.825	54			
	Length of 4th gillraker on first gill arch	41.074	54			
Corrected Total	Width (mm)	28.967	53			
	Depth (mm)	79.795	53			
	Length of first dorsal spine (mm)	9.425	53			
	Length of second dorsal spine (mm)	9.186	53			
	Length of jaw (mm)	17.170	53			
	Width of mouth (mm)	7.465	53			
	Length of head (mm)	144.071	53			
	head depth	61.630	53			
	Length of pelvic girdle (mm)	71.453	53			
	Length of pelvic spine (mm)	23.608	53			
	Length of 4th gillraker on first gill arch	2.456	53			

a. R Squared = .864 (Adjusted R Squared = .856)
b. R Squared = .947 (Adjusted R Squared = .944)
c. R Squared = .648 (Adjusted R Squared = .627)
d. R Squared = .567 (Adjusted R Squared = .541)
e. R Squared = .842 (Adjusted R Squared = .833)
f. R Squared = .769 (Adjusted R Squared = .755)
g. R Squared = .938 (Adjusted R Squared = .935)
h. R Squared = .933 (Adjusted R Squared = .928)
i. R Squared = .764 (Adjusted R Squared = .750)
j. R Squared = .653 (Adjusted R Squared = .632)
k. R Squared = .710 (Adjusted R Squared = .692)

Foraging efficiency

Bloodworm and Daphnia handling times: without interaction

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Average Log10(HT for bloodworm)	.950[a]	2	.475	35.136	.000
	Average Log10(HT for Daphnia)	.472[b]	2	.236	19.871	.000
Intercept	Average Log10(HT for bloodworm)	2.939	1	2.939	217.302	.000
	Average Log10(HT for Daphnia)	1.713	1	1.713	144.282	.000
SL_img_i	Average Log10(HT for bloodworm)	.929	1	.929	68.659	.000
	Average Log10(HT for Daphnia)	.447	1	.447	37.673	.000
Site	Average Log10(HT for bloodworm)	.015	1	.015	1.116	.298
	Average Log10(HT for Daphnia)	.030	1	.030	2.546	.120
Error	Average Log10(HT for bloodworm)	.473	35	.014		
	Average Log10(HT for Daphnia)	.416	35	.012		
Total	Average Log10(HT for bloodworm)	31.342	38			
	Average Log10(HT for Daphnia)	21.969	38			
Corrected Total	Average Log10(HT for bloodworm)	1.424	37			
	Average Log10(HT for Daphnia)	.887	37			

a. R Squared = .668 (Adjusted R Squared = .649)
b. R Squared = .532 (Adjusted R Squared = .505)