

pungitius) sticklebacks and its consequences for
morphology and behaviour.

Thesis submitted for the degree of Doctor of Philosophy at the University of
Leicester

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To My Family...

Mum & Dad

Sidney & Ivy Czeslaw & Elsie

Donna, Pauline, Gina & Riley



...for vindicating history!

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Bendacht For Cech N-Oen Legfas
(Ancient Irish: a blessing on all who shall read this)

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I never came upon any of my discoveries through the process of rational thinking.

-Einstein (1879-1955)

1: Introduction

1.1: Why are there so many species?

Why are there so many species? This is not the lament of the taxonomist late one night in his laboratory, but a question that remains pertinent to today's evolutionary biologists, some 140 years after Darwin first published his theory of evolution by natural selection in the illustrious *Origin of Species* (1859). Although at the time Darwin was considered "nothing but an intelligent pigeon fancier" (G. B. Shaw from Williams, 1961), by present standards his theory's most significant claims remain highly compelling. His ideas have provided the cornerstone upon which all modern theories of evolution are based.

Darwin (1859) proposed that inter-specific competition for resources may be a major driving force behind the 'divergence of characters' he observed. Some 100 years later this concept was taken and modified by Brown & Wilson (1956) and defined as *character displacement* (see section 1.3.5.iii for full discussion). Ironically the Galápagos island finches originally collected by Darwin provide one of the most compelling examples of character displacement. In an exhaustive study of these birds Grant & Grant have uncovered the remarkable array of morphological variation shown by the finches' now infamous beak (reviewed by Weiner, 1994; Grant, 2001). Character displacement is deemed to be the process that facilitated phenotypic variation in these finch populations. Although the character-displacement hypothesis is a theoretically persuasive explanation of how variation occurs in populations, the example of the Galápagos finches remained one of a few citable examples in the literature for a remarkably long time. People became sceptical. "The great tragedy of science" as T. H. Huxley (1825-1895) puts it so succinctly is "the slaying of a beautiful hypothesis by an ugly fact". However, evidence is at last accumulating. In only as recently as the last 10 years, 23 cases have come to light as 'strong candidates' to support the theory of character displacement (see Schluter, 2001) and more potential candidates are apparent in the most recent literature (e.g.: freshwater snails *Lymnaea peregra* and *L. ovata* (Wullschleger *et. al.* 2002); sailfin mollies *Poecilia formosa* (Gabor & Ryan, 2001)).

The work submitted here focuses on the theoretically unlikely co-existence of two ecologically similar species of stickleback, the threespine (*Gasterosteus aculeatus* L.) and ninespine (*Pungitius pungitius* L.) sticklebacks, and how, as a result of this co-existence, the morphology of one of the populations, the threespine stickleback, is altered. The work I present in this thesis

provides persuasive, though not conclusive, evidence of phenotypic differentiation potentially resulting from a process of character displacement.

1.2: Out of the jam-jar: The sticklebacks

The family Gasterosteidae includes all known species of stickleback. The most prominent features are the varying number of dorsal spines that lie in front of the dorsal fin, from which the fish take their common names. The threespine sticklebacks in particular have received more attention from researchers than almost any other species of Teleost (e.g.: see Wootton, 1976; 1984; Bell & Foster, 1994a; Behaviour, 137, 2000). Sticklebacks are not commercially important, which is a common incentive for research (although they occur in sufficient concentrations in North America that they may be taken as dog food or even for human consumption (McPhail and Lindsey 1970)). Workers were initially attracted to them because they are a hardy fish that are easy to maintain and will readily breed in aquaria. What has emerged more recently is that they offer us an excellent opportunity to study evolutionary mechanisms (see Bell & Foster, 1994a).

The Gasterostidae have a wide-ranging distribution throughout the Northern Hemisphere. The threespine stickleback in particular has been an especially successful invader of a diverse range of coastal marine water, brackish and freshwater habitats. Original taxonomic descriptions offered more than 40 synonyms for *G. aculeatus*. Now it has become common practice to ignore phenotypic differentiation and to treat the threespine as a single species complex (Bell & Foster, 1994b) though in the light of genetic evidence we may once again have to revise our taxonomy of *G. aculeatus*. Because of its expansive distribution the threespine's gene pool has consequently become fragmented which has, in turn, generated a range of phenotypic diversity (Bell & Foster, 1994a). Not only are there morphological differences between populations from different habitats but evidence from mitochondrial DNA suggests these populations are genetically distinct too (e.g.: Thompson *et. al*, 1997; Reusch *et. al*, 2001).

1.2.1: Co-existence of the threespine and ninespine sticklebacks.

The threespine and the ninespine sticklebacks are two of the more familiar fish found in Britain's freshwaters. What is striking is the similarity of these two species in aspects of morphology, behaviour and physiology compared with the other members of Gasterosteidae (McPhail & Lindsay, 1970; Wootton, 1976). The distribution of the ninespine in Britain is more restricted than that of the threespine so it is more usual to find solitary populations of threespines (see Maitland, 1972). However, threespine and ninespine sticklebacks will often be found living side-

by-side in streams or ponds. The microhabitats they are captured in are generally similar, and both species appear to prefer the lentic refuges found in small streams (Copp, 1992). According to Gause's principle (1934) this co-existence is theoretically unlikely as competition for resources (e.g.: space, food, nesting sites) between these ecologically similar species should be so intense that one would be expected to out-compete and exclude the other. However Lotka & Volterra's model of competitive mechanisms indicate that co-existence can occur between species providing intra-specific competition is more intense than inter-specific competition. The rationale for this work is to uncover the ecological mechanisms that facilitate this co-existence of the threespine and ninespine sticklebacks in Britain.

1.2.2: Character displacement in Canadian threespine sticklebacks.

More recent investigations of threespines in British Columbia (BC), Canada have uncovered divergent populations of limnetic and benthic morphs (Schluter & McPhail, 1992; McPhail, 1994). There is growing behavioural and genetic evidence (Taylor & McPhail, 1999; Rundle *et al.*, 2000) that the limnetic and benthic morphs are reproductively isolated from each other, and as such they constitute true 'biological' (Mayrian) species. The initial divergence of the threespine morphs to their benthic and limnetic forms is believed to have been due to a process of character displacement (Schluter, 1994; Taylor *et al.* 1997). It is possible that this same mechanism of character displacement has altered the morphology of threespines in Britain and facilitated their co-existence with ninespine sticklebacks. The relevance of these Canadian studies to the proposed hypothesis will be discussed in detail further on in the introduction.

1.3: In a nutshell: The ecological theory of an adaptive radiation

An adaptive radiation comprises two core processes: a rise in the rate of appearance of new species and a concurrent increase in ecological and phenotypic diversity (Schluter, 2000a). The original definition of adaptive radiation (Darwin, 1859) has been modified to account for advances in our knowledge of the processes involved. The definition of adaptive radiation I adhere to, which preserves the crucial elements of its predecessors (Simpson, 1953; Lack, 1947; Mayr 1963; Grant, 1986; Schluter, 2000a), is that proposed by Schluter (2000a).

'Adaptive radiation is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage. It occurs when a single ancestor diverges into a host of species that use a variety of environments and that differ in traits used to exploit those environments.'

Fish species as we observe them today are the *result* of an evolutionary process, the cause of which is usually obscure. The adaptive radiation theory proposes that new species emerge by one of the three major processes summarised below:

1.3.1.i: Phenotypic differentiation between populations and species caused directly by differences in the environments they inhabit and the resources they consume.

The environment subjects its inhabitants to unique interactions of physical and chemical selection pressures. The conditions in each habitat will dictate an animal's ecology, phenotype and behaviour. This is believed to be *the* major evolutionary force driving phenotypic diversification.

The radiation of the teleost fish into virtually every aquatic habitat provides many examples of phenotypic differentiation (see Wootton, 1990). Conditions in aquatic environments will impose a different set of morphological requirements from those in terrestrial systems. The physical properties of water determine methods of locomotion, respiration and sensory perception. Most notably all teleosts use gills to respire and the lateral line provides a sixth sense with which to navigate its 3D environment. The vast array of body forms can be attributed to three major forms of locomotion, either short burst acceleration (e.g. pike (*Esox lucius*)), cruising (e.g. tuna (*Thunnus* spp.)) or localised manoeuvring (e.g. butterfly fish (*Chaetodon vagabundis*)). The morphology of the teleost jaw is a particularly plastic trait and has facilitated novel trophic adaptations; a particularly good example is given by the species flocks of cichlid fish in African lakes (see Wootton, 1990). Fins also show enormous diversity amongst fish. Some have aided streamlining and thus enhanced propulsion (e.g. the finlets on tunniforms). Some aid foraging, the modified fin rays of the gurnard (*Chelidonichthys* spp.) allow them to search the benthos. Other fish use their fins as a means of communication; the dorsal fin on the angler fish (*Lophius piscatorius*) acts as a lure to potential prey; the spines of the sticklebacks act as defensive armour, and are used to warn off enemies and potential predators (Reimchen, 1994).

The importance of the environment as an evolutionary force shaping the phenotype has never been disputed. As we begin to understand the constraints various environments impose on organisms we can interpret the various phenotypic adaptations accordingly. Today the role of ecomorphological studies is particularly relevant to species conservation (Norton *et. al.* 1995). Our knowledge of how the environment interacts with and can ultimately alter morphology can be used to assess the outcome of fish species introductions, or the effects of other anthropogenic manipulations.

1.3.1.ii: Divergence in phenotype resulting from biological interactions

Other species in the biological community will exert selective pressures on the phenotype of a species through the various processes of resource competition, predation, parasitism and mutualism. Biotic interactions have the potential to affect directly the distribution, abundance and genetic composition of the individual species population as well as the entire species assemblage present in a location (see Wootton, 1990). It is not denied that these interactions have an effect on phenotype but it is for the biologists to decipher whether they have an important role as an evolutionary mechanism.

The consequences of biotic interactions such as competition and predation will have a direct selective effect at the level of the individual. Whether a fish manages to find sufficient food for itself or whether it becomes someone else's meal whilst looking will decide if the fish survives to pass its genes to the next generation. The structure of biological communities fluctuates more often than conditions in the abiotic physical and chemical environment around them. Biotic selection pressures will be present that have great potential to hasten diversification. The evolutionary significance of species interactions has a sound theoretical basis but its influence on actual biological populations remained doubtful (e.g. Grant, 1972; Arthur, 1982) until recently (Robinson & Wilson, 1994).

1.3.1.iii: Resource competition

Competition for resources such as food, territory and mates is considered the strongest selective biotic force between species (Robinson & Wilson, 1994). Populations within the same habitat are likely to be the most similar in their resource requirements and so are potentially intense competitors. Species are considered competitors if each exerts an inhibitory effect on the growth rate or equilibrium size of the other, these effects should be mutually felt (Arthur, 1982). Competition can be categorised as either interference or exploitative. Interference competition is directly inhibitive (e.g. the secretion of substances by one species that are harmful to another) whereas exploitative competition is indirect (e.g. the depletion of a common limiting resource). Populations may evolve as a consequence of these competitive interactions. A species may become more efficient at inhibiting another, or become better at obtaining the resource. Alternatively it may be able to utilise a greater or shifted range of resources (Arthur, 1982; Nakajima, 1998) though evolution of some traits can occur without resource competition (Nakajima, 1998). Competition is such a plausible evolutionary force yet it constantly comes under strong criticism; at present it remains "surrounded by a cloud of scepticism from which it has not yet emerged" (Robinson & Wilson, 1994).

The availability of food resources in particular is thought to elicit competitive interactions. It has recently been shown that the relative abundance of food influences aggression in convict cichlids (*Archocentrus nigrofasciatus*) (Grant *et. al.* 2002). If food is either scarce or in abundance aggressive encounters between the fish are minimal. A submissive fish will not risk injury by confronting a dominant fish; instead it finds an alternative food source if the preferred food is scarce. Alternatively the fish waits for an opportunity to feed (if food resource is abundant) when the dominant fish becomes distracted by some other activity such as eating or chasing other subordinates away. A similar situation is found for territorial availability (Grant *et. al.* 2002). Interspecific territoriality may reduce competition between haplochromine cichlid species with different diets, which allows co-existence and enhances competition between species with the same diet (Genner *et. al.* 1999a). However specialisations for the same resource may increase competition between haplochromine cichlid species

Levels of heterogeneity in the environment also have an effect on competition. If a food resource is patchily distributed throughout a particular location fish are expected to distribute themselves amongst the resources according to an ideal free distribution (Fretwell & Lucas, 1970). If patches of food are homogeneous they should usually encourage divergence as fish adapt to the broader resource base. Divergence will be facilitated if habitat preferences are heritable thereby lowering migration rates between patches and reducing the mixing of the gene pool (Day, 2000). The relative abundance and availability of the food resource must remain static for long enough to elicit a reaction. If one species is rapidly excluded by a superior competitor it will have little time to exhibit an evolutionary response.

A number of processes have been proposed as responses to competitively induced interactions. These include; *character convergence* where two characters become more similar in sympatric populations; *character release* where increasing variability in habitats may lead to an increase in the variability of morphological characters; *competitive release* leads to an increase in population density when no competitors are present; *ecological release* leads to either an expansion of feeding habits or an increase in the number of micro-habitats when no competitors are present; and finally *character displacement* which is the process of phenotypic divergence caused or maintained by interspecific resource competition. These are theoretical concepts and there is little evidence of the effects that any of these competitive processes may have on evolution. The one process that can be substantiated with recent evidence from natural populations is character displacement (e.g.: Schluter, 1996). I propose it is this process that facilitates the co-existence of the threespine and ninespine sticklebacks in Britain. Character displacement was originally

defined by Brown & Wilson (1956) and later modified by Grant (1972). The following definition is based on their original:

Character displacement is the process by which the mean values of a phenotypic character, in two competing species, displace away from each other in areas of sympatry, because of the presence in sympatric populations, but not in allopatric ones, of a selective pressure stemming from interspecific competition.

Grant's (1972) original definition stated that only the morphology of the species was subject to change. Although the morphology of a fish is the most likely and easily observable feature to change it has subsequently been shown that other heritable behavioural and physiological characters can be altered (e.g. advertisement vocalisations of *Bufo fowleri* and *B. americanus* (Leary, 2001)). As a consequence I have replaced the original 'morphological character' in the definition by 'phenotypic character' to remove this restriction. Slatkin (1981) observes that divergence can arise when two species are either ecologically or reproductively similar. He proposes a further sub-division of character displacement into two categories of either ecological or reproductive character displacement. When I later discuss character displacement, I specifically mean ecological character displacement.

1.3.1.iv: Predation

Other biotic factors of predation, parasitism and mutualism are thought to affect the phenotype but to a lesser extent than competition for resources (see Schluter, 2000a). Predation has a particularly strong effect on the phenotype; nothing impedes an individual's gene flow like being eaten! Fish from populations exposed to high risks of predation show particularly well-developed anti-predator responses. Anti-predator tactics in fishes include the development of morphological defences (e.g.: stickleback body armour, Bell, 1994; Godin & Valdrón Clark, 1997) cryptic coloration, predator inspection (e.g.: threespine sticklebacks, Magurran, 1986; Huntingford *et. al.* 1994; Godin & Crossman, 1994) and parental care (e.g.: mouth brooding in cichlids, male sticklebacks guard nests of juveniles). The threespine stickleback exhibits high levels of plasticity in its body armour between populations (Bell, 1994). The particular type of piscivore (avian, fish or invertebrate) will dictate the arrangement of the lateral plates along the stickleback's body (Reimchen, 1994). The threat of predation on populations of Trinidadian guppies (*Poecilia reticulata*) has had an especially strong selective effect on their present phenotype (Magurran *et. al.* 1993). Many aspects of their behavioural and morphological features can be attributed to anti-predator defences. The guppies show high levels of predator

assessment and avoidance schooling, the predator regime even has an effect on female choice of mate and other mating tactics. The implication of predation as an evolutionary mechanism has only recently been acknowledged but the threat of it has resulted in a wide variety of anti-predation strategies (see review Lima & Dill, 1989).

1.3.2: Allopatric and sympatric populations.

Allopatric populations (of different species, or populations within a species) do not occur together but have mutually exclusive geographical distributions. A sympatric population is one where the geographical range or distribution of populations of two or more species coincide or overlap. These are based on the original definitions proposed by Mayr (1942, 1999). For the purposes of the thesis I refer to sites where the threespine stickleback occurs on its own as allopatric, and ones where they co-exist with ninespines as sympatric.

1.3.3: Benthic and limnetic zones

The benthos includes the entire flora and fauna that live on or near the bottom of an aquatic system, these organisms are described as benthic. The limnetic region describes the open water, non-vegetated areas of freshwater systems. It is equivalent to the marine pelagic zone. Most commonly we associate this term with lakes but it is applicable to running water systems too. Although sites in this study are shallow freshwater streams, I use the term 'limnetic' throughout the thesis to describe the open water habitats within them.

1.4: Are some animals more likely to diverge than others?

Why should it be that some habitats harbour a richer diversity of species than others? To a certain extent the abiotic characteristics of an environment will dictate the degree of phenotypic specialisation of a fish. Speciation rates will increase if the environment is heterogeneous providing fish with the opportunity to specialise into different trophic niches. But, not every habitat offered in the environment is filled with its own uniquely adapted fish species. The extent of niche specialisation is restricted by biotic factors limiting the plasticity of the fish's phenotype. As we begin to understand the influence of abiotic and biotic factors upon the life-history of individual species, we are able to some extent to predict how likely they are to diverge and thus gauge speciation potential.

1.4.1: Influence of abiotic factors on species diversity.

The extent of species diversity in an environment can partly be explained by the influence of abiotic factors in the particular environment. For example, tropical lakes are seasonally stable,

homogenous environments. High levels of physical and chemical permanency encourage fish to specialise into small adaptive niches. In contrast temperate rivers are seasonally fluctuating and heterogeneous; fish living in rivers must contend with regular environmental disturbance and adopt a more generalistic approach.

The age of the aquatic system also has an impact on biodiversity. The colonisers of younger systems will show lower levels of diversification because the gene pool is more restricted than that of the ancestral population. It should follow then that older systems have more species because of the accumulated specialisations of the inhabitants. This follows Simpson's (1953) view that specialisation is a dominant trend in adaptive radiation. He described this trend as the 'differentiation of a more broadly adapted population.....into separate populations each more narrowly adapted to part of the original adaptive range'. If fish follow Simpson's (1953) rule of evolving from generalists to specialists they may become so specialised that they bring about the extra burden of extinction if resources suddenly become unavailable. However, it is possible that animals will retain 'ancestral' morphologies and behavioural attributes that will allow them to exploit less favourable resources and thereby avoid extinction. This was shown to be the case with a species flock (of more than 200 spp.) of cichlids collectively referred to by their Chitonga name, Mbuna cichlids in Lake Malawi (Ribbink, 1991).

1.4.2: Influence of life history on species diversity.

There are inconsistencies to these general patterns of colonisation discussed above. Distributions of species richness in African freshwaters show that larger, younger lakes have more species than older lakes. Lake Tanganyika is one of the oldest lakes in Africa (between 5-12 million years old) yet it contains fewer cichlid species (≈ 200 spp) than other African lakes (Lake Malawi is ≈ 4 million years old and contains between 700-1,500 spp.) (from Meyer, 2000). Another puzzle is why some lineages should speciate more prolifically than others. In cichlids the *Haplochromis*-like lineage is represented by more than 1000 species, whereas, the *Tilapia*-like lineage is represented by a mere 10 (Ribbink, 1991).

Some differences in fish species diversity can be explained by where on the scale of eurytopy to stenotopy they lie (Fryer, 1960; Fryer & Iles, 1969; see Ribbink 1991). A eurytope may live in a wide range of conditions during its life-history, often moving readily from one habitat to another. In contrast, a stenotope is usually confined to a single habitat; it has a narrow habitat tolerance range and usually a restricted geographical distribution. Between these extremes lie a variety of intermediates. These distinctions apply to the *whole* life-history of a species rather than *r* and *k*

selection, which is restricted to a species' reproductive strategy (Mac Arthur & Wilson, 1967). They are not synonymous with the terms specialist and generalist, which are often only applied to a particular aspect of the life history such as diet or behaviour. Speciation rates show a positive correlation with the diversity of the physical environment and where the animal lies on the eurytopy/stenotopy scale (Ribbink, 1991).

1.5: Ecomorphs and species pairs in the Northern Hemisphere

African cichlids have diversified into a remarkable number of species, many of which are ecologically and morphologically very specialised (Turner, 1999). Why should it be that we have not observed equivalent extensive radiations of fish species in the north? The geological history of freshwater habitats in the Northern Hemisphere is distinctly different from those in the south, which may explain this disparity. Southern African lakes were formed millions of years ago (e.g.: Lake Tanganyika estimated to be 5-12 million years old (from Meyer, 2000)). They are huge in comparison to the majority of temperate freshwaters, and habitats are often distributed in separate patches (Turner, 1999). All these features encourage species divergence. In contrast, freshwater habitats in the northern hemisphere are much younger (initial formation in the Pleistocene, 1.6 million years ago (Harvey Pough *et. al.*, 1989)) and most of their inhabitants were only introduced in the Holocene, the last glaciation event estimated to be 10-12,000 years ago (Taylor, 1999) a short time-scale by evolutionary standards.

In the Northern Hemisphere a number of eco-morphs have formed in some fish species, in some cases species pairs. It is proposed that these morphs have emerged a number of times in geographically isolated populations with similar selection pressures (Taylor, 1999), this phenomenon has been labelled parallel speciation (Lavin & McPhail, 1993). Evidence of species pair's formation has been shown for threespine sticklebacks, smelts (*Osmerus*), charr (*Salvelinus* spp.), trout (*Salmo* spp.) (Schluter, 1996), whitefish (*Coregonus clupeaformis*) (Lu & Bernatchez, 1999), salmon (*Salmo* spp.), and grayling (*Thymallus* spp.) (Taylor, 1999). Parallel speciation is an important evolutionary mechanism believed to be responsible for the multiple cases of divergent life-history types in northern fishes (Thompson *et. al.*, 1997). Evidence is continuously growing and workers are uncovering more promising cases of species pairs in a diversity of aquatic habitats from temperate lakes to marine coral reef systems (Taylor, 1999).

There are several features that are thought to promote ecomorph and species-pair formation in recent times. Post-glacial freshwater habitats are depauperate environments that offer novel ecological opportunity. They offer an abundance of under-utilised resources and predator-free

space, also interspecific competition is relaxed (Smith & Skulason, 1996). The flexibility of morphological features allows species to fill empty niches by character release (Taylor, 1999). With regard to the threespine stickleback species-pair formation (B.C. Canada) it is proposed that during the events of the last glaciation connections between certain habitats were temporarily broken; this allowed a brief period of allopatric divergence. About 2000 years later connections were re-established and some diverged threespine populations made secondary contact. In some cases where morphological divergence was more established, reproductive isolation built up between ecomorphs by reinforcement (Taylor, 1999).

Three main radiations of threespine sticklebacks have been recognised in areas bordering the north Pacific region (McPhail, 1994). These threespines have been categorised as parapatric anadromous (sea-run), sympatric lacustrine limnetic and benthics and stream sticklebacks. Molecular analysis has substantiated morphological evidence that the threespine stickleback's ecomorphs are indeed distinguishable forms. Reproductive isolation has built up between lake and stream forms (Thompson *et. al.* 1997) and between limnetic and benthic forms (Taylor & McPhail, 2000). Radiation of ecomorphs is not peculiar to North America. There is evidence to suggest that the threespine sticklebacks from the Japanese and Argonaut plains are from a separate lineage (McPhail, 1994). In Schleswig-Holstein, in north Germany, three major monophyletic clades have been found and can be categorised by the habitats they are found in as either stream, lake or estuarine forms (Reusch *et. al.* 2001). There is a particularly marked difference between parapatric stream and lake populations, despite there being opportunity for gene-flow between them. DNA microsatellite evidence from these north German populations suggests that colonisation occurred after the last glaciation ($\approx 12,000$ years ago). Divergence into lake and stream forms was rapid during the initial stages of colonisation and was shown to decline in the later stages. In the light of these recent discoveries it is possible that more examples of threespine ecomorphs exist in other areas of the Northern Hemisphere that were similarly effected in the last glaciation. Similar patterns might also be apparent in other members of the Gasterostidae and not just peculiar to the threespine sticklebacks.

1.6: Dichotomy between Canadian species pairs is mirrored in British sympatric populations

Are there repeatable patterns between separate radiations? The idea of a characteristic sequence of events common to many radiations is appealing but has been little tested (Schluter, 2000b). An examination of the features that have facilitated the divergence of the Canadian threespines populations into limnetic and benthic species pairs reveals a number of ecological similarities to the situation between British threespine and ninespine sticklebacks. Populations of

morphologically divergent species pairs of threespine stickleback have been found in a handful of lakes in British Columbia (B.C.), Canada (see review, McPhail, 1994). Each species pair constitutes a benthic and limnetic trophic morph, and these forms are regarded as separate ecomorphs (Rundle *et. al.* 2000). The limnetic ecomorph is slim bodied, with numerous long gill rakers, narrow mouths and long slim snouts, and these features are associated with a plankton-feeding regime (McPhail, 1994). For the majority of the time the limnetics are found in the open-water areas of the lakes, but will seasonally exploit the benthic regions, probably at nest-building time (Schluter, 1993). The benthic ecomorph is characterised by a deep body, few short gill rakers, a wide mouth, and a short broad snout and is overall generally larger than its limnetic counterpart (McPhail, 1994). Benthics rarely exploit limnetic zones in the lakes and are probably exclusive to benthic regions (Schluter, 1993). These morphological features are common amongst all the divergent threespine populations found in B.C., Canada. Populations also differ in other meristic and morphometric traits from lake to lake but these could be explained by either differing local selection regimes amongst lakes, or differences in the initial founder populations in each of the lakes (McPhail, 1994).

Evidence implies that high levels of resource competition drove initial divergence between the ecomorphs. Selection has worked most strongly on upper jaw size and gill raker number (Lavin & McPhail, 1987). Both of these characters impose a maximum size on the fish's prey choice. Upper jaw size is indicative of the maximum gape width of the mouth. Even a small increase in jaw length as shown by the benthic ecomorph, will allow the stickleback to take larger prey items (Gill & Hart, 1996). Gill raker number and length also reveal aspects of diet choice (McPhail, 1994). Limnetics with their numerous, long rakers are typically planktivorous, while benthic fish with fewer shorter rakers can include larger prey items in their diet. These morphological differences correlate with differences in prey size encountered by the fish in their respective habitats. The observed morphological divergence between the species pairs indicates association with different trophic feeding regimes. This suggests, but does not prove, character divergence as the driving mechanism promoting morphological divergence (Taylor & McPhail 1997). Behavioural experiments have observed an increase in the ecomorph's efficiency in its respective habitat, which comes at the expense of reduced fitness in the alternative habitat (Bentzen & McPhail, 1984; Schluter, 1993). Although intermediate morphotypes are able to exploit both habitats they are dominant in neither (Schluter, 1993). Inferiority of intermediate forms is a specific prediction of character displacement. Divergence is maintained through assortative mating. Mate choice is based on morphological traits that distinguish the ecomorphs, in particular body size and probably shape (Nagel & Schluter, 1998). Fish have even been shown

to express a mating preference for corresponding ecomorphs from different lakes over their sympatric partners in the same lake (Rundle *et. al.*, 2000).

The ecology and morphology of limnetic and benthic ecomorphs seem to present a similar picture to that of sympatric stream dwelling populations of threespine and ninespine sticklebacks found in Britain and there are a number of parallel features. The limnetic ecomorph might be regarded as equivalent to the sympatric threespines whereas the benthic's opposite number is the ninespine stickleback. The benthic form prefers the vegetated areas on the bottom of the lake, which reflects the preferred habitat of the ninespine stickleback. Like the ninespines the benthic fish have a reduced body armour and rely on evasive behaviour rather than morphological defences to avoid capture (Schluter, 1993). In one population in Enos Lake, BC the male benthics even adopt black nuptial colours in breeding season as do ninespine sticklebacks (Boughman, 1999).

I propose that the ninespine sticklebacks subject sympatric threespines to similar pressures of interspecific resource competition as has been demonstrated for benthic sticklebacks on their limnetic counterparts. If this prediction is correct, observations of sympatric threespine sticklebacks should reveal a marked relocation to limnetic regions within streams if competition is high.

1.7: The evolutionary path of the thesis

The focus of the thesis is whether the co-existence of the threespine and ninespine sticklebacks can be explained by character displacement. Each chapter is essentially a self contained piece of work and should be read as such. However, each follows the other sequentially, as each chapter builds on the findings and questions arising from the previous one. This was the way it developed in the laboratory and it makes sense to present it this way in the thesis.

The 2nd chapter sets the scene and describes the environmental conditions in the sites from which the stickleback populations were originally caught. In chapter 3 I describe the initial detection of morphological divergence between the threespine stickleback populations, and why character displacement might be proposed as the mechanism driving the divergence. The work in remaining chapters focuses on this presupposition and each in some part aims, to satisfy the criteria of the character-displacement theory. As the influence of the environment is ruled out (chapter 2), the next likely explanation is competition for food resources. The number and form of a fish's gill rakers can be very indicative of diet, and the information gathered here provides

further support to the hypothesis (Chapter 4). A further investigation of the stickleback's diet through stomach contents analysis is presented (chapter 5). The final experimental chapter presents a series of behavioural studies that give further insight into interactions between the threespine and ninespine fish (chapter 6). These findings, and a discussion of them, will be drawn together in the final concluding chapter (chapter 7).

1.8: Definitions of terms used in the thesis.

Adaptation: A divergent trait that originally arises and is maintained in the population through natural selection

Adaptive radiation: The evolution of ecological and phenotypic diversity within a rapidly multiplying lineage. It occurs when a single ancestor diverges into a host of species that use a variety of environments and that differ in traits used to exploit those environments.

Adaptive zone: a collection of empty niches that may be exploited by a set of species varying in phenotype but descended from a common ancestor

Allopatry: A geographical distribution of different species, or populations within a species, in which they do not occur together but have mutually exclusive distributions

Ecological character displacement: the process of phenotypic divergence caused or maintained by interspecific resource competition

Ecological speciation: the build up of reproductive isolation between recently diverged species.

Eurytope: an animal living in a wide range of conditions during its life history, often moving readily from one habitat to another.

Exaptation: Traits that arise by means other than natural selection (e.g. neutral genetic drift), and only appear in a population by chance

Niche: The role of an organism within a community

Phenotypic plasticity: the production of one or more alternative forms of morphology, behaviour or physiological state in response to environmental conditions

Species: Species are groups of organisms, which are phenotypically similar and reproductively isolated from other such groups, and are actually or potentially capable of interbreeding among themselves. Where species populations continue to interbreed at a low rate the strong restriction of gene flow is sufficient to prevent collapse of genetically distinct populations.

Stenotope: an animal usually confined to a single habitat, it has a narrow habitat tolerance range and usually a restricted geographical distribution.

Sympatry: A geographical range or distribution of species populations where two or more species coincide or overlap.

Field biology [is] the primary source of inspiration: academic biologists [will] go outdoors again, a taste for fieldwork [is] essential to maintain a focus on the entire organism in its natural environment

- Baerends (1916-1999)

Chapter 2: The sympatric and allopatric study sites.

2.1: Introduction

The work in this chapter describes how study sites were selected in order to make comparisons between populations of threespines that were either allopatric (ninespines absent) or sympatric (ninespines present). Various ecological features of these sites were measured and compared in order to test whether the allopatric-sympatric comparison was confounded by systematic variation in these features.

The environment subjects its inhabitants to unique interactions of physical and chemical selection pressures. The conditions in each habitat will dictate an animal's ecology, phenotype and behaviour. The various conditions experienced by an animal in the habitat are believed to be the major evolutionary force driving phenotypic diversification (see Schluter, 2000a). Most incidents of morphological differentiation between species are attributable to conditions in the animals' respective habitats; this scenario is incorporated within the first criterion of the 'adaptive radiation' hypothesis. Character displacement is included within the second criterion of the adaptive radiation hypothesis, namely that divergence in phenotype is a consequence of biological interactions between species living in the same habitat. Character displacement is named as the process driving divergence between populations of Canadian threespine stickleback (Schluter & McPhail, 1992); it is possible that the same process may be causing phenotypic displacement in populations of British sticklebacks. If character displacement is to be held accountable for any divergence observed between sympatric and allopatric threespine populations then it is essential that any differences between habitats, with the potential to influence phenotype are identified before such a conclusion can be drawn. If ecological differences exist between allopatric and sympatric sites it is far more likely that the observed divergence is a result of environmental variation rather than a consequence of biotic interaction. Thus it is essential to identify any potential habitat discrepancies between allopatric and sympatric sites that could be responsible for any observed phenotypic divergence. This action is necessary before considering the role of character displacement as the major diversifying force between threespine populations.

It is proposed that character displacement may be the process permitting the co-existence of the nine and threespine sticklebacks in the sympatric habitat. The aim of this chapter is to quantify

the features of the allopatric and sympatric study sites and to test whether features differ significantly between the two habitats. The proposed null hypothesis is that no ecological differences exist between allopatric and sympatric sites. The leading null hypothesis is answered by means of two subsidiary null hypotheses, specifically that 1) no abiotic (physical habitat) differences exist between sites and that 2) no biotic (invertebrate fauna) differences exist between sites.

2.1.1: The stream environment

The sites in this study constitute shallow, lowland, freshwater streams. Threespine sticklebacks living in streams have received far less attention than their counterparts found in lacustrine, riverine and estuarine habitats (Copp, 1998). Each of these different freshwater habitats offers a unique series of ecological features to which the sticklebacks have had to adapt. The freshwater, lowland stream offers its own unique set of environmental variables. Physically, freshwater environments are comprised of areas of either lotic (running water) or lentic (still) water, and in the case of rivers and streams areas of both. The major discriminating feature relevant to the ecology of the stickleback here is the relative size of the available habitat. The stream is considerably smaller in both width and depth compared with other freshwater environments. In larger rivers lentic and lotic regions are afforded far more space and zones are readily distinguishable. It is possible that animals inhabiting one of these regions (lentic or lotic) in a river will not encounter animals inhabiting the other. In essence, stream habitats display generally similar physical properties to those of rivers, but in condensed form. Available lentic and lotic habitats in streams are restricted by size and are generally situated in very close proximity to each other. One consequence of the restricted size of the stream is that competition may be enhanced for limited resources. A further consideration is that fish populations in streams may be in such close proximity with their neighbours that they might encounter each other with greater frequency than they would in a river.

Information gathered from a detailed survey (Copp, 1992; 1998) indicates some degree of microhabitat preference in each species. The findings of the survey offer some distinction between ninespine and threespine habitats. Threespine sticklebacks show a preference for sites with lentic, depositional areas that contain higher levels of silt and submerged vegetation. The ninespine fish show a preference for aquatic macrophytes and filamentous algae, and sandy/silt substrata, with little use being made of ligneous debris (Copp, 1992, 1998). Both threespine and ninespine fish show a preference for deeper areas (100-150 cm) in the stream. These areas are significantly associated with both very high and low water conductivity. The threespine

stickleback demonstrates a greater affinity for areas of elevated water conductivity and dense ligneous debris. Unlike the threespine fish the ninespine shows no preference for water conductivity or temperature. The threespine's preference reflects its tendency to exploit areas with high nutrient levels, such as downstream of treated effluent, where few other fish are found (Copp & Kovac, 2003). Threespine sticklebacks are thought to choose areas of still or relatively slow moving water (lotic) so as to minimise the energy costs of maintaining spatial position (see Wootton, 1984). The availability of lentic habitat is crucial to the daily functioning of both species of stickleback and so provides a potentially limiting factor between the two species, particularly in streams where in general, regions are predominantly lotic.

Whilst both stickleback species exhibit some distinctive micro-habitat preferences in the restricted stream environment their paths will inevitably cross. This contact is inevitable since both species show preferences for conditions only available in lotic regions of streams. The lotic environment harbours the majority of the stream's aquatic plants. This resource is of paramount importance to the sticklebacks as use of vegetal or ligneous structures by both species is generally intense in small, shallow streams. Vegetation provides an essential refuge from predators and harbours many of the invertebrates that constitute the staple prey in the stickleback's diet (Hynes, 1950; see Wootton, 1976; 1984). In streams lentic and lotic regions are close both in distance and physical structure. The close proximity of the sticklebacks is demonstrated by the fact that the two species are often caught in the same sweep of the net. Differential micro-habitat use may occur in order to reduce competition between co-existing stickleback species.

2.1.2: Influence of resource heterogeneity.

A series of mathematical models developed by Day (2000) describe the influence of resource heterogeneity on the process of character displacement. Day's primary objective was to extrapolate information on the divergence of the Canadian threespine morphs. The models were designed on Gause's (1934) principle that there is a limit to how similar species can be and still co-exist. The models showed that when fish were offered two identical environments, competition between threespines always resulted in phenotypic divergence. However, divergence did not always arise from competition when the fish were offered contrasting environments. Morphological divergence was dependent upon rates of migration between the two sites. Only when migration from one site to the other was minimal was divergence the outcome. As the model fish begin to adapt to conditions in one habitat they do so at the cost of a loss of efficiency in the other.

When these results are translated to conditions in the wild they imply that when faced with competition the stickleback makes one of two choices. Either the stickleback must generalise and migrate between two habitats, or, remain in one and adapt to the conditions within. The choice of whether to become a specialist or remain a generalist is dependent on the strength of competition from other species present in the habitat. According to Gause's principle if competition is weak the stickleback could withstand the occasional meeting with heterospecifics during migration between sites. The alternative is to become a specialist, which requires the means to adapt to the new environment in the first place. If, in one habitat, one species is a far better competitor than the other an adaptation to the alternative habitat is necessary.

2.1.3: Influence of food availability

Day's (2000) models indicate that, when ecological conditions warrant, a stickleback will move from one habitat and adapt to an alternative. One such ecological pressure comes in the form of competition for food resources. A study on juvenile convict cichlids (*Archocentrus nigrofasciatum*) (Grant *et. al.* 2002) has shown that the incidence of competitive aggression between fish is dependent upon the level of food resources available in the habitat. If food is either scarce or in abundance aggressive encounters between the fish are minimal. Aggressive interaction will mostly occur when food is at intermediate abundance. At intermediate food levels more submissive fish are more willing to enter a patch. The cost of possible aggressive interaction with a conspecific is balanced by the raised probability of obtaining food. A similar outcome is observed when the cichlid fish come into conflict over territory. A fish is likely to defend aggressively a territory when food levels are intermediate. The threespine is notoriously pugnacious and males are aggressive in defending their territory in breeding season (Rowland, 1983; see McFarland 1987). Like the convict cichlids, the levels of aggressive encounters between sticklebacks might fluctuate according to level of available resources particularly for nesting territory and materials. Evidence from threespine and ninespine populations observed at St- Ippollitts Brook, river Great Ouse, showed that competition for the lentic habitat was limited and equivocal (Copp, 1998). It is suggested (Copp, 1998) that with more space available the fish tended to avoid each other rather than fight for the same resource.

2.1.4: Selection of study sites

It is possible to satisfy the conditions of two of the criteria of the character displacement hypothesis (Brown & Wilson, 1957) through careful selection of study sites. One of the criteria states that there should be no variation in environmental conditions between allopatric and sympatric sites. A second criterion states that sampling should be conducted along several

geographically separate transects. In order to provide an unambiguous conclusion the study must satisfy the criteria of character displacement given in italics. As far as is practicable these two conditions have been adhered to and are described in outline below.

2.1.4.i.: The alteration in the mean value of the character at the allopatry/sympatry border should not be predictable from variation within either of these areas.

It is essential that morphological differences between populations should not be attributable to differences between the environments. Sites were accepted on the basis that they were physically comparable to each other. It was not practicable to gauge environmental conditions using traditional methods such as temperature and pH as sites were only occasionally visited. The equipment used to collect conventional data regarding water chemistry was not always available or reliable during the course of the study. An alternative was to obtain data from environmental monitoring agencies such as the Environment Agency, but such backwaters as were used in the study have never been monitored, therefore no data were available. As chemical conditions in shallow freshwater streams are liable to fluctuate on a daily basis (see Moss 1988) any data collected at the time of sampling was deemed meaningless. Attempts to find historical records for conditions in shallow backwater streams also proved fruitless. The most useful resource available is that of the endemic invertebrate fauna. Many invertebrates are specific to particular environments and are thus indicative of the physical and chemical conditions.

Information gleaned from the endemic invertebrate fauna was translated into information about the chemical conditions in the environment. The rationale behind this decision is that for many years information about pollution levels in freshwaters has been obtained from the presence or absence of indicator species (e.g.: Trent Biotic Index (Woodiwiss, 1964), Biological Monitoring Working Party (BMWP) revised by Walley & Hawkes, 1996)). By way of example, a dominance of chironomids and freshwater snails (*Hydrobidae* spp.) indicates high levels of eutrophication and possible pollution at a site (BMWP scoring system). These species also prefer a silty sediment that shows signs of little disturbance at the site, indicative of a low water velocity. A high number of certain mayfly nymphs (e.g. *Baetidae* spp.) are found in cleaner (less eutrophic) waters with a higher water velocity. Freshwater shrimps (*Gammarus* spp.) are typical of intermediate pollution levels where the environment has increased levels of ligneous debris on which the shrimp feeds.

2.1.4.ii: *Sampling should be conducted along several geographically separate transects*

If a character shows a significant change at only one site there will be a local explanation. If this change occurs at a number of different sites some general explanation is required. If character displacement is expected it is essential that samples are taken from a number of sites in different geographical locations to render this a plausible explanation. Geographical isolation of the populations from each other minimises the chance of migration and, thus, gene flow between sites. If incidents of parallel adaptation occur across these isolated sites it is feasible that character displacement can provide an explanation.

The criterion that states that sites should be 'geographically separate' is necessarily obscure, in that it is left to the worker to interpret what determines a geographically separate site. The nature of the life history of the focal species determines what constitutes an isolated site. The situation with geographic location of stream dwelling sticklebacks is ambiguous. By their very nature all tributaries are connected by the rivers they ultimately flow into, thus allowing potential mixing of all their inhabitants. But aspects of the life history of most species of freshwater fishes restrict them to particular zones within the river network, so much so that the zones are named after the fish communities found there (e.g.: the trout zone in high mountain streams (see Greenhalgh, 1999)). It becomes confusing with the sticklebacks as they are reported to adapt very well to a number of these different zones. Populations of sticklebacks are found throughout the river network; from the small tributary streams right through to the river estuary. This renders it impossible to say that stickleback populations connected by a particular river are truly isolated no matter how small the chances are of them mixing. However, freshwater sticklebacks are not reported to undertake such extensive migrations as their lacustrine and marine counterparts (Bell & Foster, 1994b). The populations in the study would have to migrate considerable distances to reach a second study population (e.g. > 5km). The closest neighbours in the study are found at a sympatric site in Leicester, even then they are separated by a distance of over 2 km. Sites were sampled in the spring and autumn months over the course of a year in order to monitor seasonal influence on fish morphology. (Unfortunately the foot and mouth crisis (2000) disrupted a more extensive sampling programme).

2.1.5: Chapter rationale

It is apparent that both the biotic and abiotic conditions in the environment may have subsequent repercussions on the stickleback's morphology. The aim of this chapter is to determine what differences exist between sympatric and allopatric habitats, if any. If significant differences are

observed between habitats, the relative influence of any such differences on the stickleback's morphology will be determined.

Examination of the stickleback's habitats is achieved through two separate surveys taken at each site on each sampling occasion. Firstly the physical (abiotic) habitat is inspected using survey methods based on those of the Environment Agency's 'River Habitat Survey' (Raven *et. al.*, 1997). A second survey monitors the invertebrate fauna present at each site. The presence of particular invertebrate species in the habitats is in some part indicative of physical and chemical conditions which provide further information on abiotic conditions. In addition to information about the abiotic environment the invertebrate surveys also provide valuable data on what invertebrate species are potentially available on the stickleback's menu.

2.2: Methods

2.2.1: Location of sites

Information about potential populations of sympatric and allopatric sticklebacks was found from unofficial Environment Agency records (as sticklebacks are not routinely monitored in river surveys) and word-of-mouth. A total of 27 sites were investigated in a preliminary exploration from which a final 10 were chosen. The final 10 sites (table 1) to be used in the study were accepted according to abiotic and biotic similarity, and the relative abundance of sticklebacks at the site. It is essential that the stock should be considered able to replenish itself so that sampling will not be detrimental to population size. The final study populations were situated in Leicestershire and North Yorkshire, which comprise two different geographical regions. Five populations were studied in each region. The precise location of each of the final sites included in the study is given in table 1. An OS map indicating the position of each site in relation to the others is given for each geographic region of Leicestershire and North Yorkshire (figures 1 and 2)

No.	Stream location	Region	Grid reference	Major river	Population
1	Allerstone: Friar dyke	N. Yorks	SE 873 818	Derwent	Sympatric
2	Brompton: Brompton Beck	N. Yorks	SE 945 821	Derwent	Allopatric
3	Folkton Carr: Drainage ditch	N. Yorks	TA 057 804	Hertford	Sympatric
4	Lindhead Gorse	N. Yorks	SE 944 938	Sea Cut	Allopatric
5	Knighton: Saffron Brook	Leics	SK 599 011	Soar	Allopatric
6	Rushey Mead: Melton Brook	Leics	SK 601 074	Soar	Sympatric
7	Troon Industrial estate: Melton Brook	Leics	SK 616 075	Soar	Sympatric
8	Welham: Stonton Brook	Leics	TL 759 918	Welland	Allopatric
9	Wistow: River Sence	Leics	SP 645 959	Soar	Allopatric
10	Yedingham Ings: Welldale Beck	N. Yorks	SE 902 796	Derwent	Sympatric

Table 1: Location of sites where populations of sticklebacks were obtained. 'Sympatric' indicates sites where populations of both ninespine and threespine stickleback fish were found; 'Allopatric' indicates sites where only populations of threespine stickleback were found.

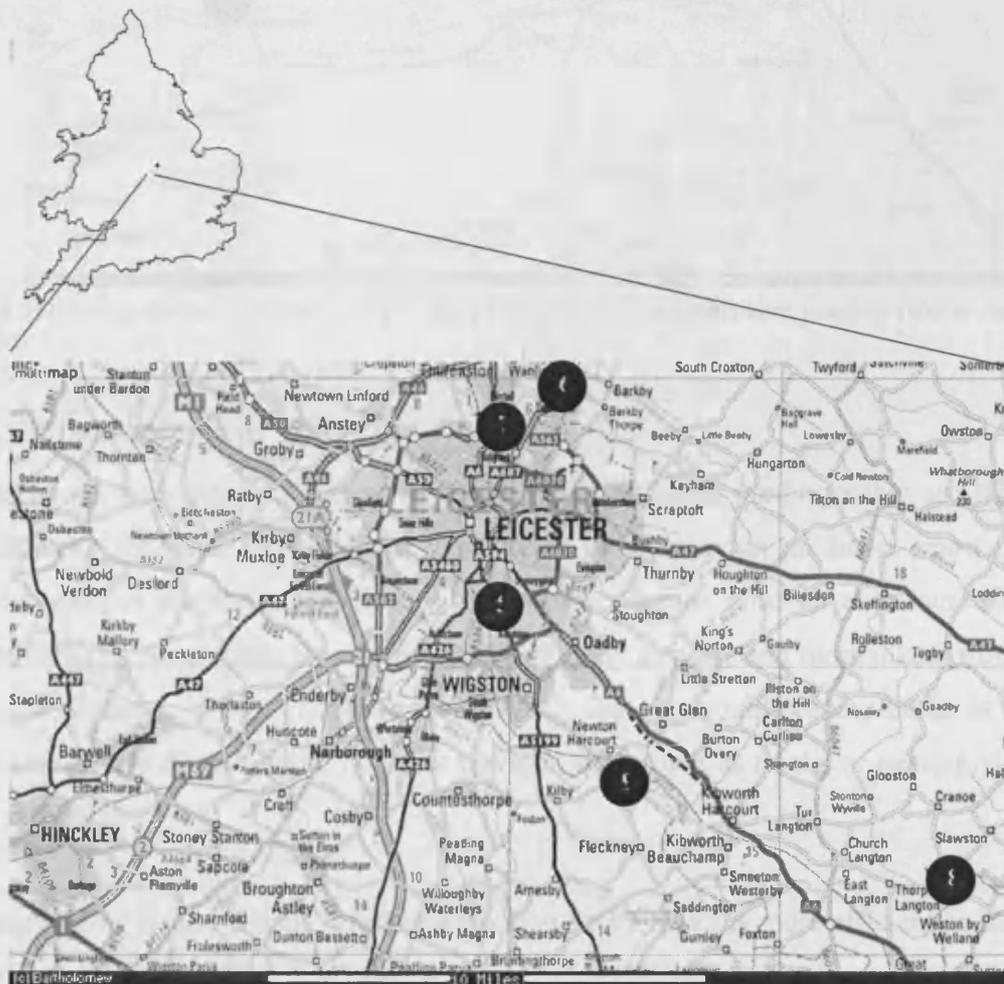


Figure 1: OS map of sites in Leicestershire (sites indicated by circular markers, numbers refer to site number (see table 1))

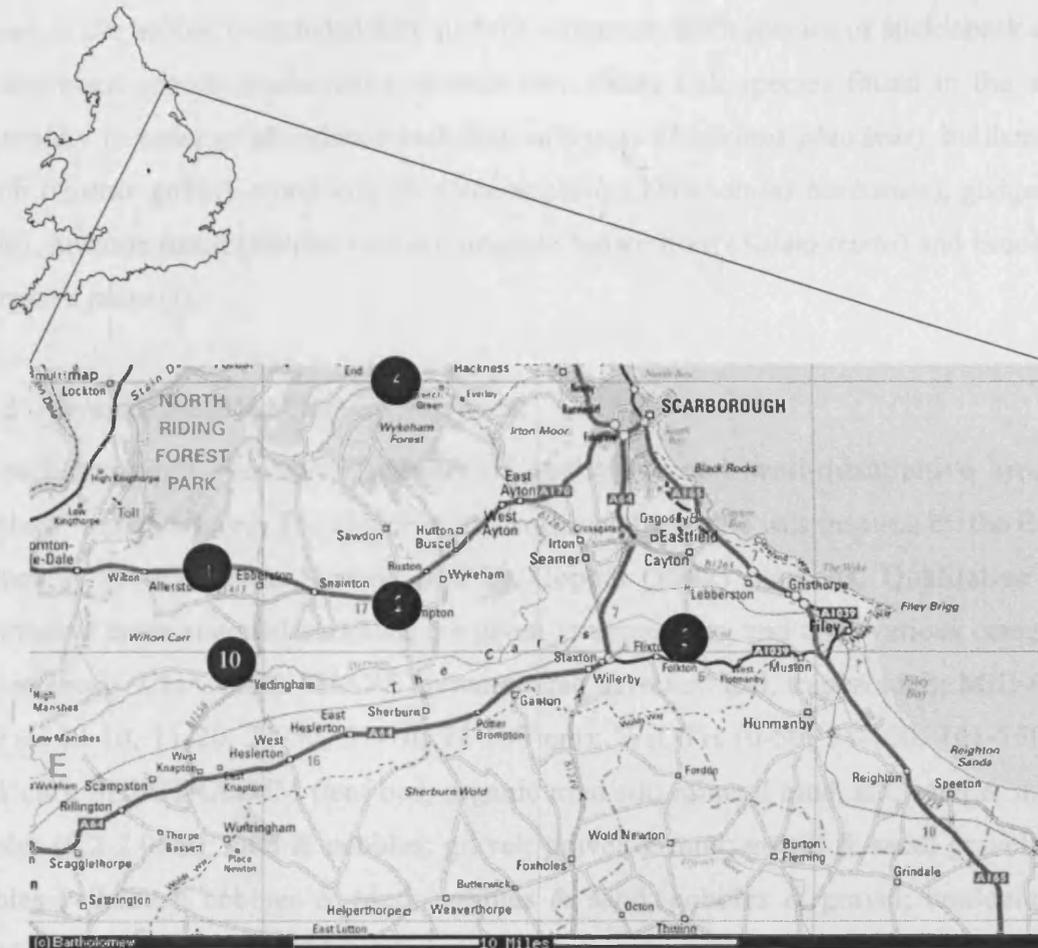


Figure 2: OS map of sites in North Yorkshire (sites indicated by circular markers, numbers refer to site number (see table 1))

2.2.2: Observations at sites

Whilst at the sites observations were made of any potential predators that are likely to target sticklebacks for food. Observations were made of local avian predators known to consume sticklebacks, in particular kingfishers and herons. Herons pose an ever-increasing threat to sticklebacks as populations have grown proportionally as more and more inland trout farms have emerged (Behrent, 1988). Though it was unlikely that we would see any birds, as our presence would doubtless disturb them, no other obvious signs such as tracks or nesting sites indicated their presence.

The method of electro-fishing employed to catch sticklebacks is more efficient at stunning larger fish (Bird & Cowx, 1993), thus making it more likely that larger freshwater piscivores would be caught. No such predators were observed at any of the sample sites. Other fish species were stunned during sampling. This offered the opportunity of observing the structure of the fish community, and therefore potential competitors for resources. The sampling strategy was not

efficient enough to give absolute counts of population size in the habitat so a list of the other species in the habitat is included here just for reference. Both species of stickleback constituted the dominant species numerically at each site. Other fish species found in the stickleback community in order of abundance included; minnows (*Phoxinus phoxinus*), bullheads/miller's thumb (*Cottus gobio*), stone loaches (*Noemacheilus (Barbatula) barbatula*), gudgeon (*Gobio gobio*), juvenile roach (*Rutilus rutilus*), juvenile brown trout (*Salmo trutta*) and brook lampreys (*Lampetra planeri*).

2.2.3: Physical Habitat Survey methods

On each sampling occasion a number of qualitative and semi-quantitative environmental variables were measured. The choice of environmental variable was inspired by the Environment Agency's 'River Habitat Survey' and by Copp's (1992) methods. Qualitative and semi-quantitative environmental variables are given in upper case, and their various categories given in parenthesis. CHANNEL SHAPE (symmetrical, asymmetrical, trapezoidal); MID-CHANNEL DEPTH (0-10, 11-20, 21-50, 51-70, 71-100 cm); WIDTH (0-50, 51-100, 101-150, 151-200, >201 cm); SUBSTRATUM (leaf bed, organic mud/silt; mineral mud, silt; sand & mud; sand & pebbles (0.2-2.0cm); mud & pebbles; gravel; gravel & mud; gravel & sand; gravel & pebbles; cobbles (>0.6cm); cobbles & mud; cobbles & sand; cobbles & gravel; boulders/concrete); FLOATING MACROPHYTES (absent, some, dense); AQUATIC GRASSES (absent, some, dense); SUBMERGED BROAD LEAF MACROPHYTES (absent, some, dense); POND WEED (absent, some, dense); REEDS/PHRAGMITES (absent, some, dense); FILAMENTOUS ALGAE (absent, some, dense); RIPARIAN VEGETATION (absent, some, dense); LIGNEOUS DEBRIS/ROOTS (absent, some, dense); WATER VELOCITY (null, weak, medium, strong); WATER FEATURES (riffle, pool, ponded, marginal deadwater).

2.2.4: Invertebrate fauna survey methods

Invertebrates were obtained by means of a series of three separate 'kick samples' on each sampling occasion, constituting a total of 3 minutes sampling at each site. A standard pond net with a mesh size of approximately 0.5 mm was used to capture any dislodged invertebrates. A minute's kick sampling was spent at either margin of the stream and a final sample was taken from the mid-point along this transect. Kick samples were taken from the area where the majority of stickleback fish were caught. Samples were taken back to the lab where they were sorted. All the captured invertebrates that could be seen were extricated from the samples, identified and counted.

2.3: Results

The work in this chapter proposed to test for potential differences between allopatric and sympatric habitats in both geographical region (Leicestershire and North Yorkshire) and threespine population (allopatric and sympatric). In addition invertebrate analysis also examined seasonal variation (spring and autumn). Work in the following section examines the physical habitat with data collected from the habitat survey; subsequent analysis investigates biotic habitat features using data collected in the invertebrate fauna survey.

2.3.1: Analysis of Habitat Survey Data.

2.3.1.i: Potential environmental influences in allopatric and sympatric sites

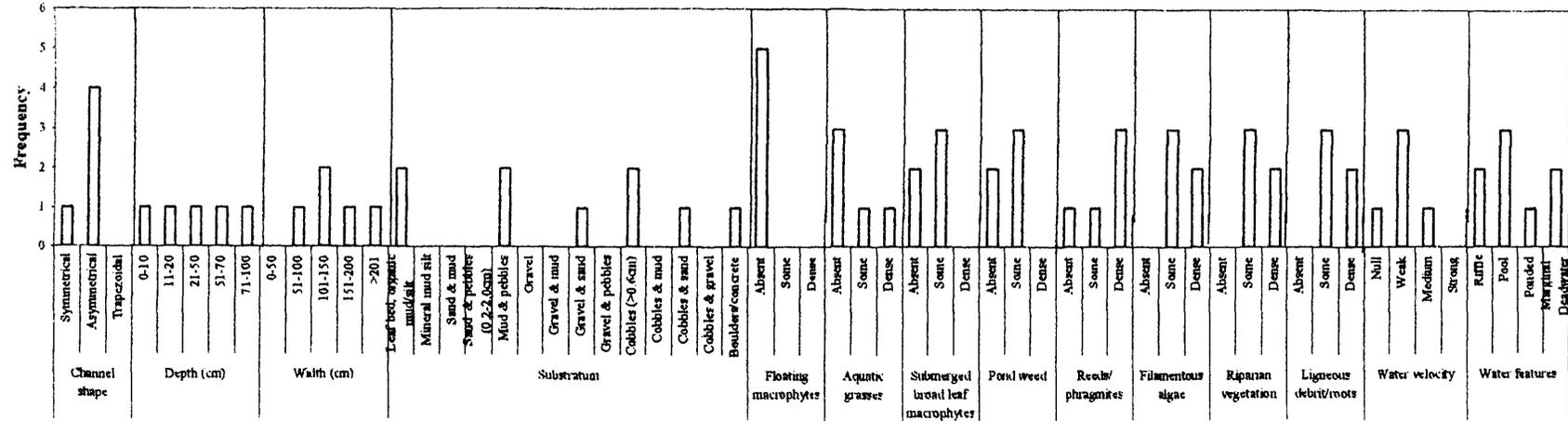
Each feature, (e.g. channel shape) was divided into a number of categories, (e.g. symmetrical, asymmetrical and trapezoidal). Every site was assessed and given a score of 1 for the most applicable variable within each category. Graphs of the raw data comparing habitat differences between allopatric and sympatric threespine populations are given in figure 3. It was not possible to statistically analyse these data as sample size would not allow for a sufficiently powerful test. In order to compare differences between the allopatric and sympatric habitat, each habitat feature was considered sufficiently different if there was a difference in three or more sites between individual categories.

Most habitat features between allopatric and sympatric sites were not conspicuously different, but there were some exceptions. The depth of water at sympatric sites was almost constantly between 21-50 cm with the exception of one site with a depth of 51-70cm; whilst almost each allopatric site was represented by a different depth category. In all sympatric sites the substrate constituted organic detritus and silt whereas this substrate was less predominant in allopatric sites where substrate was composed of larger particulate matter. Aquatic grasses were present in only one allopatric site but were a common feature in all sympatric habitats. Water velocity was considered either null or weak in sympatric sites but weak to medium in allopatric sites, though velocity was not considered particularly fast at any of the sites. The majority of the water course at sympatric sites was described as ponded therefore predominantly lotic. Riffles were found in three of the allopatric sites which provides areas of lotic habitat in these sites, but as there were still pooled regions and areas of marginal deadwater in all allopatric sites lentic habitat was available.

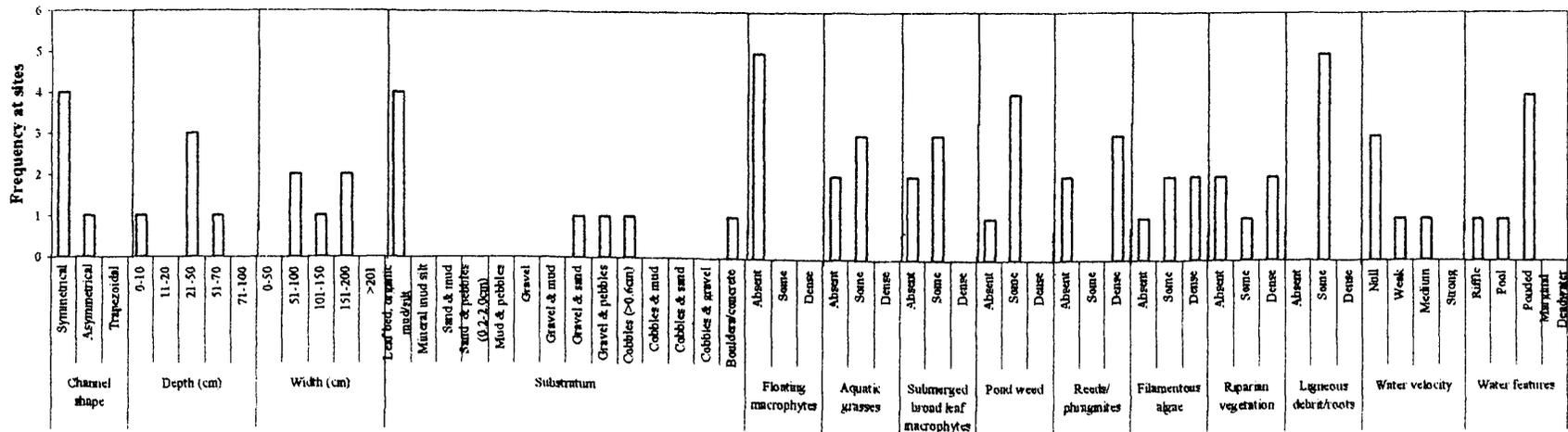
2.3.1.ii: Potential influence of geographic region

A second analysis was performed in order to find any potential differences between sites in Leicestershire and North Yorkshire. The same data as before were used in the analysis but this time grouped according to geographic region. Bar charts of the raw data are given in figure 4. The comparison in this case was that of the physical habitat features between sites in Leicestershire and in North Yorkshire.

Only two habitat features were noticeably different when sites were compared according to geographic location. The first difference showed that sites in Leicestershire were generally asymmetrical in shape but that those in North Yorkshire were usually symmetrical. The second difference indicated a difference in the nature of lotic habitat. In Leicestershire the lotic habitat comprised marginal deadwater, whereas ponded regions were more predominant in North Yorkshire.



Microhabitat variables and categories



Microhabitat variables and categories

Fig 4: Bar charts indicating occurrence of various microhabitat variables measured at sites in Leicestershire (top) and North Yorkshire (bottom).

2.3.2: Analysis of Invertebrate data

Invertebrates were collected on each sampling occasion; consequently they are grouped according to season (spring or autumn), geographical location (Leicestershire or North Yorkshire) and population (sympatric or allopatric) in the analysis. As the data for the invertebrate survey were integer values they were transformed using the square root which meant that they could now be analysed with ANOVA (GLM on SPSS 11). The null hypotheses are as follows: firstly that there is no difference in the invertebrate faunal assemblage collected from Leicestershire and North Yorkshire; secondly, that there is no difference in the invertebrate faunal assemblage collected from allopatric and sympatric sites; and, finally, that there is no difference between the invertebrate faunal assemblages collected in the spring and autumn.

The function of the invertebrate surveys was to integrate information about the abiotic environments and also to give some idea of potential food resources available to the sticklebacks. Information was gleaned from invertebrates considered to be 'typical' fauna for a particular site. The criterion used to establish whether taxa are typical is that they must be present in at least two sites with a frequency of more than 5. In circumstances where invertebrates are few in number (<5), they are not deemed 'typical' taxa and discarded from further statistical analysis. A list of all invertebrate taxa identified from the kick sampling is given in table 40 (Appendix 1). One major criticism of the kick sampling technique is that limnetic zooplankton is under-represented. The net mesh size was considered small enough to capture any potential zooplankton; this is confirmed by the presence of ostracods and hydracaria in the samples which are of a similar size to zooplankton.

2.3.2.i: Potential differences in invertebrate fauna

Only general information was required about the ecology of the invertebrates in either the sympatric and allopatric habitat as such invertebrates were identified to taxa. The data included total counts of invertebrate taxa identified from each site, on each sampling occasion. Only seven of the invertebrate taxa collected were present in sufficient numbers to be deemed 'typical' according to the criteria established in section 2.3.2; the invertebrates included in the analysis were Asellidae, Chironomidae, Erpobdellidae, Gammaridae, Glossiphonidae, Hydrobidae and Oligochaetae. A separate ANOVA was performed in turn for each taxon. The majority of the analyses gave non-significant results and only the significant results are discussed here. Table 2 below provides a summary of the significant results. A complete breakdown of the results of the separate ANOVA's for each invertebrate taxa are given in tables 43-49 (Appendix 1).

Invertebrate Taxa	Source of variation	df	F	P
Asellidae	Population * Location	1, 17	7.855	0.019
Gammaridae	Population (Sympatric/Allopatric)	1, 17	10.554	0.009
Gammaridae	Location (Leicester/Yorkshire)	1, 17	5.531	0.041
Glossiphonidae	Location (Leicester/Yorkshire)	1, 17	9.132	0.013
Oligochaetae	Season (Spring/ Autumn)	1, 17	27.958	0.000
Oligochaetae	Population * Location	1, 17	10.489	0.009

Table 2: Summary of significant results from ANOVA of abundance of 'typical' invertebrate taxa.

There was a significant interaction between population and location for abundance of Asellidae. Asellidae were found to be in significantly fewer numbers in sympatric sites in North Yorkshire than sympatric sites in Leicestershire, but, conversely, higher in allopatric sites in Yorkshire than allopatric sites in Leicestershire. Gammaridae were significantly more abundant in allopatric sites than sympatric sites, and were also more abundant in sites in North Yorkshire than sites in Leicestershire. It is likely that the significant results obtained for Gammaridae were mostly influenced by the substantially higher abundance of this taxa in two particular sites, namely sites 2 (Brompton, Allopatric, North Yorkshire) and 5 (Knighton, Allopatric, Leicestershire). The leech taxa Glossiphonidae was found to be significantly more abundant in Leicestershire than it was in North Yorkshire. Oligochaetes were significantly more abundant in the samples collected in the spring than they were in the autumn samples. There was a second interaction between population and location where oligochaetes were found to be significantly more abundant in sympatric sites in Leicestershire than sympatric sites in North Yorkshire, but significantly fewer in number in allopatric sites in Leicestershire compared to allopatric sites in North Yorkshire.

In summary the only significant difference between sympatric and allopatric sites was the higher abundance of Gammaridae in allopatric populations. Geographical location was influential with regard to numbers of Gammaridae which were in significantly higher numbers in North Yorkshire and Glossiphonidae which were significantly more abundant in Leicestershire.

Table 3 provides a summary of the BMWP scores (see Walley & Hawkes, 1996 for revised scores) and 'Average Score Per Taxon' (ASPT) for each of the sites for each season. These scores were calculated on the entire collection of taxa identified for each individual site. A BMWP score between 16-50 which is within the range that all but one of the sites falls into indicates that a site is 'polluted'. The one exception which is site 2 (Brompton, allopatric, North Yorkshire) in the autumn has a BMWP score of 8 indicating on this occasion that this site is considered 'very polluted'.

Site	Population	BMWP Spring	ASPT Spring	BMWP Autumn	ASPT Autumn
1	Sympatric	n/a	n/a	50.5	4.6
2	Allopatric	n/a	n/a	8	4
3	Sympatric	34.1	4.26	27.2	4.53
4	Allopatric	20.8	3.47	32.5	4.06
5	Allopatric	48	4.8	24.4	4.88
6	Sympatric	30.9	3.43	22	3.14
7	Sympatric	24.7	3.53	19.9	3.32
8	Allopatric	42	3.82	28.5	3.56
9	Allopatric	45.8	3.82	37.5	3.75
10	Sympatric	33.8	5.63	48.7	4.43

Table 3: BMWP scores (see Walley & Hawkes, 1996) and ASPT for invertebrate taxa found at individual sites during spring and autumn collections.

2.4: Conclusions

The aim of the work in this chapter was to establish whether environmental differences between allopatric and sympatric sites exist. Such environmental differences could harbour potential to induce morphological divergence between allopatric and sympatric threespine populations.

2.4.1: Significance of physical habitat differences for potential morphological divergence

The comparisons performed on the habitat survey data indicated that the geographical location (Leicestershire or North Yorkshire) of a site had no consistent effect on the frequency of physical features of the sites, other than sites in Leicestershire were mostly asymmetrical with a higher proportion of deadwater areas, whereas sites in North Yorkshire are generally symmetrical with the water course more likely to feature ponded regions. As the threespine stickleback is reported to prefer areas of lotic habitat (Copp, 1992; 1998) it is important that the habitat supply such regions, but, it is presumed to be of little consequence for the purposes of this study whether these areas are in the form of marginal deadwater or ponded regions.

A few differences emerged when physical habitat was compared between allopatric and sympatric sites. It is apparent that the sympatric habitat constitutes predominantly more lotic conditions than the allopatric habitat. Most of the sympatric sites had a water depth of between 51-70cm despite both stickleback species having shown a preference for areas of deeper water (100-150cm) (Copp 1992). Sympatric sites were more likely to contain a higher abundance of aquatic grasses and denser mats of filamentous algae than allopatric sites. The nature of the differences found in the stream flora indicates that sympatric sites contain a higher area of benthic habitat than allopatric sites. Water velocity was generally negligible in sympatric sites, and much of the water course constituted ponded regions. The substrate in sympatric habitats was predominantly organic detritus and silt; this is presumably a consequence of the limited

water flow. Water velocity was considered generally slightly faster in the allopatric habitats, and 3 of the 5 allopatric sites had riffled areas in the water course providing areas of lotic habitat, however, all allopatric sites were most prominently lentic. From what is reported in the literature about habitat preferences of the ninespine stickleback (Copp, 1992, 1998; see Wootton, 1976) it is known that these fish will be found in regions offering an abundance of aquatic macrophytes and filamentous algae. Ninespine fish also prefer a sandy/silty substratum which is characteristic of still or slow-moving freshwaters, as was typical of sympatric sites. All the information gathered about the physical aspects of the sympatric stream environment does more to explain the presence of the ninespines in these particular environments and possibly why they are not found at allopatric sites.

2.4.2: Significance of differences found in invertebrate fauna for potential threespine divergence

The occurrence of taxa regarded as 'typical' were found to be similar between allopatric and sympatric sites. As the named invertebrates were dominant species at both allopatric and sympatric sites it is considered that abiotic (physical and biochemical) conditions between sympatric and allopatric sites are generally similar. In addition, each of the 'typical' invertebrate species feature in descriptions of dietary intake recorded for sticklebacks (Hynes, 1950; see Wootton, 1976, 1984). Statistical analysis did however find some differences between the abundance of 'typical' invertebrates between sites. Significant differences occurred between frequencies of Asellidae, Gammaridae, Glossiphonidae and Oligochaetae, although no significant differences were found for frequencies of Chironomidae, Hydrobidae or Erpobdellidae.

Gammaridae were the only invertebrate taxa found to have a significantly different abundance between allopatric and sympatric sites. This result is likely to have been strongly influenced by the uncharacteristically high occurrence (for the sites measured) of *Gammarus* in just two of the allopatric sites, namely sites 2 (Brompton, Allopatric, North Yorkshire) and 5 (Knighton, Allopatric, Leicestershire). The foundations of the water course at these particular sites were man-made and constituted a shallow (<20cm) concrete channel. Neither of these sites contained autochthonous substrate and relied on the surrounding riparian vegetation for input of organic matter. *Gammarus* are found in ligneous debris and are intolerant of eutric water or of water with a low oxygen content characteristic of sites with slow-flowing water. Low species diversity is usually indicative of poor water quality and the BMWP scores for sites 2 and 5 would indicate some level of eutrophication but *Gammarus* can not tolerate such poor conditions, rather the reason for low invertebrate diversity might be explained by the lack of autochthonous organic

matter. Many of the invertebrate taxa present in the other sites require habitat features provided by a sandy/silty substrate; this habitat feature is lacking in sites 2 and 5 and might explain the low diversity of invertebrate taxa found at these sites.

Asellidae, Glossiphonidae and Oligochaetae were all found in significantly higher frequencies in sites in Leicestershire; in particular Asellidae and Oligochaetae were found in significantly higher numbers in sympatric sites. Each of these invertebrate taxa is indicative of habitats with slow-moving waters and silted substrate which agrees with the findings of the physical habitat survey for these sites; and each taxon is tolerant of organically enriched or polluted water and score low on the BMWP index. The sympatric sites in Leicester held amongst the lowest BMWP scores of all the sites for both seasons. The higher level of eutrophication in Leicestershire could be the unfortunate consequence of these sites being located in urbanised areas. However, *all* of the sites in *both* sampling seasons are classed as 'polluted' according to the BMWP scoring system. It is well-established that sticklebacks are highly tolerant of polluted or eutrophic conditions (Copp *et al.* 1998; Copp, 1992; VanBavel *et al.* 1997; see Wootton, 1976, 1984). Presumably all sticklebacks, regardless of site, have adapted to increased levels of pollution, for the purposes of this study it is considered of little consequence that the sites in Leicestershire harbour a small but significant number of invertebrate taxa tolerant of poor water conditions.

2.4.3: Consequences of habitat differences on potential threespine divergence

The conclusion drawn from this chapter is that although some significant differences are found to exist between threespine sites, the differences that emerged have done more to explain the presence of the ninespine stickleback in the sympatric sites. Most interestingly the evidence gathered points to a reduction of both lotic (slow flowing) and limnetic (open water) habitat at sympatric sites. This may prove relevant further on in the study as threespine fish are reported to utilise more of the resources in the limnetic habitat than the ninespine fish (Copp 1992, 1998; see Wootton 1976). This situation where the limnetic habitat is reduced poses a potential paradox: if the environment does influence morphology it is expected that the sympatric threespine fish should present adaptation to the benthic and lotic environment; however if sympatric threespines indicate a preference for the benthic habitat this could bring them into competition with the ninespine fish.

"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' ('I found it!') but rather 'hmm....that's funny...'"

— Isaac Asimov (1920-1992).

Chapter 3: Examining stickleback morphology

3.1: Introduction

Interspecific competition is deemed to be one of the major ecological processes driving speciation and the origin of biodiversity. The competitive exclusion principle is based on Gause's demonstration that complete competitors cannot co-exist (Gause, 1934). This places a limit on how ecologically similar the niches of coexisting species can be before one or the other of them will be excluded from the habitat. Whilst this concept is intuitively clear it does not account for the many examples of apparently ecologically similar species that co-exist in natural environments. In light of the situation found in natural communities a more appropriate question to ask is 'how different do two species have to be in order to co-exist in the same environment?' (MacArthur, 1972). Animals most frequently interact over shared food resources, and are deemed competitors when this interaction causes a reduction in population growth rate in both species. In order to co-exist species must increase their competitive ability by adapting their features and partitioning resources. There are a number of ecological processes that can facilitate coexistence, for example a degree of separation in time or space can reduce the strength of competition and allow coexistence. Another alternative is that a shared natural predator can reduce competition for food by reducing population density. It is assumed that none of these mechanisms applies to stickleback in these particular study streams. The research in this chapter aims to explore whether interspecific competition has led to divergence that may have allowed co-existence between the ecologically similar threespine (*Gasterosteus aculeatus*) and ninespine (*Pungitius pungitus*) sticklebacks. When two ecologically matched species occur sympatrically interspecific competition between them sometimes results in divergence of certain characteristics (e.g. Sympatry of eastern (*Sitta tephronota*) and western rock nuthatches (*S. neumayer*) in Iran, Brown & Wilson 1956). This phenomenon forms the basic premise of Brown and Wilson's (1956) theory of ecological character displacement. As discussed in the introductory chapter their theory has since been reconsidered and is defined as follows:

Character displacement is the process by which the mean values of a phenotypic character, in two competing species, displace away from each other in areas of sympatry, because of the presence in sympatric populations, but not in allopatric ones, of a selective pressure stemming from interspecific competition.

Over the years this hypothesis has met with much scepticism from ecologists since very few examples are apparent in nature (see Schluter, 2001). Only a handful of other studies implicate character divergence as the promoter of divergence between two species. The reason for this may be twofold. Either character displacement does not commonly occur in nature, or, if it does, it is difficult to demonstrate convincingly.

In Britain populations of threespine stickleback occasionally co-occur with populations of ninespine sticklebacks. As these fish are ecologically similar, high levels of competition might be expected. As competition has not resulted in the elimination of either one of them from the habitat it can be assumed some ecological process allows them to co-exist. In this chapter I provide evidence to show that the phenotype of threespine sticklebacks living together with ninespines (sympatric) has diverged from that of threespines on their own (allopatric). This morphological divergence could lead to the sympatric threespines exploiting a narrower range of food types than their allopatric counterparts, but with greater efficiency. I propose that character displacement is the major process driving this divergence.

3.1.1: The case for the sticklebacks

Whilst revelations made about Canadian threespine stickleback populations have obviously inspired the current train of thought in the thesis, in the preliminary observations a sceptical eye was also kept open. Huxley's (1825-1895) observation that a "beautiful hypothesis" is often slain by an "ugly fact" serves as a haunting reminder that nothing in nature can be taken for granted.

Since the threespine and ninespine sticklebacks are closely related, share similar niches and are often found living in sympatry, they are ideal candidates for investigating the hypothesis of character displacement. An essential condition of the hypothesis is that any phenotypic change must confer a competitive advantage to the species. If morphological differences are found between populations, the differences should mean that the fish are better able to compete for resources. If the fish are phenotypically different and, yet these changes hold no specific competitive advantage, there could be any number of explanations. However, character displacement would not be one of them. In the following discussion I provide a review of the various ecological features of the threespines' life-history.

3.1.2: Circumstances that indicate potential character displacement: Same habitat

Standard textbook descriptions (e.g.: Wootton, 1976, 1984; Bell & Foster, 1994) tell us that threespine sticklebacks show a preference for the limnetic region in aquatic habitats. This zone consists of open water areas typified by the lack of any permanent structures, although ligneous debris may create ephemeral structure. Wind and other agents produce a spatially heterogeneous distribution of zooplankton upon which the fish graze. Meanwhile, the shyer ninespine finds its niche in vegetated regions (see Wootton, 1976). This zone is more structurally complex because it usually contains high densities of aquatic vegetation. The ninespine forages in the substrate so its diet consists mainly of larger invertebrate prey (see Wootton, 1976). These two zones are easy to visualise in larger water masses such as lakes. However, this study used populations of fish from shallow streams, where the average depth was <50cm, making it unlikely that vegetated and limnetic zones exist as discrete sub-habitats. In fact, in these small streams, both species of stickleback are commonly caught in the same sweep of the net. Apparently sticklebacks do not discriminate between zones either!

3.1.3: Circumstances that indicate potential character displacement: Similar dietary habits

Both the threespine and ninespine sticklebacks are generalist feeders (e.g. Hynes 1950) and the diets of both are very similar (see Wootton 1976). Being a generalist will have facilitated successful invasions into a number and diversity of freshwater habitats. Both species consume a range of prey; both micro and macro-invertebrates, the only apparent limitations being the availability of prey, or gape size of the mouth (Gill & Hart 1996). These conclusions about the stickleback's diet were drawn from an array of sources by Hynes (1950) and would appear to be an oversimplification of conditions at individual sites. Not all the invertebrates listed as potential prey will be present at every location and the sticklebacks will be expected to adapt to local conditions accordingly. However, a mutual attraction for the same food resources might induce competition between the nine and threespine sticklebacks.

Diet choice has a large potential impact on phenotype. One only has to look to the diversity of the mouthparts of the haplochromine cichlids for evidence of this (see summary Wootton 1990). Jaw structure is one of the most malleable of the teleost features and is rapidly altered to adapt to changing circumstances. If food resources are fundamentally different between allopatric and sympatric sites the observed changes in the threespine's phenotype may be as a direct consequence of an altered dietary regime, and not because of the presence of the ninespines in the habitat. Because the choice of what and how prey is taken has a big impact on the subsequent phenotype a detailed analysis of the subject is given in separate chapters. An analysis of the

invertebrate assemblages at individual sites is included in chapter 2 and details of dietary intake for the different stickleback populations are given in chapter 5.

3.1.4: Focus of previous morphological investigations

An initial dilemma faced when conducting morphological studies is choosing which specific features of the fish's anatomy to measure. In a study of trophic interactions any chosen feature must provide useful information about how the stickleback manoeuvres itself and utilises the resources in a particular environment. On a practical level these features must be relatively easy to measure, as large samples of fish have to be processed. A number of studies that have previously investigated divergence in sticklebacks (Lavin & McPhail, 1985; 1986; Schluter, 1993; Hatfield & Ptolemy, 2001) offer some insight into which particular features are important for detecting any potential divergence between populations. This provides us with a useful starting point for subsequent studies. However, these studies also highlight possible holes in our knowledge, which gives us the opportunity to try to rectify the problem in future studies.

3.1.4.i: Focus on jaw morphology

The main focus of Lavin & McPhail's studies on the trophic morphology of threespine sticklebacks (1985; 1986) was those features associated with jaw morphology. It was observations made on these particular characters that led to the original proposal that the process of character displacement has led to the observed divergence of the Canadian threespine populations. The focus on jaw morphology appears to have been an obvious yet sensible starting point as competition so often revolves around food resources. One way that fish might alleviate the effects of potential competition is to apportion food resources amongst the populations by altering their diets. Lavin & McPhail (1986) describe the differences in habitat availability between lakes in BC, namely three main types; dominant open water, littoral and intermediate. One difference between these habitats is the kind of food on offer, the most obvious difference being the contrast in the size of prey. The dominant prey harboured in open water habitats are microscopic, free-swimming zooplankton, which contrasts with the larger, usually less mobile invertebrates available in benthic habitats. Lavin & McPhail's (1984) study concluded that selection worked most strongly on features related to food size, these features being upper jaw size and gill raker number. Stomach-sample analysis confirmed that the fish from different habitats did indeed favour different diets. These findings validate the presupposition that the jaw morphology of sticklebacks readily adapts to the most effective size for the dietary regime.

The correlation between the threespine's jaw morphology and the size of prey taken has since been confirmed in later studies (e.g.: Gill & Hart, 1996; Day *et. al.* 1994; Robinson, 1999). Robinson (1999) reported that the mouth shape of progeny from the same parental morphs is readily altered when raised on different dietary regimes centred on two different sizes of prey. Threespines raised initially on bloodworms then after 30 days given amphipods had significantly larger mouths than their siblings that were raised solely on *Artemia*. Further trials indicated that fish fed better when offered the food they were raised on than when they were provided food their counterparts were used to. A similar conclusion to Robinson's was reached in a previous experiment by Day *et. al.*(1994). Day *et. al.* (1994) measured the morphological response of the divergent threespine pairs when raised on either limnetic or benthic prey. These experiments found that both of the limnetic and benthic threespines exhibited morphological plasticity in an adaptive direction; each species closely resembled the other when raised on the latter's diet.

Gill & Hart's (1996) study approached the situation of how jaw morphology relates to food size from a different angle by observing feeding behaviour. Threespines were distinguished on the basis of either having a small or large mouth. Each fish was then paired with another from the corresponding category. In various treatments, each pair was offered either a small or large prey (various size categories of *Asellus*). The fish's behaviour was categorized as spotting, pursuing, handling or consuming the prey. This study found that mouth size was an important competitive factor as the fish that consumed the prey was the one with the most suitably sized jaw for the size of prey offered. However, this study noted significantly a second factor, which was the ability of the fish to reach the prey first. This helped to determine which fish in the pair would be the consumer. These characteristics determined the fish's 'competitive ability'. Here we begin to acknowledge that the stickleback's ability to consume different sizes of prey is not just a product of its particular jaw morphology. The jaw is involved in only the last stages of handling and consuming prey; the prey has to be caught in the first place. Prey is caught through means of hunting and pursuing, a procedure involving the whole of the fish's body. These hunting behaviours are dependent on the ability of the fish to manoeuvre itself in its environment. Different types of prey and different environments require different methods of hunt and pursuit. The study of Gill & Hart (1996) tells us that there are fundamental differences between sticklebacks other than those we have observed in jaw morphology. The collection of more information about the phenotype of the stickleback's entire form will elaborate our understanding of what factors determine competitive ability.

It is possible that the differences observed in jaw morphology are a result of environmental influences rather than a result of character displacement. Features around the jaw may be quickly reversed in just a few generations depending on the surrounding environmental conditions. Some years may bring an abundance of food in the environment thus reducing competition and the effects of density dependence on a population, resulting in a loss of divergence in these particular morphological characters. Such an effect was observed in the Enos lake, BC population in 1999 (Kraak *et. al.*, 2001). In this particular year statistical analysis of a sub-sample of the population showed a higher than expected proportion of hybrids between the limnetic and benthic morphotypes which lead to the conclusion that the species pair had begun to collapse (Kraak *et. al.*, 2001). There is a possibility that in this particular year that food sources were in abundance, reducing competition and relaxing divergence between the threespine morphs. However, in the absence of long-term morphological data Kraak *et. al.*'s hypothesis cannot be corroborated. Either way this evidence confirms that reliance upon jaw morphology alone is not sufficient to claim character displacement in threespine populations. It does not wholly convince us that the divergence of jaw structures is not just the result of a temporary plastic response to environmental conditions at a particular time. What is required is some further evidence of morphological adaptation to the rest of the stickleback's morphology that would serve to substantiate the observations on divergence of jaw morphology. Direct genetic inheritance of jaw morphology features could be tested by back-crossing allopatric and sympatric threespines and raising the subsequent F1 generation in identical environments. If the F1 generation show a 1:1 of jaw features genetic inheritance of these characteristics is implied.

3.1.4.ii: Alternative morphological features measured

Lavin & McPhail (1985; 1986) and later Schluter's (1993) studies also include measurements of standard length and a depth measurement along the deepest axis of the body. These data inform us that the limnetic fish are thinner at this particular dimension for their length and led to the conclusion that the limnetic morphs are more streamlined than their benthic counterparts. However, measurements such as these are limited in their applicability (see Howe, 2002). Standard length is a notoriously variable feature even within populations so it is difficult to extract significant results even if they do exist. Limiting analysis to two measurements of the remainder of the sticklebacks form also tells us rather little about the *shape* of fish in potentially divergent populations. The fact that limnetic populations are generally smaller and skinnier than benthic ones could be because we are observing two different year classes, the limnetic fish being the younger of two potential cohorts. Any subsequent difference in jaw morphology, again where the limnetic form has a smaller, narrower mouth might lead us to conclude that the

morphological differences we are observing are the result of normal allometric growth responses. To begin to eliminate any such discrepancy we must make measurements that will provide information about the form of the entire stickleback.

Schluter (1993) also incorporates measurements of weight in his analysis. This is not a stable variable. Sticklebacks are diurnal foragers, their main activity is in the first hours of daylight (see Wootton, 1976). Consequently a fish's weight in the morning will be heavier than in the evening after it has had a day to digest its food. Thus there will be an effect of the time of day the fish are caught for measurements of body mass. Further complications arise from fish in spawning condition. All in all, weight is often too variable to be used as a comparative character.

3.1.4.iii: Alternative methods for assessing divergence

A rather different approach to investigating potential character displacement was taken by Robinson (2002), who investigated potential divergence of brook sticklebacks (*Culaea inconstans*) found co-existing with ninespines in BC. In a similar way to this study, the ninespine was deemed to be the competitive force, equivalent to that of the benthic species morph. However, there are many apparent flaws in the methodology used to carry out this investigation that inevitably raise more questions than answers. Only one allopatric and one sympatric population of brook sticklebacks were used in the investigation. Robinson proposed that the morphology of the sympatric brook sticklebacks represented a post-displacement form. This sympatric form is better able to compete with the co-habiting ninespine sticklebacks as it has presumably become more efficient in the open water areas of the environment. To test this proposal brook sticklebacks from the allopatric population were translocated to cages in the sympatric site. This site also contained cages that held samples of the resident brook sticklebacks, and others that contained the resident ninespines. In addition there were cages of mixed populations, so the ninespines were mixed with either sympatric or allopatric brook sticklebacks. The fish were left in these cages for 30 days after which they were weighed in order to assess growth rate in each of the different experimental conditions.

An area of concern is that allopatric and sympatric brook sticklebacks were represented by only one population respectively. A more convincing argument for character displacement is when similar morphological divergence happens, in parallel, at a number of different locations. It is possible that the experiment was in some way biased to a higher survival/growth rate of both species of fish from the sympatric population as they remained in their original location. It would have been fairer to transplant all the fish involved to a neutral territory. Although the cages the fish were held in were sufficiently large they still restricted movement of the fish in the

environment. This is more significant for the brook sticklebacks who like their threespine cousins are adept at moving round the open water areas of the habitat. The static cages presumably held potential prey for the benthic ninespines but reduced the amount of limnetic prey that the brook sticklebacks rely upon. Limnetic prey has a patchy distribution that requires the brooks to actively hunt for a potential meal. In the cages fish had to wait until a potential meal inadvertently swam into their cage. It is more likely then that the brook sticklebacks will go hungry, and this may be a reason why in the results the mortality rate of the captive allopatric and sympatric brook sticklebacks was much higher than for the ninespines. Overall the paper probably tells us more about the effects of restricting the brook stickleback's movement in its environment than the effects of competition and subsequent character displacement from the heterospecific ninespines.

Having considered what morphological features have been measured in previous studies it is apparent that there are some obviously useful traits such as those centred on the jaw, but on their own these have limited applicability. What is obvious from Gill & Hart's (1996) study is that other aspects of the stickleback's body form are involved in allowing it to exist in different environments. The current focus on just a few jaw characteristics ignores what might be happening to the rest of the stickleback's form. If jaw shape is so readily altered it is possible that the rest of the stickleback's form is doing likewise. As more and more single features change there may be an additive impact on the whole fish, which alters how it functions in its environment. As the phenotypic features slowly change the stickleback becomes more specialised in a particular environment. This specialisation is reached as the fish rather than exploit a wide range of resources from its environment becomes more efficient in a sub-region of the habitat. Morphological specialisation is only effective if the habitat has sufficient resources to support this new form. If morphology is altered it strongly suggests that there is a permanent ecological pressure forcing it to do so.

3.1.5: Objectives of this chapter

The primary objective of this chapter is to examine how the presence or absence of a heterospecific competitor is associated with phenotypic differences in the threespine stickleback. In each of the shallow, lowland tributary streams included in the study the threespines constitute the most abundant species (pers. obs.). The major difference between sites is the presence or absence of the ninespine stickleback (see chapter 2). If the ninespines offer a strong competitive presence in the benthic regions of the habitat then I would expect there to be a subsequent convergence of traits of the sympatric threespine's phenotype. It is documented that the

ninespine stickleback is an efficient competitor in the highly vegetated benthic region of the habitat (see Wootton, 1976), presumably any divergence amongst sympatric threespines should manifest itself as a threespine more adapted to foraging for small prey items in open water. If this assumption is correct there is an expected concurrent relaxation of pressure in allopatric populations where the ninespines are absent, and morphology should be more variable within individual populations. If character divergence is to be implicated, these patterns of convergence and divergence of threespine populations in the presence and absence of the ninespines respectively should be apparent regardless of geography. The results should show evidence of parallel convergence of similar traits in the sympatric populations in both the Leicester and North Yorkshire populations in order to discount the effects of other biotic and abiotic influences on the threespine stickleback's form.

3.2: Methods:

This study was carried out in the early summer (June) and autumn (September) of 2000. Samples of sticklebacks were electro-fished from five sites each in Leicestershire and North Yorkshire. Isolated, allopatric populations of ninespine stickleback were not uncovered in any of the areas searched. Fish were given an overdose of the anaesthetic MS222, and taken back to the lab for morphological analysis. The various morphological dimensions, as indicated in figures 1 to 3, were measured using callipers; finer features were measured with a graticule on a binocular microscope. The length of each dimension was measured to the nearest 0.1mm. Data were analysed using SPSS 10 for Windows.

3.2.1: Aspects of morphology that were measured.

A number of different measurements were taken that encompassed the full length of the stickleback's body. These are depicted diagrammatically in Figures 5 and 6. Each of the measurements avoids regions of the body prone to fluctuate regularly, such as around the stomach region, which will readily distend with fullness of stomach or gonad growth. In addition each of the features chosen was considered to be a 'landmark' on the stickleback's body. For example the dimension named 'spine 1' is always measured along the axis from the base of the first spine to the ventral surface. In this way the same regions are measured on each fish reducing the opportunity for mistakes. It also means that the reader clearly understands which dimensions are measured and can be easily translated to other studies for comparison.

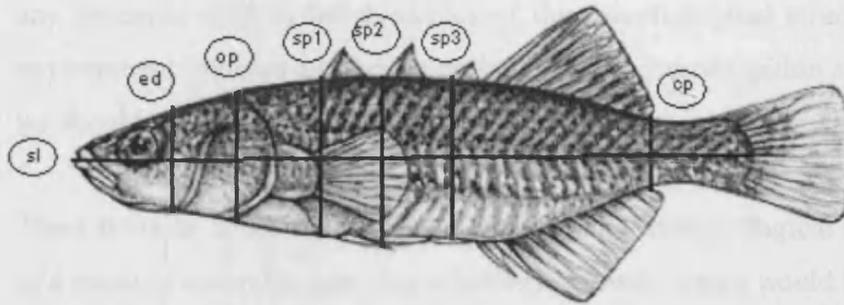


Figure 5: Diagram indicating body dimensions measured. Key to abbreviations used: sl = standard length; ed = eye diameter; op = depth over operculum; sp1 = depth over spine 1; sp2 = depth over spine 2; sp3 = depth over spine 3; cp = depth over caudal peduncle. Stickleback picture adapted from PA fish tables.

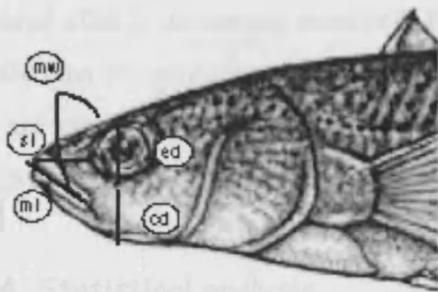


Figure 6: Diagram indicating jaw and head features measured. Key to abbreviations used: mw = mouth width; sl = snout length; ml = mouth length; ed = eye diameter; cd = chin depth. Stickleback picture adapted from PA fish tables.

3.2.4. Statistical analysis

3.2.2: Examining environmental effects of trophic regime on morphology.

A study running concurrently with this one aimed to measure the effects of trophic regime on toothwear in threespine sticklebacks (Purnell et. al. in prep). The methodology involved raising juvenile sticklebacks under controlled conditions designed to simulate benthic and limnetic trophic niches. Juveniles in 'limnetic' conditions were raised solely on *Daphnia* and those in 'benthic' conditions were fed chironomids. The fish were maintained in these conditions for 9 months after which time they were sacrificed for tooth-wear analysis. Before the teeth of the fish were examined it was possible to take measurements of various morphological traits as described in section 3.2.1. These data were used to test the hypothesis that the trophic environment during development has no effect on the morphology of the threespine stickleback. Data were analysed using ANOVA on SPSS 11 for Windows.

3.2.3: Handling of raw data

The stickleback populations were grouped in the analysis according to the original population (i.e. allopatric, sympatric or ninespine), and the season they were sampled in (i.e. spring or autumn). The distinction between sampling dates is maintained to take account of the fluctuating structure of the populations at these different times. The autumn samples will likely contain both adult fish and the juveniles that were spawned from the earlier spring samples. Potentially the

autumn samples contain two separate year classes, which may have repercussions on the results obtained from the morphological analysis. Maintaining this distinction also allows us to observe any potential shift in the dynamics of the morphological structure of the population. If the environment imposes a selection pressure on individuals within a range of morphological forms we should expect to see a less homogenous structure in the juvenile population (autumn sample).

There remains doubt as to whether any potential morphological divergence observed would be as a result of naturally occurring allometric growth, which would be due to differences in *size*, as opposed to changes in the actual *shape* of the sticklebacks. It was considered that any differences in *size* between populations will conceal potential changes in *shape*. In this analysis potential effects of size are removed; this is in order to remove any doubt that the differences in populations observed are due to changes in *shape* as opposed to changes in *size*. The effects of size are removed by regressing each individual trait against standard length, the residuals of which are logged (\log_{10}) and used in subsequent statistical analysis.

3.2.4: Statistical analysis

Much of the biometric analysis follows that conventionally used in morphometrics, but one method in particular deserves further explanation. The data were first analysed using multi factor ANOVA followed by 'canonical discriminant function analysis' (DFA) (see Manly 1986). Previous studies (e.g. Schluter, 1993) have relied on Principal Components Analysis (PCA). Both DFA and PCA are similar in principle; the major way they vary is in how they group series of data. PCA is used to identify groups of data points. This method is useful if the researcher believes he may have two (or more) individual groups contained in an isolated sample as is the case for the threespine fish in BC, Canada. For groups of data where groups of individuals can be defined *a priori*, such as in the case of this study (fish are from known allopatric and sympatric populations), DFA is a preferable method. In this case the analysis sees how well individuals 'fit in' to the groups they were naturally allocated to. All data were analysed using SPSS 11 for Windows.

3.3: Results

The objective of this chapter is to establish whether threespine sticklebacks from sympatric populations are a different *shape* from those living allopatrically. Thus the primary null hypothesis is that all threespine fish have identical morphologies regardless of the presence on ninespine fish in the habitat.

3.3.1: Are morphological differences apparent?

The foremost priority in the analysis is to establish whether differences in morphology actually exist between allopatric and sympatric threespines. And, if differences exist, which of the features measured contribute most to this morphological divergence. The null hypothesis proposed is that the sticklebacks from different populations all have the same shape. The data were initially analysed using multi-factor ANOVA. Ninespine sticklebacks are included in the analysis despite their possessing morphology sufficiently different from that of the threespines to be classed as a separate genus. Their inclusion is warranted here so as to evaluate exactly how different, or indeed how similar, their morphology is from that of the threespine populations, at least for those features included in the analysis. Table 4 gives a summary of mean values for each of the morphological features measured on each of the stickleback populations for each season. An entire breakdown of the descriptive statistics collected from fish from individual sites is given in tables 50-53 (Appendix 2)

Character	Sympatric threespine		Allopatric threespine		Ninespine		SE	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Standard length	34.788	23.029	36.943	26.444	34.725	28.798	0.729	1.675
Body depth over eye	5.627	3.59	5.904	4.064	4.980	4.126	0.274	0.169
Body depth over operculum	7.370	4.503	7.645	4.500	6.327	5.014	0.401	0.167
Body depth over spine 1	8.043	5.258	8.516	5.822	6.767	5.493	0.522	0.164
Body depth over spine 2	8.384	5.516	8.943	6.263	6.873	5.634	0.618	0.232
Body depth over spine 3	7.255	4.431	7.752	5.110	5.602	4.636	0.650	0.201
Body depth at caudal peduncle	1.756	1.069	1.946	1.251	1.169	0.983	0.234	0.079
Mouth width	2.970	1.637	3.161	1.972	2.921	2.185	0.073	0.159
Mouth length	2.323	3.716	2.501	4.324	2.309	4.881	0.062	0.336
Eye orbit	2.577	5.248	2.739	5.969	2.432	5.829	0.089	0.221
Chin depth	1.932	2.277	2.078	2.803	1.648	2.945	0.126	0.203
Snout length	2.721	3.996	2.977	4.959	2.246	4.587	0.214	0.280

Table 4: Mean value (mm) of morphological feature measured for each population of stickleback, for each season, with standard error of difference between means.

The results from the multi factor ANOVA for both the spring (table 54, appendix 2) and autumn samples (table 58, appendix 2) are significantly different for *all* the characters measured with the exception of mouth width in the spring sample ($df=1, 490$; $F=2.63$; $P=0.073$) for this reason mouth width in the spring sample is omitted from further *post-hoc* analysis. This result might seem unsurprising due to the inclusion of the ninespine stickleback in the analysis. Traditionally a significant multi factor ANOVA can be followed with a series of separate univariate ANOVAs on the individual traits (see Manly, 1986). These ANOVAs are 'protected' against inflated type 1 error by the initial multi factor ANOVA (i.e. if the null hypothesis cannot be rejected by multi factor ANOVA any subsequent tests are ignored because any significance must be a type 1 error

because the null hypothesis has been accepted) (see Field, 2000)). *Post-hoc* analyses (univariate ANOVA) between the three stickleback populations show that the significant differences shown in the multi factor ANOVA is *not* because ninespine fish are included. A table of mean values for each morphological feature measured on each stickleback population for each season is given in tables 50-53 (appendix 2). The following discusses the results of the *post-hoc* univariate ANOVA, taking just two of the groups at a time, for each season, spring and autumn respectively. Only the most relevant results are summarised in the following section, though a complete list of the analysis is given in tables 55-57, 59-61 (appendix 2). Non-significant results are also supplied. This is because non-significant results indicate common features shared between stickleback populations.

Comparison between the ninespines and their sympatric threespines showed that all traits are significantly different with the exception of standard length ($df=1, 336; F=0.004; P=0.951$) and mouth length ($df=1, 336; F=0.032; P=0.859$) in the spring sample. In the autumn samples there were no differences with regard to body depth over the chin ($df=1, 334; F=1.945; P=0.164$), snout length ($df=1, 334; F=3.281; P=0.071$), mouth length ($df=1, 334; F=3.030, P=0.083$, and body depth over spine 2 ($df=1, 334; F=0.871; P=0.351$) and body depth over spine 3 ($df=1, 334; F=2.262; P=0.134$). Body depth over spine 1 was almost significant ($df=1, 334; F=3.650; P=0.057$). The remaining traits were all significantly different. Interestingly in both seasons sample mouth lengths did not significantly differ between the two species (spring: $df=1, 336; F=0.032; P=0.859$; autumn: $df=1, 334; F=3.030; P=0.083$). This contradicts the prediction that the features around the mouth would be amongst the first to change.

When the ninespines are compared to the allopatric threespine population there are more significant differences than were shown for the sympatric population. In fact, for the spring sample, the two populations show significant differences for *all* the measured features. In the autumn populations all features are significantly different, with the exception of body depth over the eye ($df=1, 331; F=1.680; P=0.196$) operculum ($df=1, 331; F=1.041; P=0.308$), and spine 1 ($df=1, 331; F=0.597; P=0.440$), and once again mouth length ($df=1, 331; F=2.1054; P=0.148$).

Comparison between allopatric and sympatric threespines shows that in the spring sample a number of features were significantly different (see table 55, Appendix 2) with the exception of body depths over the eye ($df=1, 316; F=2.329; P=0.128$), operculum ($df=1, 316; F=1.070; P=0.302$), and spines 1 ($df=1, 316; F=2.876; P=0.091$), 2 ($df=1, 316; F=3.466; P=0.064$) and 3 ($df=1, 316; F=1.942; P=0.164$). The most interesting set of results comes with the comparison of

morphological characters for the autumn sample where *all* measured traits are significantly different between the allopatric and sympatric threespine populations.

3.3.2: Are differences due to geographical region?

The second criterion of the character displacement hypothesis, which examines the influence of geographic region, is dealt with here. In order to satisfy the second criteria of character displacement the following null hypothesis was posed; do these shared traits reflect common ancestry or geneflow, rather than independent parallel evolution? The same data as before were analysed but in this case each population was examined separately (i.e. ninespine, sympatric or allopatric threespine) and groups were defined according to geographic region (i.e. either North Yorkshire or Leicester). These data were analysed using ANOVA on SPSS 11 for Windows. Full details of all these results are provided in tables 62-67 (appendix 2).

Character	Leicestershire		North Yorkshire		SE	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Standard length	36.375	23.789	33.865	22.561	1.255	0.614
Body depth over eye	5.777	3.749	5.540	3.492	0.119	0.129
Body depth over operculum	7.468	4.484	7.312	4.515	0.078	0.016
Body depth over spine 1	8.111	5.115	8.004	5.345	0.053	0.115
Body depth over spine 2	8.453	5.251	8.344	5.680	0.054	0.215
Body depth over spine 3	7.196	4.308	7.289	4.506	0.046	0.100
Body depth at caudal peduncle	1.723	1.048	1.776	1.083	0.026	0.018
Mouth width	2.905	1.823	3.007	1.522	0.051	0.150
Mouth length	2.374	3.210	2.293	4.027	0.040	0.409
Eye orbit	2.660	5.196	2.529	5.28	0.066	0.042
Chin depth	1.930	2.24	1.933	2.300	0.001	0.030
Snout length	2.904	3.927	2.615	4.039	0.144	0.056

Table 5: Mean value (mm) of morphological features measured for each population of sympatric threespine stickleback according to geographic location, for each season, with standard error of difference between means.

Character	Leicestershire		North Yorkshire		SE	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Standard length	39.76	26.45	32.051	30.165	3.855	1.858
Body depth over eye	5.652	4.045	4.624	4.173	0.514	0.064
Body depth over operculum	7.165	4.8	5.882	5.139	0.641	0.169
Body depth over spine 1	7.765	4.995	6.237	5.783	0.764	0.394
Body depth over spine 2	7.795	4.98	6.384	6.015	0.706	0.517
Body depth over spine 3	6.422	3.982	5.166	5.017	0.628	0.517
Body depth at caudal peduncle	1.253	0.797	1.125	1.091	0.064	0.147
Mouth width	3.313	2.26	2.713	2.141	0.300	0.060
Mouth length	2.154	3.867	2.2	5.472	0.157	0.803
Eye orbit	2.617	5.357	2.334	6.104	0.142	0.373
Chin depth	1.846	2.707	1.542	3.084	0.152	0.189
Snout length	2.473	3.981	2.125	4.939	0.174	0.479

Table 6: Mean value (mm) of morphological features measured for each population of sympatric ninespine stickleback according to geographic location, for each season, with standard error of difference between means.

Character	Leicestershire		North Yorkshire		SE	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Standard length	33.339	25.070	42.648	27.986	4.656	1.458
Body depth over eye	5.347	3.926	6.785	4.219	0.719	0.146
Body depth over operculum	6.779	4.691	9.015	5.338	1.118	0.324
Body depth over spine 1	7.477	5.540	10.162	6.138	1.343	0.299
Body depth over spine 2	7.843	5.901	10.685	6.669	1.421	0.384
Body depth over spine 3	6.539	4.867	9.673	5.384	1.567	0.258
Body depth at caudal peduncle	1.692	1.149	2.35	1.365	0.329	0.108
Mouth width	2.815	1.900	3.71	2.057	0.448	0.081
Mouth length	2.158	3.870	3.043	4.834	0.443	0.482
Eye orbit	2.535	5.255	3.062	6.771	0.264	0.758
Chin depth	1.875	2.620	2.4	3.008	0.263	0.194
Snout length	2.629	4.703	3.527	5.246	0.449	0.272

Table 7: Mean value (mm) of morphological features measured for each population of allopatric threespine stickleback according to geographic location, for each season, with standard error of difference between means.

The traits measured on the allopatric threespine populations sampled in the spring were all significantly different between the two regions with the exception of eye orbit ($df=1, 147$; $F=0.906$; $P=0.343$). These significant differences were also found between the autumn samples with the exclusion of body depth over the eye ($df=1, 171$; $F=3.497$; $P=0.063$) and mouth width ($df=1, 171$; $F=3.733$; $P=0.055$). This situation is mirrored in the results of the ninespines analysis when the populations are examined for differences between the two regions. The spring samples of ninespine sticklebacks showed significant differences between regions for *all* the traits measured. The majority of traits examined in the autumn sample were also significantly different, although in this case body depth over the eye ($df=1, 162$; $F=0.586$; $P=0.445$) and operculum ($df=1, 162$; $F=2.331$; $P=0.129$), mouth width ($df=1, 162$; $F=1.367$; $P=0.244$) and mouth length ($df=1, 162$; $F=0.001$; $P=0.975$) were found to be the same between populations. Consideration of these results alone would lead to the conclusion that these populations of fish have different morphologies according to geographical region, thus rejecting the conditions of character displacement. However an interesting phenomenon is observed in the examination of the sympatric threespine populations. Where these fish are concerned the majority of results show that there are *no* significant differences between geographical regions for the traits measured. The spring samples of sympatric threespine fish show *no* significant difference between traits with the exception of standard length ($df=1, 158$; $F=4.202$; $P=0.042$), which as discussed in the introduction, is a notoriously fluctuating feature, and snout length ($df=1, 158$; $F=5.309$; $P=0.022$). The autumn sample showed slightly less homogeneity as body depth over the eye ($df=1, 159$; $F=3.964$; $P=0.048$), mouth width ($df=1, 159$; $F=14.664$; $P=0.000$) and mouth length ($df=1, 159$; $F=18.432$; $P=0.000$) were all significantly different. The remaining traits were all shown to be non-significant. These results imply that for the traits measured the sympatric

threespine populations show more homogeneity between geographical location than either the allopatric threespine or the ninespine populations.

3.3.3: Discriminant Functions Analysis (DFA)

ANOVA is a useful technique for telling us that significant differences exist between individuals for individual traits. An ANOVA's applicability is limited if we wish to decipher how a number of individual traits combine and interact together to form a characteristic morphology. When all these changes in morphology combine in an individual their cumulative effect is far greater on the individual, regardless of whether they produce a significant result when traits are treated as separate entities. DFA is an analytical technique that allows us to examine what the additive effect of a number of changes to individual traits has on the overall form of the fish. In addition it makes it easier to visualise in what way the traits belonging to one population of stickleback differ from those in the other stickleback populations. A stepwise method of DFA was used, employing Mahalanobis distance. This is deemed the most appropriate method for extracting information specifically relating to potential changes in the *shape* of individuals (see Manly, 1986). The relative positioning of the groups in the resulting plot tells us something of the nature of any potential differences we might observe. If in the final outcome the individual groups lie along the $x=y$ axis it implies that the only differences that exist between the populations are a result of allometric growth responses.

All three populations of stickleback (i.e. allopatric, sympatric and ninespine stickleback) are analysed together. Once again the spring and autumn samples are kept separate. These results are included in tables 8 through to 15. Eigenvalues are included for each site for both seasons. The percentage variance tells us how much influence each function has on the overall result and a significant Wilks' Lambda confirms between-group differences. The figures given as 'standardised canonical discriminant function coefficients' tell us to what extent individual traits have contributed to the overall group classification. A large result, whether positive or negative, indicates a larger contribution of that particular trait to the final group structure. If the discriminant function contains a mix of both positive and negative co-efficients *size* differences can be discounted and the resulting groupings are classified according to *shape* (Manly, 1986). The plots of the group centroids allow us to visualise these coefficients (figures 7 and 8). The summary table (tables 10 & 11 and 14 & 15) at the bottom of each respective plot indicates how well each case (i.e. individual fish) is correctly classified into its original group (i.e. population).

3.3.3.i: DFA of individual sites in the spring

Function	Eigenvalue	% of variance	Wilks' Lambda
1	6.533	68.6	0.000
2	1.133	11.9	0.000

Table 8: Discriminant function analysis between individual stickleback populations, results for spring sample.

Dependent Variable	df1	df2
Body depth at eye	0.277	-0.284
Body depth at operculum	0.252	0.178
Body depth at spine 1	-0.045	0.065
Body depth at spine 2	0.329	0.150
Body depth at spine 3	0.197	0.409
Body depth at caudal peduncle	0.579	0.143
Mouth width	-0.693	-0.158
Mouth length	-0.650	1.065
Orbit diameter	0.056	0.192
Chin depth	0.155	-0.584
Snout length	0.566	-0.739

Table 9: Standardized canonical Discriminant Function Coefficients (df) of log₁₀ residual data from individual populations at individual sites, results from spring sample

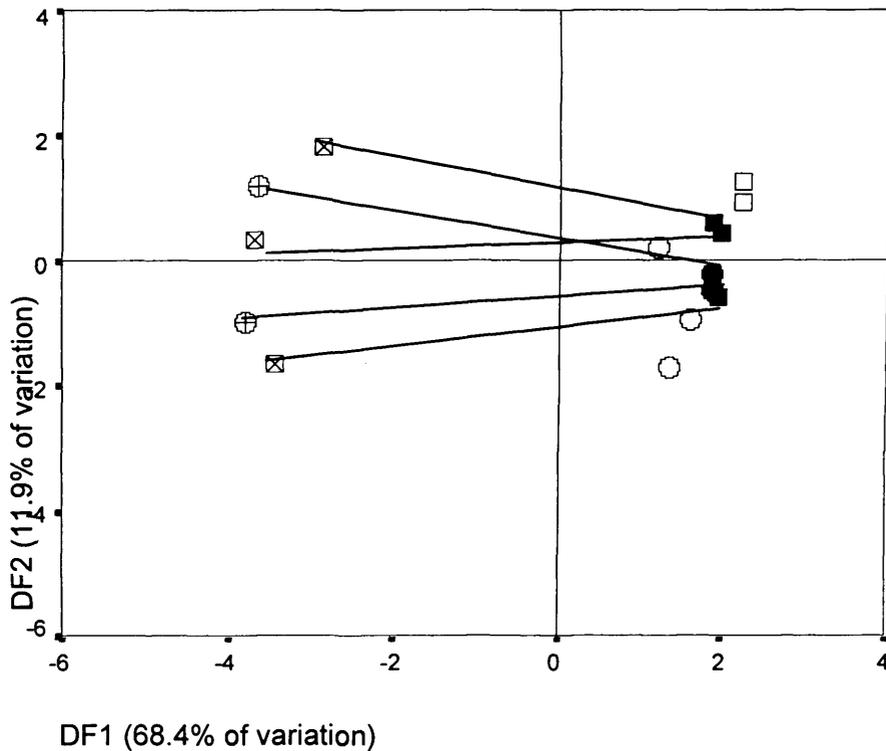


Figure 7: Analysis of log₁₀ residuals of the fish collected in the spring. Circular markers indicate fish collected in Leicestershire; square markers indicate fish collected from Yorkshire. Filled markers denote sympatric threespine fish; empty markers indicate allopatric threespine fish. Partially filled markers indicate sympatric ninespine fish. Lines join sympatric threespines and ninespines from the same site.

Site & population	% correctly classified to original site	% misclassified within sympatric	% misclassified within allopatric	% misclassified within ninespine	% correctly classified to population
1 sympatric	51.6	9.6	38.7	0	61.2
3 sympatric	46.7	19.5	33.3	3.3	66.2
6 sympatric	41.2	23.5	32.3	2.9	64.7
7 sympatric	41.4	30.9	27.5	0	72.3
10 sympatric	65.7	17.3	17.2	0	83.0
2 allopatric	45.2	19.3	35.5	0	64.5
4 allopatric	70.4	18.5	11.1	0	81.5
5 allopatric	43.8	37.5	18.8	0	62.6
8 allopatric	61.5	11.4	23.0	3.8	84.5
9 allopatric	75.0	3.1	18.7	3.1	93.7
11 ninespine	70.0	0	2.5	27.5	97.5
12 ninespine	53.3	0	0	46.7	100
13 ninespine	88.5	0	0	11.5	100
14 ninespine	50.0	0	0	50.0	100
15 ninespine	82.9	0	2.9	14.3	100

Table 10: Summary of classification results, indicating correct classification to original site, misclassification to alternative sites within the original population, misclassification to alternative populations and correctly classified to original population (i.e. allopatric, sympatric or ninespine stickleback) spring sample. 59.2% of original grouped cases correctly classified

Site, population & location	% correctly classified to original site	% misclassified within Leicestershire	% misclassified within Yorkshire	% correctly classified to location
1 sympatric, Yorks	51.6	25.8	22.5	74.1
3 sympatric, Yorks	46.7	16.6	36.6	83.3
6 sympatric, Leics	41.2	44.0	14.7	85.2
7 sympatric, Leics	41.4	34.4	24.0	75.8
10 sympatric, Yorks	65.7	11.6	22.9	88.6
2 allopatric, Yorks	45.2	12.9	41.9	87.1
4 allopatric, Yorks,	70.4	3.7	25.9	96.3
5 allopatric, Leics	43.8	40.7	15.6	84.5
8 allopatric, Leics	61.5	26.8	15.2	88.3
9 allopatric, Leics	75.0	6.2	18.7	93.7
11 ninespine, Yorks	70.0	15.0	15.0	85.0
12 ninespine, Yorks	53.3	26.7	20.0	73.3
13 ninespine, Leics	88.5	0	11.5	88.5
14 ninespine, Leics	50.0	13.3	36.7	63.3
15 ninespine, Yorks	82.9	2.9	14.3	97.2

Table 11: Summary of classification results indicating correct classification to original site, misclassification to alternative sites within the original geographic location, misclassification to alternative geographic location and correctly classified to original geographic location (i.e. Leicestershire or Yorkshire), spring sample. 59.2% of original grouped cases correctly classified

Figure 7 (spring collected fish) shows that the first discriminant function (DF1, x axis) divided ninespines from *all* the threespine fish. With the exception of the uppermost ninespine site (site 15, Yeddingham) there is little differentiation between ninespine populations (all data points lie on or just above -4 on the x axis). At this stage allopatric threespines are clearly divided into geographic region (location of data points on the x axis sites in Leicester <2 sites in North Yorkshire >2). There is no discernable division between the sympatric fish (all points lie approximately on 2).

The second discriminant function (DF2, y axis) separates the ninespines but still without any obvious geographical influence. The wider scatter of the ninespine groupings (on the y axis the data points lie between 2 and -2) indicate that these fish populations are clearly more divergent in shape amongst themselves than are the sympatric threespine fish amongst themselves (on the y axis the data points lie between 1 and -1). DF2 expands the division between allopatric populations in Leicestershire; this is interpreted as a strong environmental influence at each of these individual sites. The division between the allopatric sites in Yorkshire is not as marked as are their counterparts in Leicestershire but they still lie in a definable group, away from the sympatric fish. The sympatric group lies between Leicestershire's allopatric group and North Yorkshires allopatric group. DF2 has also begun to separate the sympatric populations into geographical location although this division is not entirely successful as one of the Yorkshire populations (site 1, Allerstone) has a position that is almost indistinguishable from the Leicestershire fish. The lines joining ninespine and threespine fish from the same site are roughly parallel and suggest that some local feature at individual sites is affecting both species in a similar way.

Interpretation of the DF co-efficients and the plots of the data points (see Manley, 1986) indicates that in comparison to *all* threespine fish the ninespines have narrower heads (eye and operculum) and shallower bodies (width at spine 2 and width at caudal peduncle). The ninespines have the widest and longest jaw (mouth width and mouth length). These observations are based on the co-efficients arising from DF1. Furthermore those ninespines with positive DF2 scores have shallower head depth (chin depth and body depth at the eye) with shorter snouts and longer mouths than the ninespines fish with negative DF2 scores. There is an element of individual environmental effects at each site as generally the position of the ninespines on the DF2 axis corresponds with the position of its co-existing threespine.

Allopatric threespines from Yorkshire are distinguished from allopatric fish in Leicestershire by having deeper body depth (depth over spine 2 and depth over caudal peduncle and depth at spine 3). The Yorkshire fish have a shallower head depth (depth at eye and chin depth) and shorter snouts, with a narrower mouth width but a longer mouth. Allopatric fish from Leicestershire have the narrowest body depth of *all* the threespine fish (depth at spines 2 and 3, depth at caudal peduncle, eye and operculum) and also have the shortest snout length but the largest mouth (mouth width and length).

Regardless of geographical location sympatric fish are more similar in shape to each other than they are to either of the groups of allopatric populations. Sympatric threespines have the longest snouts and smallest, narrowest mouths of *all* the threespine fish. The allopatric fish show some consistency in the depth of the body between spines 2 and 3 meaning that if the body is deep over spine 2 it will also be deep over spine 3, with the converse holding if the body is shallow over spines 2 and 3. This is not true for the sympatric threespines. The body depth of the sympatric fish is greater than that of the allopatric fish over the caudal region and spine 2 but then sympatric fish are shallower over spine 3. These differing traits indicate that the sympatric fish essentially have a diamond shaped profile. The sympatric fish also have the deepest head region of all the fish as measured by the body depth at the operculum and eye. In addition the two isolated threespine sympatric Yorkshire sites are distinct from all the remaining threespine populations as they have longer mouths and shorter snouts than all the other threespine fish.

3.3.3.ii: DFA individual sites in the autumn

Function	Eigenvalue	% of variance	Wilks' Lambda
1	4.599	54.3	0.000
2	1.827	21.6	0.000

Table 12: Discriminant function analysis between individual stickleback populations, results for autumn sample.

Dependent Variable	df1	df2
Body depth at eye	-0.133	0.279
Body depth at operculum	-0.478	0.166
Body depth at spine 1	0.058	0.059
Body depth at spine 2	0.875	-0.129
Body depth at spine 3	0.273	0.235
Body depth at caudal peduncle	0.374	0.236
Mouth width	-0.711	0.213
Mouth length	0.008	-1.131
Orbit diameter	-0.031	-0.059
Chin depth	-0.079	-0.142
Snout length	0.349	0.359

Table 13: Standardized canonical Discriminant Function (df) Coefficients of log₁₀ residual data from individual populations at individual sites, results from autumn sample.

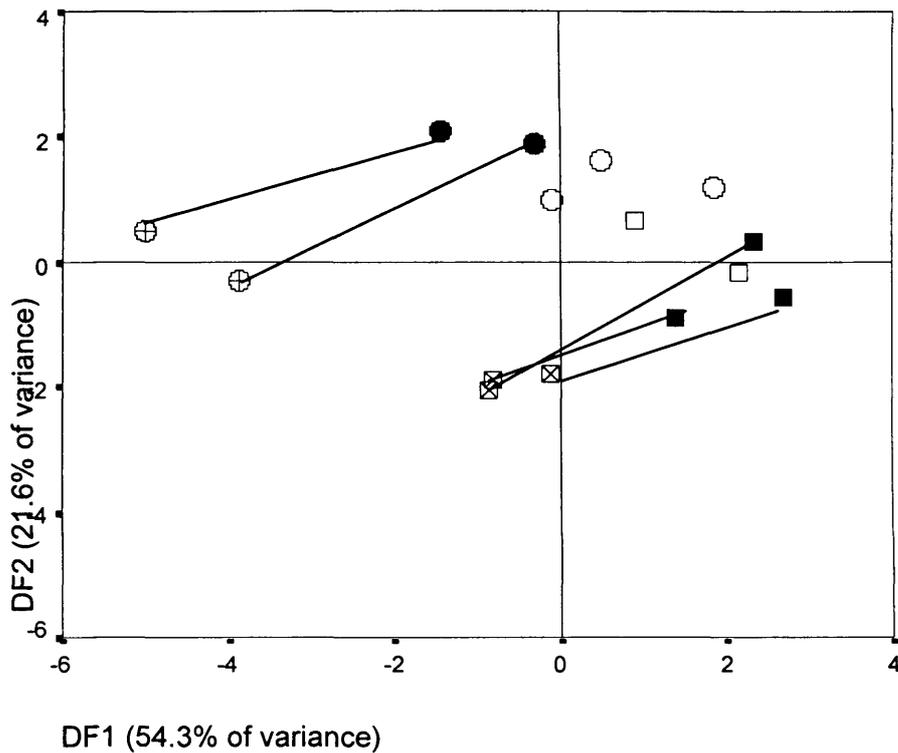


Figure 8: Analysis of \log_{10} residuals of the fish collected in the autumn. Circular markers indicate fish collected in Leicestershire; square markers indicate fish collected from Yorkshire. Filled markers denote sympatric threespine fish; empty markers indicate allopatric threespine fish. Partially filled markers indicate sympatric ninespine fish. Lines join sympatric threespines and ninespines from the same site.

Site & population	% correctly classified to original site	% misclassified within sympatric	% misclassified within allopatric	% misclassified within ninespine	% correctly classified to population
1 sympatric	58.1	21.9	21.8	3.1	75.0
3 sympatric	78.4	5.4	10.8	5.4	83.8
6 sympatric	62.1	13.7	24.0	0	75.8
7 sympatric	78.1	12.5	96.4	0	90.6
10 sympatric	60.0	10.0	30.1	0	70.0
2 allopatric	81.0	4.8	14.3	0	95.3
4 allopatric	64.1	30.8	5.1	0	69.2
5 allopatric	45.2	22.6	25.9	6.4	71.1
8 allopatric	46.7	16.6	23.3	13.3	70.0
9 allopatric	56.7	10.0	26.7	6.6	83.4
11 ninespine	48.3	10.3	3.4	37.9	86.2
12 ninespine	78.4	2.7	0	18.9	97.3
13 ninespine	90.3	0	0	9.7	100
14 ninespine	89.7	0	0	10.3	100
15 ninespine	62.2	2.7	5.4	29.7	91.9

Table 14: Summary of classification results, indicating correct classification to original site, misclassification to alternative sites within the original population, misclassification to alternative populations and correctly classified to original population (i.e. allopatric, sympatric or ninespine stickleback) (i.e. allopatric, sympatric or ninespine stickleback) autumn sample, 66.9% of original grouped cases correctly classified

Site, population & location	% correctly classified to original site	% misclassified within Leicestershire	% misclassified within Yorkshire	% correctly classified to location
1 sympatric, Yorks	53.1	6.1	40.6	93.7
3 sympatric, Yorks	78.4	0	21.6	100
6 sympatric, Leics	62.1	27.5	10.2	89.6
7 sympatric, Leics	78.1	18.8	3.1	96.6
10 sympatric, Yorks	60.0	10.0	30.1	90.1
2 allopatric, Yorks	81.0	9.6	9.5	90.5
4 allopatric, Yorks,	64.1	5.1	30.8	94.9
5 allopatric, Leics	45.2	16.2	38.7	61.4
8 allopatric, Leics	46.7	29.9	23.3	76.6
9 allopatric, Leics	56.7	23.4	19.9	80.1
11 ninespine, Yorks	48.3	0	51.6	100
12 ninespine, Yorks	78.4	0	21.6	100
13 ninespine, Leics	90.3	6.5	3.2	96.8
14 ninespine, Leics	89.7	10.3	0	100
15 ninespine, Yorks	62.2	10.8	27.0	89.2

Table 15: Summary of classification results indicating correct classification to original site, misclassification to alternative sites within the original geographic location, misclassification to alternative geographic location and correctly classified to original geographic location (i.e. Leicestershire or Yorkshire), **66.9%** of original grouped cases correctly classified

Figure 8 (autumn collected fish) shows that DF1 does not separate the ninespine and all threespine fish as did the DF1 in the spring sample. This DF1 shows that ninespine fish from Yorkshire are not distinguishable from sympatric threespine fish caught in Leicester. However this DF1 separates ninespines and sympatric threespines into geographic region, which is the converse of the situation in the spring sample and this division is enhanced by DF2. On this occasion allopatric threespines are nested between the geographical groups of sympatric threespines and show no distinct grouping on either DF1 or DF2 that would coincide with geographical location. DF1 and DF2 account for more similar levels of variability (DF1 = 54.35 of the variation; DF2 = 21.6% of the variation) than was found for the spring samples, which explains why the major discrimination occurs along the $-x=-y$ axis.

In this analysis the ninespine fish are clearly distinguishable by geographical region. Furthermore, the relationship between ninespine and threespine fish from the same sympatric site indicates that the morphology of the fish is being influenced in similar ways by some local feature. However, interpretation of canonical function co-efficients and the plots of the data points (see Manley 1986) indicates that the ninespine fish in both geographic regions are the same *shape* as each other but that the ninespine fish from Leicestershire are a smaller version of those ninespine fish from Yorkshire. Ninespines in Leicestershire have the shallowest body depth at spines 2 and 3 and depth at the caudal peduncle of *all* the fish, but have the largest head measured by body depth at the eye and at the operculum. These shape changes are consistent amongst the ninespine fish in Yorkshire, but differentiation is to a lesser extent. The ninespines in Leicestershire and Yorkshire also have the largest mouth width and length of all the fish; however there is a subtle geographical difference in that the ninespines in Leicestershire have

the greatest mouth *width* of all the fish and the ninespines in Yorkshire have the greatest mouth *length* of all sticklebacks.

The least discrimination occurs between the allopatric threespine fish; which indicates that they are the most similar in shape. The allopatric fish are deeper over the third spine than any other stickleback. The depth over the second spine is greater than those sympatric threespines in Leicester, but smaller than that found amongst sympatric threespines found in Yorkshire. This implies that the depth of the allopatric threespines body over spines 2 and 3 is more constant than that found for the sympatric threespines. The allopatric fish have the shallowest caudal region. The allopatric fish have a smaller head than sympatric fish from Leicester but larger than those found in Yorkshire. Mouth width of the allopatric threespines is greater than in the sympatric threespines in Yorkshire, but mouth length is smaller; the converse applies to fish found in Leicestershire, whereby the allopatric fish have a smaller mouth width but a greater mouth length than the Leicester sympatric threespines. The allopatric threespines are not clearly separated from the sympatric threespine fish. In the autumn data the allopatric fish appear intermediate in shape between the geographical groups of sympatric threespine fish.

Sympatric threespine fish in Leicester have long snouts with the smallest mouth length of *all* the sticklebacks, but these fish have the largest mouth width of all the threespine fish, this small mouth length but large mouth width corresponds with that found for the sympatric ninespines found in Leicester. In contrast sympatric threespines found in Yorkshire have a longer mouth length but a narrower mouth width as was found amongst the sympatric ninespines in Yorkshire. Sympatric threespines in Leicester are wide over the eye but are shallower over the operculum. The fish are also deep over spine 3 but are shallowest of all sticklebacks measured over spine 2. The sympatric fish in Yorkshire have a smaller head and opercular depth than the sympatric Leicestershire fish. In contrast the sympatric fish from Yorkshire have the deepest body depth over the 2nd spine and are shallowest over the 3rd. The sympatric fish have the deepest caudal depth of all the sticklebacks. The depths over the 2nd and 3rd spines still indicate that the sympatric threespine fish have a more diamond shaped profile, but that the deepest mid-body depth is more posterior in the fish from Leicestershire than is found for the sympatric threespines in Yorkshire.

Changes in body shape generally concur between the two sampling seasons. For ease of clarity modifications to the sympatric threespines form are depicted in figure 10 and are offered in contrast to the ninespine and standard threespine form, offered as an allopatric form. The sympatric form is based on the findings of the sympatric populations in Yorkshire, sympatric fish from Leicestershire are similar in form but the deepest portion of the body lies over the 3rd spine as opposed to the 2nd as depicted.

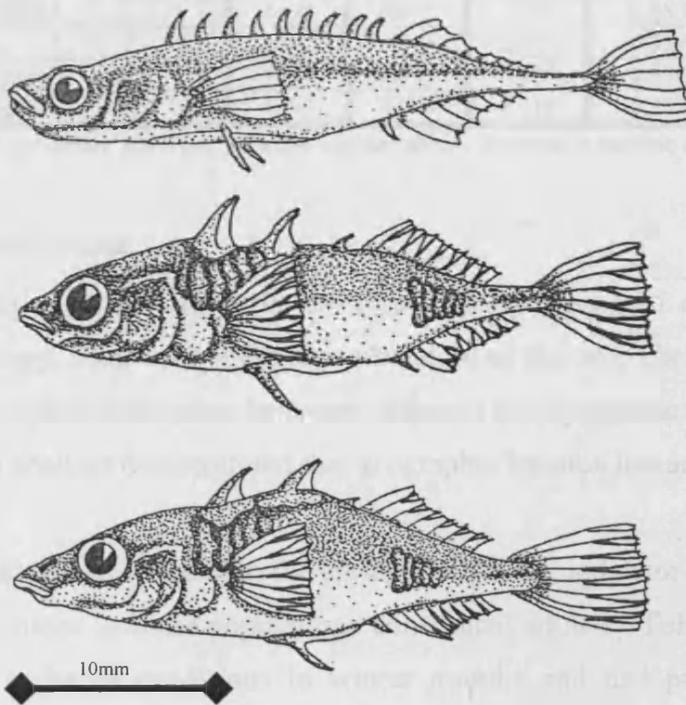


Figure 9: Impressions of ninespine (top) allopatriic threespine (middle) and sympatric threespine (bottom). Ninespine and allopatriic threespine redrawn from Wootton 1984, sympatric threespine modified from original drawing (Wootton, 1984).

3.3.4: Are these traits phenotypically plastic?

Data from Purnell *et al.*'s (unpublished) studies were analysed in order to test whether traits are phenotypically plastic in response to feeding regime. The null hypothesis posed here is that threespine fish raised on different food types are the same shape, for those traits measured. If the results show that there are significant differences in shape between sibling fish raised on different food sources we can assume that morphology is plastic and influenced by feeding environment. For this analysis threespine fish are grouped according to dietary regime. Those fish raised solely on *Daphnia* are regarded 'limnetic' whilst those raised on chironomids are classed as 'benthic'. With the exception of body depth over the operculum ($df=1, 97; F=7.931; P=0.006$) all traits produced non-significant differences between groups (table 17). The conclusion drawn is that the threespine fish have the same morphology regardless of which trophic regime they are raised on.

Dependent variable	df	MS	F	Sig
Standard length	1, 97	0.016	2.457	0.120
Depth over eye	1, 97	0.023	2.914	0.091
Depth over operculum	1, 97	0.07	7.931	0.006
Depth over spine 1	1, 97	0.026	1.790	0.184
Depth over spine 2	1, 97	0.032	2.266	0.135
Depth over spine 3	1, 97	0.011	0.905	0.344
Caudal peduncle	1, 97	0.007	0.0252	0.617
Mouth width	1, 97	0.026	2.437	0.122
Mouth length	1, 97	0.041	3.074	0.083
Orbit	1, 97	0.016	3.653	0.059
Depth of chin	1, 97	0.011	0.467	0.496
Snout length	1, 97	0.024	1.716	0.193

Table 16: univariate ANOVA, between subject effects: limnetic V benthic dietary regime.

3.4: Conclusions

Competitive interactions between species may lead to character displacement in either morphology, behaviour or some combination of the two. The results from this study show some morphological differences between allopatric and sympatric threespines, although discriminant function analysis demonstrated that geographic location has an equal effect (figures 7 & 8).

The sticklebacks sampled in the spring are a better indicator of the effects of competition from the ninespines as these populations constituted adult 1+ fish that had over-wintered and been subject to harsh conditions in winter months and had presumably undergone Darwinian selection. The discussion that follows refers to the spring data only (figure 7).

Interpretation of results from DFA indicates that the ninespine fish have narrower heads and shallower bodies in comparison to *all* threespine fish. The ninespine fish also have the widest and largest mouths of all the sticklebacks. The body of the sympatric threespine fish appears as 'diamond' shaped due to a deeper mid-body depth. In addition the sympatric threespine fish have the deepest caudal peduncle. This is in contrast to the allopatric and ninespine fish, which appear to have a more constant depth along the axis of the body, indicating a more elongated form. The sympatric threespine fish have the longest snouts and the smallest mouths of all the sticklebacks. Amongst the threespine fish the sympatric threespines form a more cohesive group indicating that these fish are more similar in shape to each other than they are to either the allopatric threespines or the ninespine fish. Allopatric threespines are readily distinguished by geographic region. The allopatric fish have essentially the same basic shape but it was found that the fish in North Yorkshire have a deeper body than those fish in Leicestershire. Amongst the sympatric threespine fish discrimination by geographic region only becomes apparent on analysis of the second discriminant function.

These results (figure 7) indicate that the sympatric are less heterogenous in form than the allopatric threespines. Some, but not all aspects of the sympatric threespines morphology are more different from the ninespines than is that of the allopatric threespines. The prediction of altered morphology in response to competition for shared resources is only partially supported. The sympatric fishes' current morphology indicates a partial shift into a different region of the environment as compared to that of their allopatric counterparts, as predicted in the original hypothesis. But there is no unambiguous separation of sympatric from allopatric threespines. In addition the effect of geographic location on the morphology of the different threespine populations suggests that phylogeny has an important influence (figure 8).

3.4.1: How morphology is indicative of foraging behaviour.

When morphological studies show a positive correlation between organismal design and environmental variation subsequent conclusions suggest some *post-hoc* explanation of the observed association. Often these explanations are never experimentally tested but are accepted regardless, and such *post-hoc* explanations have been criticised as being 'just so stories' (Walker, 1997). In his paper Walker (1997) describes a series of biomechanical models that explain what specific morphological requirements are necessary to enable a fish to function efficiently in a given aquatic habitat. Such biomechanical models can then be used to derive *a priori* hypotheses, which can subsequently be tested with comparative data. The suggested models take account of specific behavioural requirements of the threespine sticklebacks as they either forage for food or evade predators, the conclusions of which are summarised in the following discussion. Where applicable, Walker's models have been related to the allopatric and sympatric stickleback morphology and used to derive a functional explanation of the observed differences between the two forms. Although the multivariate DFA did not reveal a clear separation of sympatric threespines from allopatric groups, some of the individual traits were significantly different and these will be interpreted below.

One of the more familiar behaviours in the stickleback's repertoire is the stereotyped c-start (Eaton *et. al.* 1977; Walker 1997). This behavioural response allows the fish to rapidly accelerate away from its current location. The most effective body shape to achieve controlled, high-powered turns are deepest at the middle, such as was found for sympatric threespine fish. The c-start response relies on the fish's ability to efficiently accelerate through a body of water. A fish achieves acceleration as a result of the caudal peduncle and tail sweeping through water accelerating the mass of water behind it. The displaced water exerts a reactive force and propels the fish forward. The mass of displaced water is proportional to the depth of the caudal body and

tail, thrust is maximised when greatest depth is distributed in the caudal region (Walker, 1997). Sympatric threespine populations are shown to possess a deeper caudal peduncle, which implies that they are adapted to accelerate in quick bursts of activity than either the allopatric threespines or the ninespines. This adaptation is an advantage when foraging on zooplankton where it is necessary for the fish to strike at the erratically moving prey.

Both viscous and inertial forces also serve to impair movement through a water body (Walker, 1997). Inertial forces are found to be higher on those fish with elongated, shallow bodies. A shallow fish with round cross section incurs less frictional drag than a deeper bodied fish with an elliptic cross section. Both the ninespine and allopatric threespine fish show a more constant depth along their entire body length so presumably encounter less frictional drag. In a highly vegetated benthic habitat agility is more of a prerequisite than speed. The ninespine and allopatric threespines morphological profiles are deemed most appropriate for fish that require the ability to manoeuvre themselves around structurally complex habitats. Manoeuvrability encompasses the ability to regulate a steady forward motion and an increased control over acceleration, braking, turning, rising, falling and hovering behaviour.

All the behavioural responses described are not exclusive to any particular region of an environment. Stickleback fish are capable of employing any combination of these behaviours at any time. However, different habitats impose different foraging and predation regimes. When a fish spends a majority of its time in one particular habitat, it is beneficial to specialise in one of these behavioural responses. Based on Walker's biomechanical models (1997) from these findings it can be surmised that fish most like ninespine and allopatric threespine fish, with a constant large mid-body depth specialise in manoeuvring, which is most effective in structurally complex vegetated habitats. Whilst those with a large, mid-body depth like the sympatric threespines are most suited to rapid changes in direction so are labelled escape specialists; behaviour which is more suited to the marginal and open-water regions.

3.4.2: How morphology relates specifically to stickleback foraging behaviour

The threespine populations show some significant divergence around the head and mouth region; although these differences are not always as distinctive as the divergence observed between other aspects of the threespine's morphology. This should serve as a caution, that more than just mouth features should be measured in future investigations of potential divergence. These results indicate that the sympatric fish have a more slender head and smaller mouth in contrast to the wider mouth and deeper buccal cavity (inferred from chin depth measurements)

of the allopatric fish. The ninespine's mouth is wider and their snouts are shorter when compared to both populations of threespine fish. The implication here is that these contrasting adaptations around the head and mouth presumably allows each population a degree of specialisation to foraging on different prey types.

For ease of explanation of the function of different mouth morphologies the buccal cavity and mouth is likened to a cone. A long narrow cone is more effective for planktivorous feeding, whereas a short wide one is better for foraging on the benthos (Lavin & McPhail, 1986; Schluter, 1993). The most abundant food source for sticklebacks in the open water is zooplankton, most commonly copepods. This prey source has a patchy distribution and locomotion is generally unpredictable. The most effective method of capturing such evasive prey is through the 'ram' method (see Wootton, 1990; Norton & Brainerd, 1993). This behaviour requires high body acceleration strikes on the copepods. The sympatric fish's enlarged caudal region will serve to increase acceleration during pursuit, and the smaller narrower mouth will be adequate for handling small prey. In comparison benthic prey are found either partially or completely buried in the substrate. The foraging techniques required to prey on benthic invertebrates are different to that described for zooplankton. No great acceleration is required as these prey are relatively sedentary, however it is necessary to extract them from the sediment. The greater volume of the ninespines buccal cavity allows the fish to 'suck' the prey from the substrate. The ninespines mouth is larger than the sympatric threespines, which means its choice of prey is less restricted by means of size. This is an advantage as on the whole benthic invertebrates are generally larger than zooplankton. The allopatric fish's buccal cavity and mouth morphology is intermediate between that of the ninespine and the sympatric threespines. This implies that unlike the sympatric fish the allopatric fish are less restricted about the size of prey they eat, and as such can consume a wider variety of prey types.

The functional and ecological significance of variation in snout length is poorly understood (Walker, 1997). It has been suggested that long snouts preclude binocular vision (Hobson & Chess, 1978). If this is true it places the sympatric threespine fish at a disadvantage, particularly since their reliance upon their sight is apparently higher in open water (Schluter, 1993). The theory is questionable (Walker, 1997) and is contradicted by the fact that a longer snout appears to be a fundamental feature of adaptation to the limnetic zone (McPhail, 1994). From the earlier discussion it is apparent that if the fish does not increase snout length (effectively making the cone shape longer) it compromises feeding efficiency. As such there will likely be some compromise between maximum snout length and impairment of sight.

A further observation made on the sympatric threespine populations is the high degree of homogeneity within and between groups in the spring populations (but not the autumn). This situation might also be explained as a limnetic adaptation. Studies have shown that fish in exposed waters often find some benefit in forming shoals (Lindstrom & Ranta, 1993; Peuhkuri, 1998a; 1998b; Krause *et. al.*, 1998; Ward *et. al.* 2002). The shoal effectively works together to give added protection from predators. Threespine sticklebacks have been observed in dense shoals, particularly those belonging to the 0+ age group (Copp, 1992). Naturally formed foraging groups have a more homogenous phenotypic composition than artificially formed groups; phenotypic heterogeneity as well as group size will increase with food availability (Ranta & Lindstrom, 1993). Joining a shoal can have one major disadvantage in that a large mass of fish is far more noticeable to a predator than a single individual. In addition a fish that is in some way conspicuous from the others in its shoal (i.e. by size) suffers from what has been termed the 'oddity effect' (Peuhkuri, 1997). The foraging activity of a stickleback much larger than its shoaling partners has been shown to be much lower than that of large individuals in a shoal dominated by large fish or those in a size assorted shoal (Peuhkuri, 1997). An 'odd' fish is highly likely to be picked out from a group and targeted by a predator. For this reason it benefits an individual if it is able to merge with the group so it is essential that it looks the same as the other fish. Fish that shoal together have been found to show high levels of morphological uniformity, presumably as a consequence of the 'oddity' effect.

No explanation has been found in the literature to interpret the observation that sympatric threespines have a larger opercular depth than the allopatric fish. The operculum covers the fish's gills which are the fish's respiratory equipment. It should therefore follow that a larger operculum should be covering larger gills. It has already been mentioned that conditions in open-water require far higher levels of activity from the sympatric fish (i.e. high burst acceleration) than is required by their allopatric counterparts in their habitat. Although high powered burst-swimming is powered largely by the white anaerobic muscle it may be possible that a larger gill area will compensate for the increased O₂ demand placed on the fish by higher activity levels (see Wootton, 1990). These speculations offer some intriguing possibilities for future research.

3.4.3: Is divergence a plastic response?

Reviews of recent stickleback literature reveal a number of morphologically divergent threespines (see Bell & Foster, 1994a). The divergent sticklebacks are characterised according to the habitat they are found in such as stream type, lacustrine, riverine and marine. In each of these cases ecological conditions in each environment has determined the divergent morphology.

This evidence suggests that the threespine's phenotype is fairly malleable allowing them to adapt readily to conditions in the environment. The fact that threespine sticklebacks are found in such a diversity of aquatic environments might be symptomatic of an innate plasticity, and that what we are observing is in fact an expression of this plastic reaction to its environment. However, several studies have shown (Houston, 2001; Reusch *et. al.*, 2001; Vamosi & Schluter, 1999; Taylor & McPhail, 1999; Thompson *et. al.*, 1997; Taylor *et. al.*, 1997) that there is a high degree of heritability amongst those traits measured. This would indicate that differences between populations are more likely to represent genotype than phenotypic plasticity. The apparent importance of phylogeny demonstrated in this study supports this (figure 8).

3.4.4: Possible parallel divergence?

One of the more interesting aspects of the analysis was the consistency of form between the different sympatric threespine populations. This comparison of sympatric form is not restricted to sites in the UK but may parallel that observed across continents with threespine sticklebacks in Canada. Where cross-comparison has been possible, the divergence of the jaw morphology of the sympatric threespines is similar to that found for the 'limnetic' morphs in the Canadian lakes (Lavin & McPhail, 1985, 1986; McPhail, 1994; Schluter, 1993). The Canadian studies take measurements that focus mainly on the morphology of the jaw, and include only standard length and body depth at the second spine as a means of standardising the jaw measurements. The results of these studies conclude that the 'limnetic' morph has a smaller, narrower mouth, which serves to increase its foraging efficiency for limnetic prey. Such evolutionary repetition of the same traits suggests diversification has proceeded by extensive parallel selection.

3.4.5: Consequences of morphological divergence.

The ninespine's form renders it highly efficient in the structurally complex, densely vegetated regions of the habitat. Its large mouth offers little obstacle in the way of what size of prey can be taken, so in these regions it can forage on a wide variety of invertebrates. Being more permanently hidden by vegetation the ninespines are less exposed to avian piscivores, although they may be more susceptible to 'ambush' predators such as pike, who lurk in vegetation waiting for a potential meal to pass by. However, in the habitats sampled for this study no potential piscivorous fish were ever caught (see Chapter 2), presumably the threat of predation is low for the ninespines. The ninespine's morphology renders it an effective inhabitant of the vegetated region. From the evidence gathered it is more than apparent that the ninespines are expected to be the strongest competitor in the vegetated regions of the habitat.

Where no direct competitive threat exists the threespine stickleback is found in the vegetated benthic region and littoral zones of the habitat, occasionally venturing into open water to forage (Hart, 2003; see Wootton, 1984, 1976). This is assumed to be the habitat preference adopted by the allopatric population. The intermediate mouth size of the allopatric fish allows the fish to handle a range of prey sizes and should not exclude many invertebrates from its diet. The stickleback's form does not prevent it from swimming in the open-water to forage for food but it is unlikely that the fish would venture too far from an available refuge to which it can 'jump' for cover if danger threatens. If ninespine fish were present with these allopatric threespine populations it is possible they would encroach into each other's niche.

The sympatric threespine's morphology is best adapted to a relatively limnetic existence. This shift in form may be as a direct consequence of competition from the ninespine fish in the benthic habitat as no other obvious differences are apparent between allopatric and sympatric environments (see chapter 2). But this interpretation depends on two key assumptions that need to be stressed. First, it is assumed that there is competition for resources between the two stickleback species where they occur sympatrically (suggested, but not directly tested here). Second, classification of sites as sympatric or allopatric describes their present condition; there is no information on whether these sites were sympatric or allopatric in the past when adaptive evolution is believed to have occurred.

The greatest threat that the open water holds for a stickleback is that they are fully visible through 360°. By foraging on zooplankton, the primary source of prey in open water, the stickleback maximises this exposure and the stickleback renders itself an easy target from both piscivorous fish and birds. When the list of potential predators is as large as the sticklebacks this choice of habitat would seem foolhardy. To alleviate some of the pressures of predation the sympatric fish have afforded themselves greater protection by increasing spine length. In addition they are also able to accelerate faster, out of the way of potential harm because of the increased depth of the caudal peduncle. It is possible that gill surface area is increased thus increasing O₂ uptake, allowing these fish to maintain higher activity levels for longer. Their presumed allegiance to a shoal increases their chances of finding food sooner. It also decreases the chances of the fish becoming a meal themselves as vigilance is increased and the fish should be aware of any potential danger sooner. Greater acceleration also serves well in helping them to pursue evasive planktivorous prey. The longer cone shaped mouth allows them greater handling efficiency when they ultimately catch their prey.

An increasing number of studies report cases of species pairs formed through trophic divergence amongst northern temperate fish species (Taylor, 1999). In each of these cases divergence has followed a predictable path and differentiation has resulted in species pairs of benthic and limnetic/pelagic morphs. In general the benthic morph in the pair will have a deeper body and wider mouth specifically adapted for foraging in the benthic region, and, conversely the limnetic/pelagic morph of the species pair will have a narrower, more streamlined body and smaller mouth specifically adapted for foraging on planktivorous prey. The sympatric threespines in the recent study have not gone down this route they have developed a deeper body and deeper caudal peduncle but also have a narrower mouth than their allopatric counterparts. Most of the cases of character divergence described in the literature are for fish in lakes and it would appear that life in a small stream has imposed different selective pressures on trophic morphology leading to the unique combinations shown in this chapter.

It is better to know some of the questions than all of the answers.

— James Thurber (1894-1961)

Chapter 4: Gill-rakers

4.1: Introduction

Gill architecture can provide valuable insight into a fish's diet. Certainly gill-raker architecture has been shown to be an important divergent trait in many species pairs (Smith & Skulason, 1996) including the Canadian pairs of threespine stickleback (Schluter & McPhail, 1992). The divergent morphology of the sympatric threespine fish indicates a possible propensity to include more zooplankton in their diet than the allopatric threespines (chapter 3). This chapter investigates whether this divergence is also apparent in the gill-raker architecture. Because the gill-rakers are an internal morphological feature related to food handling, analysis of such meristic traits can provide supplementary information about a possible divergence that is driven by and centred on food resources. This chapter proposes to test the null hypothesis that there is no difference either in number or length of gill-rakers between sympatric and allopatric threespine populations.

4.1.1: Gill architecture and function

The gills are situated in the fish's bucco-pharyngeal cavity (at the back of the throat) and comprise three main structures; the gill filaments, used in respiration, the gill-rakers, and the gill arch to which the rakers and the filaments are attached (fig 10). The gill-rakers are small protuberances emerging from the gill-arch. It is assumed that the rakers main function is to act as a sieve, the spaces between neighbouring rakers acting as the pores of the sieve, thus preventing the loss of ingested food particles as the fish expels water over the filaments and out through the operculum (Vandewalle *et. al.*, 2000). This method of collecting food describes the gill-rakers as a 'dead-end' filter (Hoogenboezem *et. al.*, 1993). With this approach to prey retention, it is believed that the size of the space between the rakers defines the minimum size of prey retained. The tiny food particles that are too big to pass through the pores are assumed to become trapped on the rakers and later swallowed by the fish.

The problem with the dead-end filtration hypothesis is that when the fish comes to swallow the retained food particles it must do so without re-suspending the particles and undoing all of the previous filtering (Brainerd, 2001). Some fish overcome this problem of resuspension by using mucus to trap particles on the gill-rakers or the roof of the mouth. Other fish species may alternatively use cross-flow filtration (see Cheer *et. al.* 2001). Here the food particles remain in suspension and rarely come into contact with the gill-rakers. Cross-flow filtration is especially efficient at low Reynolds numbers (when particle size ranges from 40µm to 1mm), where the

gill-rakers fail to serve as a non-porous barrier to such small particles. It is assumed that particles smaller than the pores of the gill-raker sieve, which would usually be lost by dead-end filtration methods, are retained by cross-flow filtration. Tests on a series of fish species observed cross-flow filtration *in situ*. Examples are the gizzard shad (*Dorosoma cepedianum*), goldfish (*Carassius auratus*) and the ngege tilapia (*Oreochromis esculentus*) (Sanderson *et. al.*, 2001). It was found that particles rarely contacted the gill-rakers (no contact by 95% of particles) and certainly no accumulation of particles was observed. The role of inter-raker spacing in determining the size of prey retained by planktivorous fish is deemed controversial from evidence obtained by such studies. As the species studied do not follow dead-end filtration methods to retain particles, the gap widths between rakers do not necessarily serve as size thresholds to the minimum size of prey. It is proposed that the spaces between the rakers are as large as possible, whilst still allowing prey to be diverted from the filtrate streamlines to the crossflow streamlines. This perspective shifts the focus from particle size as a mechanical threshold for retention to particle size as a hydrodynamic threshold that affects the magnitude of lift and shear (Sanderson *et. al.* 2001).

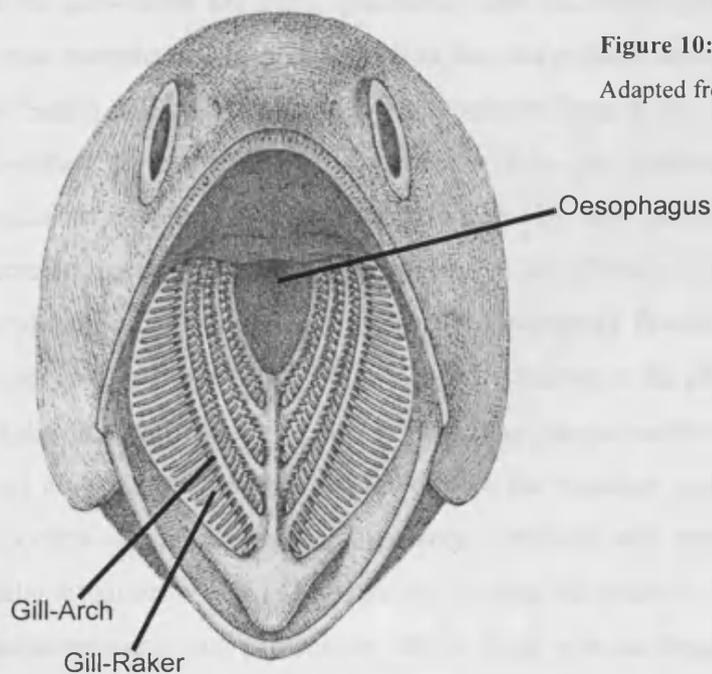


Figure 10: Situation of gill-rakers on the gill arch.

Adapted from Cheer *et. al.* (2001)

4.1.2: Gill-rakers as a diagnostic taxonomic feature

Information gathered about the meristic features of gill-rakers can provide useful information about a fish's diet. This information is given regardless of knowledge of exactly how the gill-rakers function as a retentive mechanism for food. The diets of fish species are naturally diverse; these differences in diet are apparent in the structure and number of gill-rakers. Numbers of gill-rakers are often used as a diagnostic feature in fish classification. Some of the most recent fish

species to be identified provide examples of how gill-rakers are a useful taxonomic feature. Classification includes a description of raker number in the new acropomatid fish (*Acropoma argentistignia*) (Okamoto & Ida, 2002) and a labrid fish (*Cirrhilabrus bathyphilus*) (Randall & Nagareda, 2002). Gill-raker architecture has also been used to reclassify other species of fish, for instance a catfish species (*Pangasius sabahensis*) (Gustiano *et. al.*, 2003), the goatfish *Parupeneus insularis* (Randal & Myers, 2002), *Polydactylus multiradiatus* (Motomura *et. al.*, 2002) and *Atherinomorous aetholepis* (Kimura *et. al.*, 2002).

Gill-raker architecture has proved a useful tool in identifying divergent morphotypes in a number of fish species. The incidents of divergence in species in the Northern Hemisphere often follow similar patterns, in that displacement is dimorphic (see Taylor, 1999). The most recent examples of divergence amongst fish in the northern hemisphere include a landlocked charr (*Salvelinus alpinus*) complex (see Smith & Skulason, 1996; Alekseyev *et. al.* 2002); whitefish, Pacific salmon (see Smith & Skulason, 1996) and six species of Loricariid fish (Delariva & Agostinho, 2001). Intralacustrine forms have been reported amongst the whitefish, which differ in adult size, and the gill-rakers are most commonly used to discriminate between forms. Dimorphic sockeye salmon morphs are so prevalent that they have been assigned the names sockeye and kokanee (see Smith & Skulason, 1996). The sockeye form is the larger of the two and has many more gill-rakers than the smaller kokanee. Often the number and length of the gill-raker show significant correlation with the fish's prey. By way of example there is evidence of sympatric speciation amongst charr (Alekseyev *et. al.* (2002). This divergence has resulted in three morphological types recognised as the commonly found small and large morphs and a third known as the dwarf form. Large charr are known to be piscivorous whilst the dwarf and small morphs are benthophagous. It was found that the proportion of plankton in the diets of dwarf and small charr is positively correlated with the number and length of the gill-rakers, while the proportion of benthic prey is negatively correlated with raker length (Alekseyev *et. al.*, 2002). A similar situation is observed in the six Loricariid species. Those species that feed mainly on fine detritus possess long gill-rakers whilst those species feeding on coarser material have shorter rakers (Delariva & Agostinho, 2001).

Studies on the gill-raker morphology of a benthophagous fish *Goniistius zonatus* revealed that, in addition to the number and length of gill-rakers, the *space* between rakers sometimes correlates with prey size (Matsumo & Kohda, 2001). *G. zonatus* are found in either one of two habitats; one where the dominant prey are considered small or a second where the prey is constantly large. The inter-raker spacing of *G. zonatus* is found to be significantly smaller when the fish is found in habitats where small species constitute the dominant prey. A similar situation was found

within a group of co-existing fish in Liangzi Lake, China (Xie *et. al.* 2002). Gill-raker spacing was found to be influenced by the inclusion of copepod species in the fish's diet. An inverse correlation was found between inter-raker spacing and increased consumption of copepods, whereby fish that predominantly feed on copepods have smaller inter-raker spaces. However, it is essential to bear in mind that the spaces between gill-rakers will also increase as the fish ages. This effect of an increase in inter-raker spacing is observed in kokanee salmon of different ages (Lipsey & Stockwell, 2001). The spacing between the rakers of younger kokanee is small enough to retain zooplankton of all sizes. As fish increase in size, so too do the spaces between the gill-rakers. As the fish get older, prey retention decreases, until only the largest zooplankton are retained by the oldest (age 3) fish.

There is strong evidence to suggest a high level of correlation between prey size and gill-raker architecture. Certainly as gill-raker number increases with a concomitant decrease in inter-raker spacing, prey size is also shown to decrease. It is all too easy to jump to the conclusion that the reason smaller prey are retained must be because the pores of the gill-raker sieve are smaller, therefore trapping the prey. However, recent observations made on the mechanisms of cross-flow filtration (Sanderson *et. al.*, 2001) highlight the significance of the physical properties of water working with particles at such low Reynolds numbers. This does not undermine the significance of any potential correlation between raker architecture and prey size. It is still possible to speculate on probable retentive qualities of the rakers where evidence of the functional mechanisms of the filtration procedure is lacking.

4.1.3: Adaptation of gill-raker architecture in threespine stickleback divergence.

Threespine sticklebacks characteristically possess somewhere between 17 to 25 long and slender gill-rakers, the ninespine has fewer (10-15) but nevertheless slender rakers (see Wootton, 1976). Based on observations on other fish species that prey size correlates with raker number, it is presumed that those sticklebacks which possess a higher number of rakers will be more adept at consuming smaller prey items. A number of papers examining potential character divergence in Canadian threespine sticklebacks report changes in gill-raker architecture between the divergent morphotypes in the Canadian threespine populations (e.g.: Lavin & McPhail, 1985; Lavin & McPhail, 1986; Schluter & McPhail, 1992; Schluter, 1993; McPhail, 1994; Hatfield & Ptolemy 2001). In these populations, gill-raker number and length were measured along with gape width, body depth and body length (Schluter & McPhail, 1992). These five traits were selected for analysis as they are considered *a priori* to be amongst the most strongly related to foraging efficiency, diet and habitat use. Principal components analysis revealed that of the traits

measured, gill-raker length was the strongest differentiating component and raker number the third strongest; in this study stickleback populations were distinguished according to these traits. Overall it was found that the limnetic threespine morph had more numerous gill-rakers, smaller mouths (narrower gapes), and more slender bodies than did sympatric benthics. Solitary threespine populations were also included in the analysis; their gill-raker morphology was found to be intermediate between the limnetic and benthic morphs. The differences in gill-raker morphology were also found to influence prey size. The benthic threespines with fewer, shorter gill-rakers consume the largest prey and conversely, the limnetic fish with more, longer gill-rakers take the smallest prey. Solitary populations with intermediate gill-raker architecture exhibit a choice of intermediate sized prey. These studies on the Canadian threespine pairs have considered the adaptive significance of raker architecture and have assumed that more numerous gill-rakers serve to retain smaller zooplankton found in the limnetic zone. In the light of recent evidence (Brainerd, 2001; Cheer *et. al.* 2001) the relationship between gill-raker number and prey size is however less straightforward than was previously assumed. Little is known about exactly *how* differences observed between the gill-rakers of divergent stickleback populations serve to increase foraging efficiency. We should acknowledge the correlation between morphology and prey size in these Canadian sticklebacks, but should consider any comments on the role of the rakers in influencing prey size choice as purely speculative.

4.1.4: Heritability of rakers

The recent revelations surrounding divergence in Canadian threespine sticklebacks and the role that gill-raker architecture has played in this, has inspired further investigation into the basis of genetic variation in the gill-rakers. Preliminary investigations examined the threespine's genetic map to investigate the genetic basis of recently evolved changes observed in the Canadian stickleback species pair (Peichel *et. al.*, 2001). It is evident that the substantial changes in spine length, armour plate number and gill-raker number that have been observed are controlled by genetic factors that map to independent chromosome regions. Additional work is required in order to define the number and type of genetic changes that underlie morphological diversification during stickleback evolution. Further studies measuring heritability and 'evolvability' of features amongst sticklebacks in the natural environment found a presence of additive genetic variability for the lower gill-rakers of threespine sticklebacks (Hermida *et. al.*, 2002). It is evident from these studies that the genetic architecture underlying gill-raker number and size is somewhat complex. The number of long gill-rakers appears to be determined by a large number of loci, each with small effects; however the number of short gill-rakers is

controlled by only two quantitative trait loci (QTL) that map to different linkage groups (Meyer, 2002).

Whilst there is evidence to indicate a strong genetic component in raker architecture, indicating that traits are heritable, a study on gill-raker number in sea bass (*Dicentrarchus labrax*) (Loy *et al.*, 1999) serves as a warning that the environment still has a strong influence on raker morphology in each generation. Variation in the number of gill-rakers was found between sea bass raised at different salinities. A number of meristic traits were observed, including vertebrae and fin rays, and it was shown that raker number is dramatically influenced by the environment during early phases of embryogenesis. In this study, juvenile and adult sea bass sharing a common gene pool, but reared at different salinities, showed a marked tendency toward a reduction in the number of gill-rakers in freshwater. Whether the primary cause of variability in gill-raker number is genetic or environmental is unclear. Loy *et al.* (1999) suggest that the reduced number of gill-rakers might be symptomatic of the pleiotropic effect of those genotypes best suited to freshwater. Whatever the cause of variation, Loy *et al.*'s (1999) study warns us that the use of gill-rakers in systematic studies deserves special attention. This is because raker variation may not be adaptive but purely ecophenotypic, and environmental conditions may act well beyond embryo development.

4.1.5: Chapter Rationale

The external morphology of the sympatric threespines (chapter 3) indicates that these fish are effective zooplanktivores and is consistent with the presupposition that divergence is driven by competition for food resources. If indeed zooplankton constitutes these fishes main prey item it is expected that an internal adaptation will be evident in the gill-rakers. This gill-raker adaptation is expected to manifest itself in an increased number of longer and more slender rakers, which will serve to increase the sympatric threespines' handling of the small zooplanktonic prey.

4.2: Methods

Gill-raker architecture was measured on the same fish as were used in the previous morphological analysis (chapter 3). Again fish were grouped according to original population (allopatric and sympatric threespine), geographic location (Leicestershire or North Yorkshire) and season (spring or autumn).

The gills lie at the back of the fish's throat. In order to gain access to the gill-rakers it is necessary first to remove the operculum. An initial incision was made at the mid-point of the

lower jaw. The incision was carried on along the lower jaw until the anterior end of the bony girdle was reached. The incision then followed underneath the operculum on the left side of the fish's body, along the top of the head to a point just above the most posterior edge of the eye. From this position the incision continued in a diagonal line until the original starting point on the lower jaw was reached. The direction of these incisions can be followed in figure 11. The gills were left *in situ* as removal often resulted in damage to a part of the gill. In some instances a soft opaque substance was found on the surface of the gill. It is unclear as to the origins of this substance. At the time it was considered possibly the debris of chemicals used in the preservation procedure, although in retrospect this may have been a secretion of mucus. Unfortunately records were not kept of which fish were found with this unknown substance covering the rakers. After taking care to remove any remnants of this soft substance the gill-rakers on the first gill arch were counted, and the 5th and 8th rakers measured (the most anterior raker is defined as the 1st). The 5th and 8th gill-rakers were chosen because preliminary observations noticed that these rakers were always included amongst the largest of all the gill-rakers on the first gill arch. Raker counts and measurements were conducted under a standard binocular dissecting microscope using an eye piece graticule.

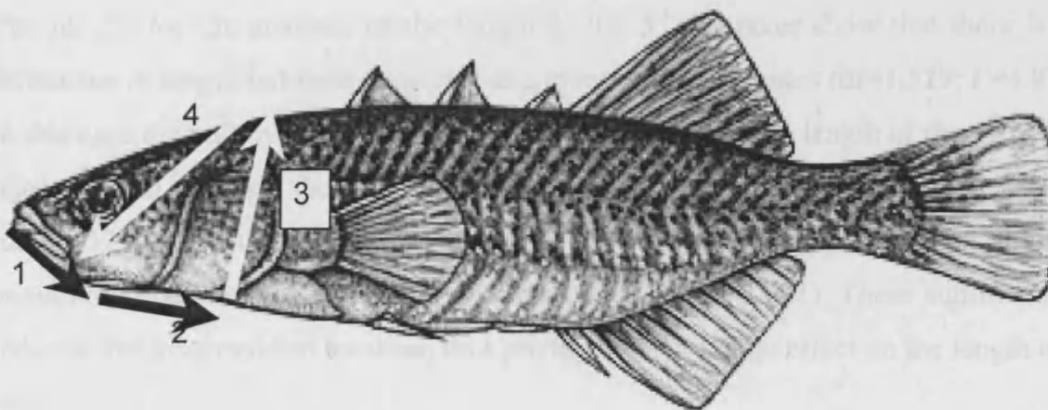


Figure 11: Diagram of the threespine stickleback indicating the direction of incisions made to gain access to the gills (original diagram adapted from PA fish Tables (2003))

4.3: Results

Threespine fish were grouped according to population (allopatric or sympatric), geographic location (Leicestershire or North Yorkshire) and season (spring or autumn). The analysis aimed to determine any potential divergence amongst gill-raker length and gill-raker number between sympatric and allopatric fish. All raw data were log transformed and regressed against log standard length; the consequent residuals were used in subsequent analysis. The analysis tested the following null hypotheses: that no difference exists between threespine populations with

regard to either gill-raker length or gill-raker number. A summary of the descriptive statistics for both gill-raker number and length is given in table 17. A full breakdown of the descriptive statistics for individual sites for both spring and autumn is given in tables 68 and 69 (appendix 3).

Descriptive statistic	Raker number		Length of 5 th raker (mm)		Length of 8 th raker (mm)	
	Allopatric	Sympatric	Allopatric	Sympatric	Allopatric	Sympatric
Min	14	14	0.1	0.048	0.1	0.048
Max	22	24	1.2	0.8	2.2	1.063
Mean	17.769	19.040	0.338	0.281	0.534	0.476
St Dev	1.439	1.428	0.182	0.135	0.262	0.190

Table 17: Descriptive statistics of gill-raker features for allopatric and sympatric threespine populations.

4.3.1: Potential divergence amongst gill-raker length and number.

The transformed data collected on the lengths of the 5th and 8th gill-rakers were analysed using univariate ANOVA (GLM) on SPSS 11 for Windows. This analysis tested the null hypothesis that there is no difference between gill-raker lengths amongst sympatric and allopatric threespine populations. The results of these analyses are given in tables 18 and 19.

The results for the analysis of the length of the 5th gill-raker show that there is a significant difference in length between allopatric and sympatric threespines (df=1,529; F=4.870; P=0.028). In this case the null hypothesis that there is no difference in the length of the 5th gill-raker can be rejected. The analysis shows further significant interactions between population and location (df=1, 529; F=19.099; P<0.001) location and season (df=1, 529; F=5.526; P=0.019) and population, location and season (df=1, 529; F=12.946; P<0.001). These significant interactions indicate that geographical location, thus phylogeny has a large effect on the length on the 5th gill-raker.

With regard to the length of the 8th gill-raker none of the individual factors have any significant effect, however there are significant interactive effects again between population and location (df=1, 529; F=21.899; P<0.001) and population, location and season (df=1, 529; F=5.362; P=0.021). The length of the 8th gill-raker appears to be most strongly effected by a combination of population and location, again indicating that phylogeny is the strongest determining factor for the length of this particular gill-raker.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	0.092	4.870	0.028
Location (Leicester/Yorkshire)	1	0.007	0.376	0.540
Season (Spring/ Autumn)	1	0.036	1.875	0.171
Population * Location	1	0.362	19.099	0.000
Population * Season	1	0.064	3.383	0.066
Location * Season	1	0.105	5.526	0.019
Population * Location * Season	1	0.246	12.946	0.000
Residual (error)	522	0.019		
Corrected Total	529			

Table 18: Results of univariate ANOVA (GLM) comparing length of the 5th gill-raker between allopatric and sympatric threespine stickleback populations. Significant results indicated in bold type.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	0.000	0.003	0.956
Location (Leicester/Yorkshire)	1	0.002	0.135	0.714
Season (Spring/ Autumn)	1	0.006	0.518	0.472
Population * Location	1	0.274	21.899	0.000
Population * Season	1	0.004	0.337	0.562
Location * Season	1	0.012	0.968	0.326
Population * Location * Season	1	0.067	5.362	0.021
Residual (error)	522	0.012		
Corrected Total	529			

Table 19: Results of univariate ANOVA (GLM) comparing length of the 8th gill-raker between allopatric and sympatric threespine stickleback populations. Significant results indicated in bold type.

The transformed data collected on gill-raker number were analysed using univariate ANOVA (GLM) on SPSS 11 for Windows. This analysis tested the null hypothesis that there is no difference in gill-raker number between sympatric and allopatric threespine populations. The result of this analysis is given in table 20. There were more significant differences found between the threespine sticklebacks for gill-raker number than were found for gill-raker length. Individually both population (df=1, 532; F=91.867; P<0.001) and location (df=1, 532; F=6.638; P=0.010) proved significant, therefore both factors have an effect on gill-raker number. In addition significant interactions were observed between population and location (df=1, 532; F=24.767; P<0.001), population and season (df=1, 532; F=6.672; P=0.010) and location and season (df=1, 532; F=5.539; P=0.019). The null hypothesis that there is no difference in gill-raker number is rejected. It is concluded that both co-existence with ninespine fish and geographic location, thus phylogeny, have equally important influence on gill-raker number.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	0.097	91.867	0.000
Location (Leicester/Yorkshire)	1	0.007	6.638	0.010
Season (Spring/ Autumn)	1	0.003	2.560	0.110
Population * Location	1	0.026	24.767	0.000
Population * Season	1	0.007	6.672	0.010
Location * Season	1	0.006	5.539	0.019
Population * Location * Season	1	0.003	2.722	0.100
Residual (error)	524	0.001		
Corrected Total	532			

Table 20: Results of univariate ANOVA (GLM) comparing gill-raker number between allopatric and sympatric threespine stickleback populations. Significant results indicated in bold type.

4.3.2: DFA examining direction of gill-raker divergence

Discriminant function analysis was performed on the log-residual data obtained from the gill-rakers in order to determine the direction divergence took from these features. Data were analysed on SPSS 11 for Windows. Table 21 gives details of the eigenvalues, table 22 summarises the discriminant function co-efficients, figure 12 provides a plot of the group centroids and table 23 provides a summary of classification results to original population.

Function	Eigenvalue	% of variance	Wilks' Lambda
1	0.415	62.1	0.000
2	0.191	28.6	0.000

Table 21: Discriminant function analysis comparing gill-raker features between individual threespine stickleback populations.

Dependent Variable	df1	df2
Length 5 th gill-raker	-0.006	0.766
Length 8 th gill-raker	0.186	0.325
Gill-raker number	0.979	-0.060

Table 22: Standardized canonical Discriminant Function Coefficients (df) from individual threespine populations comparing gill-raker features.

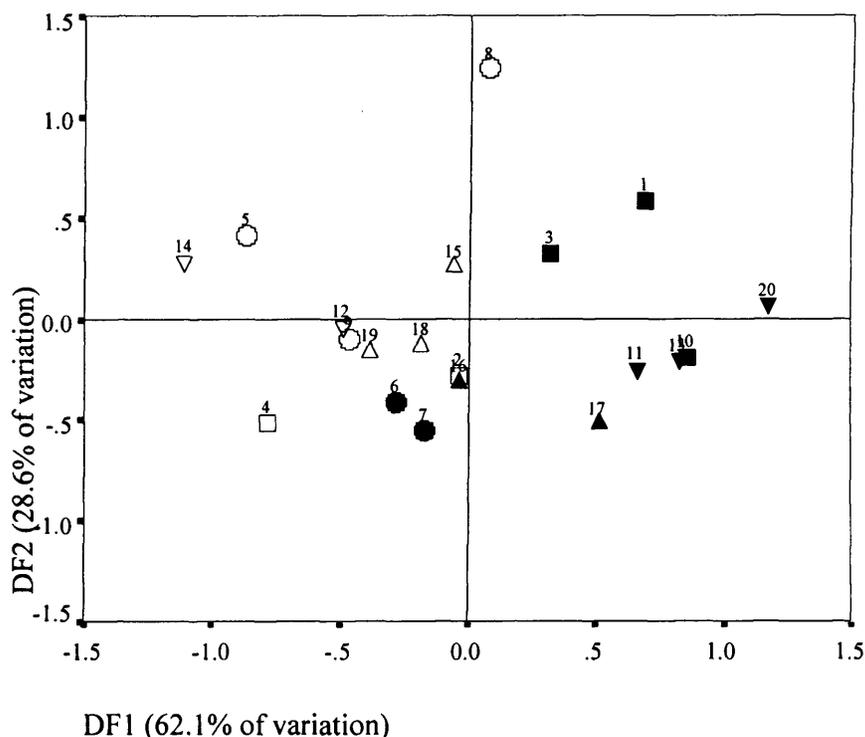


Figure 12: Analysis of \log_{10} residuals of the gill-raker features from threespine sticklebacks. Filled markers denote sympatric threespine fish; empty markers denote allopatric threespine fish. Circular markers indicate fish collected in Leicestershire in the spring; square markers indicate fish collected from Yorkshire in the spring; triangles indicate fish collected in Leicestershire in the autumn; upturned triangles indicate fish collected from Yorkshire in the autumn. Numbers 1-10 indicate site numbers in the spring; site number + 10 indicates site number in the autumn.

Site, population & season	% correctly classified to original site	% misclassified within sympatric	% misclassified within allopatric	% correctly classified to population
1 sympatric, spring	45.2	25.8	29	71
3 sympatric, spring	10.7	50.1	39.2	60.8
6 sympatric, spring	0	29.2	70.8	29.2
7 sympatric, spring	0	53.4	46.6	53.4
10 sympatric, spring	21.4	67.9	10.8	89.3
2 allopatric, spring	13.8	41.3	44.7	58.5
4 allopatric, spring	35.7	17.8	46.7	82.1
5 allopatric, spring	3.7	14.8	81.4	85.1
8 allopatric, spring	46.4	25	28.6	75
9 allopatric, spring	17.9	21.4	60.7	78.6
1 sympatric, autumn	0	63.1	36.9	63.1
3 sympatric, autumn	17.2	48.1	34.4	65.3
6 sympatric, autumn	0	39	60.7	39
7 sympatric, autumn	3.8	65.2	30.7	69
10 sympatric, autumn	25	67.9	7.2	92.9
2 allopatric, autumn	6.7	26.7	66.7	73.4
4 allopatric, autumn	55.9	17.5	26.4	82.3
5 allopatric, autumn	0	33.3	66.6	66.6
8 allopatric, autumn	0	38.2	62	62
9 allopatric, autumn	0	34.4	65.4	65.4

Table 23: Summary of classification results, indicating correct classification to original site, misclassification to alternative sites within the original population, misclassification to alternative populations and correctly classified to original population (i.e. allopatric or sympatric threespine stickleback) spring sample. **16.9%** of original grouped cases correctly classified

The first discriminant function is strongly influenced by gill-raker number ($df1=0.979$) and most clearly separates sympatric threespine populations from North Yorkshire for both seasons from the remaining threespine populations. This separation indicates that these sympatric fish have a greater number of gill-rakers and analysis in the previous section (4.3.1) indicates that this distinction is significant (see table 20). With the exception of one site (site 7 Leicestershire, Autumn) the sympatric fish from Leicester remain almost indistinguishable from the remaining allopatric fish on the first discriminant function. The second discriminant function, mostly effected by length of the 5th gill-raker ($df2=0.766$), manages to distinguish sympatric fish from Leicestershire from the allopatric populations into their own sub-group. This further separation is in accordance with the significant difference found for length of the 5th gill-raker found in section 4.2.1 (see table 18) and implies that the sympatric threespines have a shorter 5th gill-raker than the allopatric fish.

Overall allopatric and sympatric threespine populations are distinguishable as lying either side of the $x=y$ axis with exception of site 2 (Allopatric, Leicestershire, spring). Individual sites are loosely aggregated with other sites within the same categories of population, location and season, this is in accordance with the significant interactions between these factors as seen in section 4.3.1. Interpretation of the discriminant function co-efficients indicates that the sympatric

threespines generally have more numerous gill-rakers, and that this feature contributes the most to the distinction between the two threespine populations ($df_1=0.979$; $df_2=-0.060$). In the majority of sympatric threespines the 5th gill-raker ($df_1=-0.006$; $df_2=0.766$) is shorter than was found in the allopatric fish. This difference in length was shown previously to be significant (see table 18, section 4.3.1). Two sympatric sites provide an exception (sites 1 and 3, both sympatric, North Yorkshire, Spring) where fish in these sites have amongst the longest 5th gill-raker. The length of the 8th gill-raker is longer in the sympatric fish but not significantly so (see table 19, section 4.3.1). The null hypotheses stated that there would be no difference between either gill raker number or length of the 5th gill raker, these can both be rejected. It was correctly predicted that the sympatric threespine fish would have an increased number of gill-rakers, but the shorter length of the 5th gill raker in these same fish was unexpected and the converse of the outcome previously suggested. This study provides evidence of significant effects of geographical location which implies that phylogeny also influences the morphology of gill-raker architecture.

4.4: Conclusions

Although significant results were obtained during analysis of gill-raker architecture they did not indicate differentiation in the same direction as predicted from studies on the Canadian threespine pairs (Schluter & McPhail, 1992). These previous studies on sympatric threespine morphs showed an increase in both gill-raker number and length and suggested that both adaptations were required to make foraging on zooplankton more effective. In this study gill-raker number was significantly higher which is in accordance with Schluter & McPhail (1992) but also found that the length of the 5th gill-raker in sympatric threespines is shorter generally. Previous studies have shown a correlation between prey size and number of gill-rakers (see section 4.1.2), zooplanktivorous fish being found to have more gill-rakers than benthophagous fish. As the sympatric fish were found to possess significantly more rakers than the allopatric fish, this can be taken as an indicator that the sympatric threespines eat more zooplankton. Further information about the length of the gill arch is required in order to investigate a potential relationship between gill arch length and raker number. Such data was not gathered in this study as work describing the mechanisms of inter-raker spacing was only made available after the original data had been collected (e.g.: Xie *et. al.* 2002; Lipsey & Stockwell, 2001; Matsumo & Kohda, 2001). Presumably if more rakers are packed into the same length of gill arch it will serve to decrease the size of the spaces between the gill-rakers, in turn increasing the efficiency of cross-flow filtration.

There is little doubt that the rakers certainly function in some capacity as a retentive mechanism. It has been presumed in the past that the rakers act as a sieve, where an increase in both raker number and length must serve to increase the filtering capacity of the rakers by decreasing the pore size. It was presumed that the longer more numerous rakers that are observed in the Canadian limnetic morph enhance the fish's ability to retain smaller planktivorous prey (Schluter & McPhail, 1992), though this assumption has never been tested. In the light of recent information examining the function of gill-rakers and their role in cross-flow filtration (Brainerd *et. al.* 2001; Cheer *et. al.* 2001) Schluter & McPhail's (1992) conclusions must be regarded as speculative. New information has come to light that alters the function of the gill-rakers as a retentive mechanism for prey. At low Reynolds numbers the majority of particles (95%) do not come into contact with the gill-rakers and instead are ingested via the cross-flow of water that passes by the rakers (Sanderson *et. al.*, 2001). In these circumstances it is the size of the spaces between the rakers that improve the cross-flow mechanism. These findings alter our perspective on the function of the gill-rakers. Apparently an increase in gill-raker number will improve prey retention (via cross-flow filtration) and is therefore a more important adaptation to gill architecture than is an increase in raker length. The findings in this chapter certainly suggest divergence of gill-raker architecture between sympatric and allopatric threespine fish but the function of these differences is unclear. Further information about inter-raker spacing would offer some insight into the possible function of a decrease in the pore size between rakers and poses some intriguing prospects for further study.

There are two possible outcomes: If the result confirms the hypothesis, then you've made a measurement. If the result is contrary to the hypothesis, then you've made a discovery.

Enrico Fermi (1901-1954)

Chapter 5: Stomach contents analysis

5.1: Introduction

Character displacement theory states that morphological divergence should be driven by competition for food resources. Morphological and meristic analysis of the threespine's form (chapters 3 and 4) indicate differentiation of the sympatric threespine fish's morphology and suggest that this divergent form is adapted for more efficient foraging on zooplankton. Work in this chapter specifically examines whether a potential division of food resources exists between stickleback fish in the sympatric habitat. This is examined through the analysis of invertebrate prey found in the contents of the fish's stomachs. The null hypothesis to be tested in this chapter is that there is no difference in the invertebrate fauna consumed by either ninespine, allopatric or sympatric threespines. Previous discussions have suggested that the sympatric threespines are more effective planktivores than their allopatric counterparts; it should then follow that zooplankton will be a more dominant dietary component of the sympatric threespine fish's diet than it is for the allopatric sympatric fish.

5.1.1: Dietary preferences of the threespine and ninespine sticklebacks

Of the few accounts that appear in the literature specifically investigating the contents of the stickleback diet (Hynes, 1950; see Wootton, 1976, 1984; Bolger *et. al.* 1990; Campbell, 1991) it is apparent that there is considerable heterospecific overlap in dietary preferences between threespine and ninespine fish. Both stickleback species are generalist which means that they will prey upon whatever invertebrate species are available in a habitat in a particular season or geographic location. Threespine and ninespine sticklebacks consume a wide range of prey; both micro and macro-invertebrates, the only apparent limitations being the availability of prey, or gape size of the mouth (Gill & Hart 1996). Having such an inclusive diet has meant that the sticklebacks can readily accommodate naturally occurring seasonal fluctuations of invertebrate fauna assemblages. Amongst the invertebrate taxa listed that typically feature in the stickleback diet are ostracods, annelids, amphipods, gastropods, pelyceps (bivalves), most insect larvae and pupae, harpacticoid copepods, chydorid cladocerans phantom midge larvae, *Chaoborus* spp., calanoid copepods, and most cladocerans (including *Daphnia* spp.), cyclopoid copepods and chironomid larvae (Hynes, 1950; see Wootton, 1976, 1984; Bolger *et. al.* 1990; Campbell, 1991; Schluter, 1994).

5.1.2: Are Hynes' (1950) descriptions of dietary analysis still appropriate today?

Hynes' (1950) observations on the dietary preferences of the stickleback remain the most complete source available to us to date. His original lists were formed through collating the findings of a number of other studies based on the stickleback diet. At the time of Hynes' studies however, the threespine sticklebacks were regarded as one homogenous group, all the data collected from individual populations were combined together to provide us the given description of the stickleback diet. Given that in the past 10 years a number of distinct threespine eco-morphotypes have been reported it would appear that Hynes' (1950) original analysis of diet might be an oversimplification. We are now aware that a number of distinguishable threespine morphotypes exist in various freshwater bodies. Morphological divergence in these populations is believed to be driven through competition for food resources (e.g.: Lavin & McPhail, 1985; Schluter, 1993). By combining the total spectrum of prey consumed by *all* threespine sticklebacks we might be missing some important information regarding their divergence. Invertebrate assemblages are certainly not uniformly distributed throughout the entire freshwater range; there will be noticeable differences in invertebrate diversity at individual sites according to the nature of the freshwater body and geographic location.

Despite there being few studies specifically on the stickleback's dietary preference since Hynes' publication many other aspects of diet have been examined. A number of more recent studies have investigated how the morphological divergence observed in Canadian threespine populations subsequently alters what invertebrate prey are included in the diet (Schluter, 1994; Day *et. al.* 1994; Robinson 2000). These studies have found that the limnetic threespine morph is an effective planktivore and that the benthic morph forages on larger benthic invertebrates. Each morphotype would be expected to fare better in its original habitat than it would when forced to forage in that of its morphological counterpart. Workers suggest that the resulting morphological divergence that we observe now is a product of the 'ghost of competition past' (Davic, 1985) in that competition for food resources in the past facilitated the original divergence between the limnetic and benthic morphs. Subsequent divergence would presumably have brought about a reduction in competition for food resources. Other studies have found a correlation between morphology of the jaw and prey size (Gill & Hart, 1996) whereby fish with a larger gape width than their counterparts are able to take larger prey items. Such studies highlight the significance of a correlation between diet and morphology.

5.1.3: Analysis of the stomach contents of threespine and ninespine populations

Stickleback diet was investigated through examination of the stomach contents of wild caught fish. This approach was deemed appropriate, as it would give an idea of what the stickleback fish eat in their natural environment. Analysis of environmental variables in chapter 2 indicated that whilst the sympatric sites appear to offer more benthic habitat there are few differences between allopatric and sympatric sites in terms of the invertebrate faunal assemblages. The divergent morphology of the sympatric threespine fish indicates potential specialisation to a diet predominantly consisting of zooplankton; this in turn would serve to alleviate competition with the ninespine fish whose diet is reported to be predominantly benthic (see Wootton, 1976). This line of reasoning is tested through the examination of the following questions; firstly, is there significant overlap between the diets of the threespine and ninespine sticklebacks indicating a source of potential competition? And, secondly, are there differences between the stomach contents of allopatric and sympatric threespine sticklebacks that indicate divergence in diet? The following null hypothesis is offered; that there will be no significant differences in the stomach contents between ninespine, allopatric and sympatric threespine stickleback populations.

5.2: Methods

The fish used in the analysis were those caught in September 2000 (the autumn sample). These populations were selected as previous morphological analysis (chapter 3) indicated that fish in these populations constituted a greater range of sizes and would therefore give a more detailed impression of diet choice. If resource partitioning occurs between ninespine and sympatric threespine sticklebacks, these pairs of fish should show the greatest divergence in diet. It is borne in mind that the invertebrate faunal assemblage will naturally change over the seasons and that the contents of the stickleback stomach only represent a 'snapshot' of what the fish are feeding on at the time of capture.

Gape width of the smaller fish in the populations analysed will restrict the maximum size of prey they are able to handle. Consideration was given to the fact that a possible disproportionate representation of a size class would lead to bias in the results of the stomach analysis. In order to reduce the effects that body size might have the sticklebacks were categorized into groups according to size (size class 0= 15-20mm; size class 1= 20.1-25mm; size class 2= 25.1-30mm; size class 3= 30.1-35mm; size class 4= 35.1-40mm; size class 5= 40.1-45mm; size class 6= 45.1-50mm). Each stickleback population (i.e. allopatric, sympatric and ninespine) now comprises 6 successive size classes. As sample size was small in size classes 4, 5 and 6 statistical analysis was not possible so these fish were omitted from further analysis.

5.2.1: Analysis of stomach contents

Dissection of the stickleback's stomach involved an initial incision starting at the anal opening along the ventral side to the posterior portion of the lower jaw, as shown in figure 13. Two further incisions were made, one from the anal opening to the top of the body, just below the 3rd dorsal spine and a second from the jaw line (where the original incision ended) to the top of the body, finishing just posterior to the operculum. These three incisions produced a 'flap' of epidermis which was removed by making a final incision along the dorsal side of the body from the base of the 3rd dorsal spine to just behind the operculum. The body cavity of the stickleback was now fully exposed. As much of the digestive system as possible was then removed from the body cavity in order to avoid disrupting the contents of the stomach. This involved cutting through the oesophagus, located adjacent to the gills, and cutting through the rectum just before the anal opening. So extricated, the digestive system was placed into water. The contents of the stomach were extracted under a binocular dissecting microscope. A small incision was made in the stomach lining and the contents gently teased out. Many of the prey items consumed by sticklebacks have a chitinous exoskeleton, which remains intact in the stomach. Invertebrate prey species were identified by the remnants found in the stomach. In all cases it was possible to identify invertebrates to genus, or if not, at least to family level. It was also possible to give an approximation on the total number of invertebrates found in the stomachs by counting up these various remnants of exoskeleton (i.e. if 2 *Gammarus* pleiopods are found, count 1 *Gammarus*). The content of the intestine was ignored as the digestive process had broken down the invertebrate exoskeleton to such an extent that no valid identification was possible.

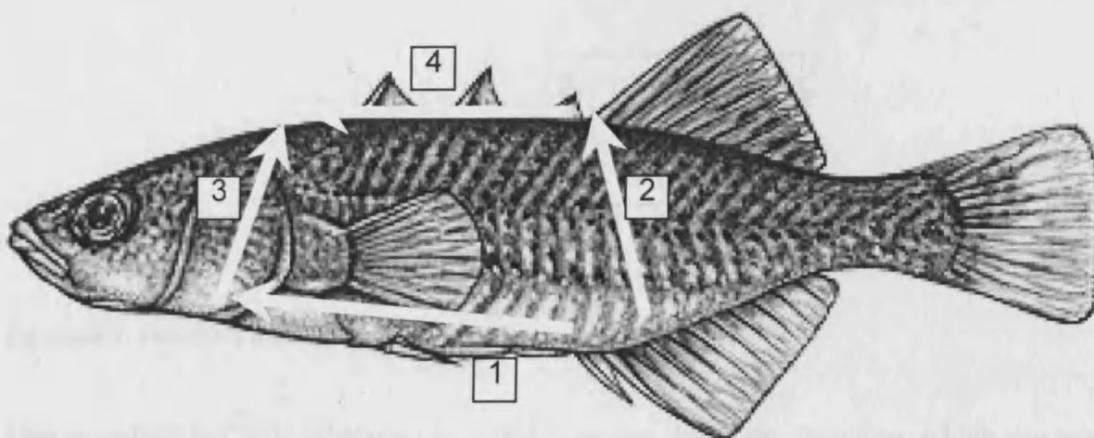


Fig 13: Diagram of the threespine stickleback indicating the direction of incisions made to gain access to the body cavity (original diagram adapted from PA fish Tables (2003). See text for explanation.

5.2.2: Analysis of invertebrates collected from stomach samples

During the sorting procedure it became apparent that some invertebrate species were very common in the stomach contents. It was also apparent that all of the more popular prey items were available to all sticklebacks at all sites (chapter 2), so any differences in stomach contents would not be because of limited availability of a particular prey item. In addition many invertebrate species that are classed in the same family comprised individual species of variable size. Certainly one large Chironomid will satiate a stickleback's hunger more than a smaller specimen. There could be some disparity in the results if all sizes of individual representatives of a species were classed together. In order to account for this potential disparity, certain invertebrate species were further classified according to size. (i.e.: Ostracoda (small Ostracoda <0.5mm; large Ostracoda >0.5mm), Chironomidae (small Chironomid <0.5mm; medium Chironomid >0.5mm <1mm; large Chironomid >1mm), Asellidae (small Asellus <1mm; large Asellus >1mm) and Gammaridae (small Gammarus <1mm; large Gammarus >1mm)).

A study by Tirasin & Jørgensen (1997) examines the sensitivity of a number of alternative methods of analysing the contents of stomach samples. They offer suggestions of how to analyse either number of prey (N), which is indicative of feeding behaviour, weight (W) of prey, indicative of the nutritional value of prey, or frequency of occurrence (F) which tells us of the uniformity of group selection. Standard error is found using Cochran's formula (Equation 1) (from Tirasin & Jørgensen, 1997). As data about invertebrate prey were collected according to counts of individual species in the stomach sample, W is replaced by N in the formula. Where N_{ij} is the proportion of prey taxon i in the stomach contents of individual fish j .

$$SE(\hat{N}_1) = \frac{\sqrt{\sum_{j=1}^{n_j} \left(N_{ij} - \hat{N}_i \sum_{i=1}^{n_i} N_{ij} \right)^2}}{n_j (n_j - 1)} \cdot \frac{1}{n} \sum_{i=1}^{n_i} \sum_{j=1}^{n_j} N_{ij}$$

Equation 1: Cochran's formula from Tirasin & Jørgensen (1997)

One oversight in Cochran's formula is that it ignores intra-site variation, which can account for a large proportion of variation in the samples (Tirasin & Jørgensen, 1997). One recommendation to overcome this problem of intra-site variation is to use Efran's (1979) bootstrapping method. Though, to be truly representative, this method requires an adequate sample size, usually between 20-30 individuals per site, anything less is insufficient and the results are unreliable (Tirasin & Jørgensen, 1997). When broken down to individual size classes at each site,

stickleback sample sizes fall below that recommended for Efron's bootstrapping procedure, so further analysis could not be performed. However, intra-site variation is considered of little consequence to this particular study as previous analysis of the stickleback population's respective habitats (chapter 2) indicated little variability in the invertebrate assemblage between sites.

5.3: Results

The objective of this chapter is to establish whether the morphological and meristic divergence observed with sympatric threespine populations serves to alleviate competition with the co-existing ninespine stickleback by allowing the sympatric fish to forage on alternative prey. The overarching hypothesis states that differences will be found between the invertebrate species found in the stomach contents of allopatric and sympatric threespines and ninespine fish.

5.3.1: Cochran's analysis

The percentage abundance and standard error of the results of the invertebrate taxa identified and counted from the stomach samples are given in tables 24, 25 and 26 for each population of sympatric threespine and ninespine sticklebacks and allopatric threespine sticklebacks respectively. Standard error has not been calculated for taxa where the percentage proportion in the diet was less than 1%, or where taxa were only represented by one individual. The most abundant invertebrate species found in the stomach contents of *all* three stickleback populations (i.e.: Cyclopoidia, Daphnidae, Ostracoda, Chironomidae, Hydrobidae and Oligochaetae) are represented in error bar graphs for each individual stickleback population.

5.3.1.i: Results of Cochran's analysis for the sympatric threespine.

The stomach contents of the sympatric threespine fish largely consists of Cyclops (between 28-50%) and Chironomid (between 11-25%), however in larger fish the proportion of each prey type is altered. In the smallest fish (class 0) Chironomids constitute the most abundant prey item but in the larger fish Cyclops become the most prominent prey consumed. Oligochaetes make up a large proportion of the diet of medium sized fish (classes 2 & 3). The invertebrates consumed by the sympatric fish are predominantly limnetic zooplankton.

Sympatric threespine:												
Invertebrate taxa	Class 0			Class 1			Class 2			Class 3		
	%	Upper CI	Lower CI									
Cyclopodia	28	37.1	18.9	17.4	21.9	12.9	31.8	62.1	1.5	48.9	76.2	21.6
Daphnidae	2.5	11.8	0	2.1	10.5	0						
Ostracoda	6.7	10.5	2.9	8.6	13.3	3.9	18.7	29.4	8	8	46.3	0
Lge ostracoda	5.7	12.3	0	2.3	37.6	0	1.9	17.4	0			
Sml chironomid	19.5	35.8	3.2	2.7	17.8	0	2.8	47.1	0			
Med chironomid	25.2	32.6	17.8	9	12.8	5.2	11.2	28.2	0	13.3	2.9	0
Lge chironomid	1.4	13.5	0	0.9	-	-	0.9	-	-	13.3	32.6	0
Snail	1.8	14.6	0	1.7	2.5	0				2.2	-	-
Oligochaetae	4.3	10.2	0	50	68.6	31.4	23.4	48.9	0			
Sml asellus	4	-	-	0.5	-	-						
Lge asellus				0.2	-	-	1.9	55.2	0	2.2	-	-
Sml gammarus				0.2	-	-						
Lge gammarus	1.1	27.9	0							4.4	-	-
Baetidae										2.2	-	-
Hemiptera				0.2	-	-				2.2	-	-
Coleoptera	3.5	11.3	0	4.2	48.6	0	0.9	-	-	2.2	-	-
Zygotera							6.5	12.5	0	2.2	-	-
Trichoptera												

Table 24: Results of Cochran's analysis indicating percentage abundance with confidence interval of individual invertebrate taxa identified from the stomach contents of sympatric threespine stickleback populations.

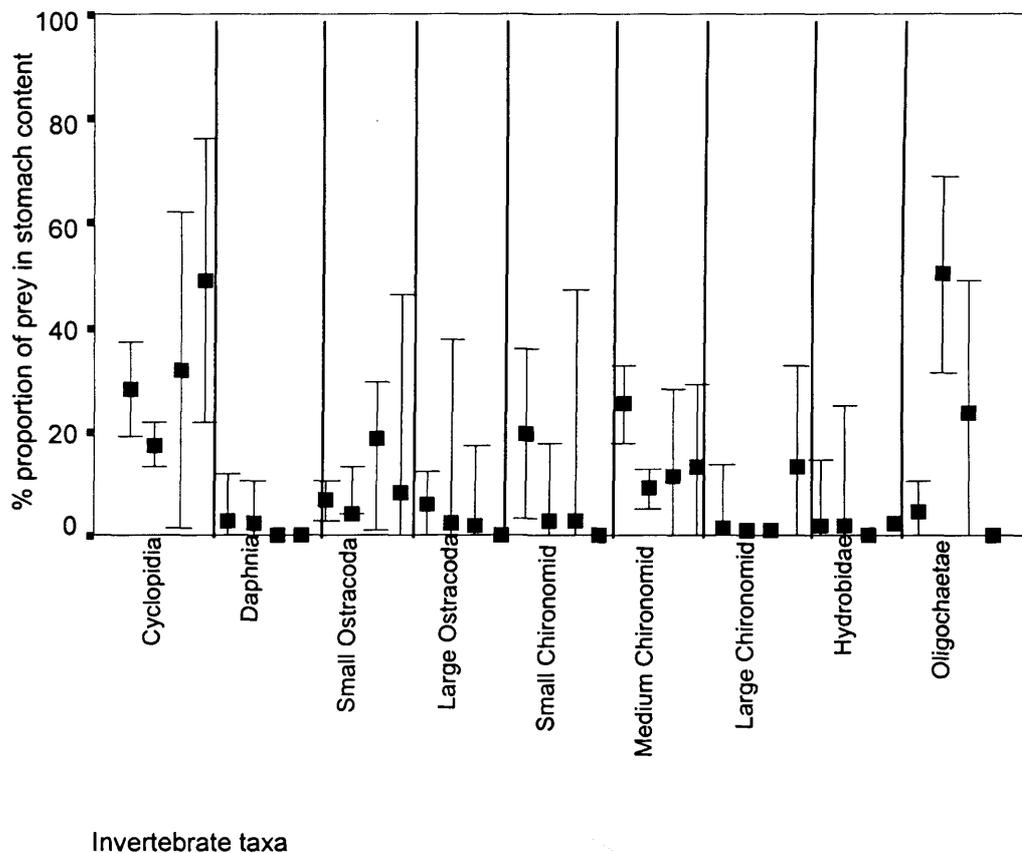


Figure 14: Error bar chart representing composition of invertebrate prey species identified from stomach samples of the different size classes of sympatric threespine fish. For each prey category the marker closest to the y-axis represent size class 0, size classes' increase in ascending order from left to right.

5.3.1.ii: Results of Cochran's analysis for the sympatric ninespine

Sympatric ninespine												
Invertebrate taxa	Class 0			Class 1			Class 2			Class 3		
	%	Upper CI	Lower CI									
Cyclopodia	41.8	62.6	21	35.5	48.1	22.9	24	34.6	13.4	10.6	24.2	0
Daphnidae	9.1	49.7	0	1.5	21.7	0	0.8	-	-			
Ostracoda	10	15.5	4.5	12.4	17.1	7.7	7.6	15	0.2	16.9	28.4	5.4
Lge ostracoda	7.3	23	0	10.4	16.5	4.3	16.7	25.8	7.6	27.1	46.2	8
Sml chironomid												
Med chironomid	24.5	34.4	14.6	9.7	14.1	5.3	20.2	28.5	11.9	10.6	14.9	6.3
Lge chironomid	0.9	-	-	1.5	13.6	0	1.9	11	0	5.1	9.4	0.8
Snail	4.5	16.7	0	4.6	9.5	0	5.7	11.4	0	3.4	10.8	0
Oligochaetae	0.9	-	-	9.3	58.9	0	4.6	27.4	0	5.5	7.1	3.9
Sml asellus				13.5	27.4	0	11	27.6	0	11.4	27.8	0
Lge asellus				0.4	-	-	3.8	11.7		6.8	35.3	0
Sml gammarus				0.4	-	-		27.3	0			
Lge gammarus							3	-	-	1.3	7.5	0
Baetidae												
Hemiptera				0.8	-	-	0.8	-	-			
Coleoptera	0.9	-	-							1.3	20.4	0
Zygoptera												
Trichoptera												

Table 25: Results of Cochran's analysis indicating percentage abundance with confidence interval of individual invertebrate taxa identified from the stomach contents of sympatric ninespine stickleback populations.

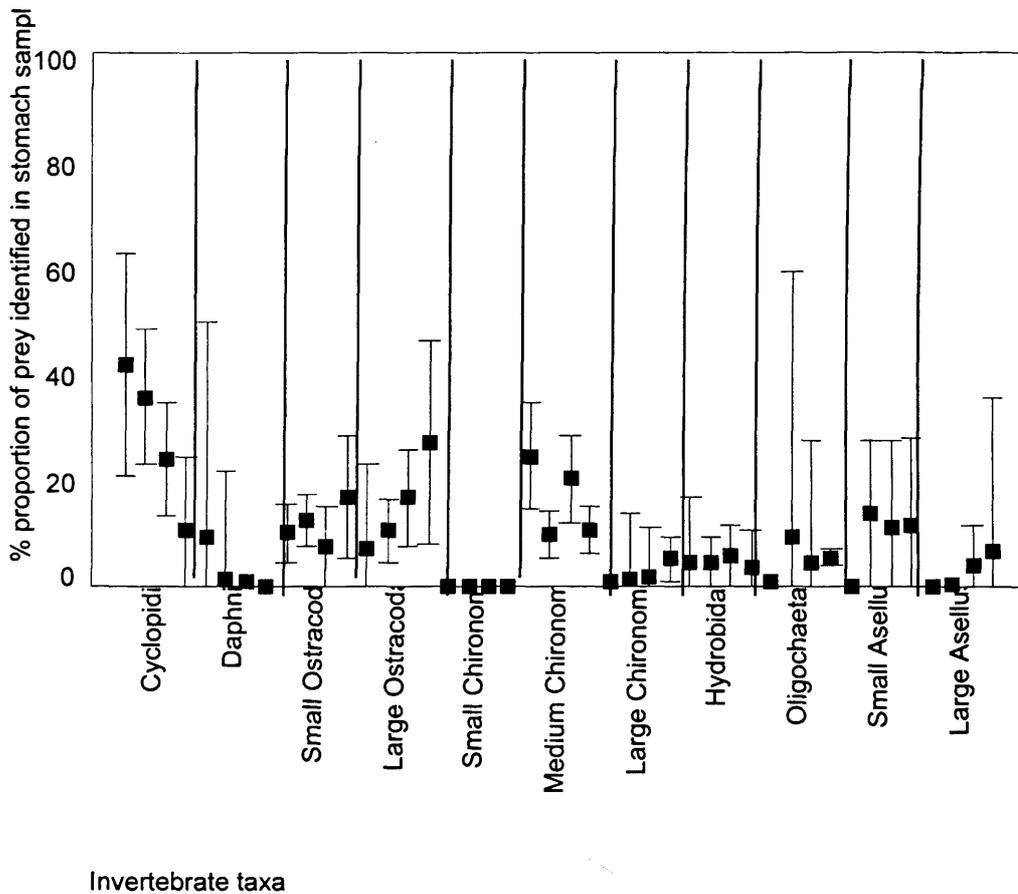


Figure 15: Error bar chart representing composition of invertebrate prey species identified from stomach samples of the different size classes of sympatric ninespine fish. For each prey category the marker closest to the y-axis represent size class 0, size classes' increase in ascending order from left to right.

The ninespine stickleback's diet is the most diverse of each population examined. Again Cyclops are one of the major dietary components, but where the sympatric threespine fish increased their consumption of Cyclops as they increased in size the ninespine fish reduce their intake. Ostracoda are a dominant prey source, the percentage proportion in the diet remains relatively constant for all size classes of fish. Whilst Chironomids constitute a high proportion of the ninespine fish's diet they are not as prominent as they are in the threespine fish's diet (both allopatric and sympatric threespines). The ninespines are the only sticklebacks that forage on *Asellus*. With the exception of the smallest fish (class 0) smaller *Asellus* constitutes ~11% of the ninespines diet. The prey items consumed by the ninespine fish are predominantly benthic but include a large proportion of zooplankton.

5.3.1.iii: Results of Cochran's analysis for the allopatric threespine.

Allopatric threespine:												
Invertebrate taxa	Class 0			Class 1			Class 2			Class 3		
	%	Upper CI	Lower CI									
Cyclopida	14.3	20.6	8	6.3	7.7	4.9	5.1	11.9	0			
Daphnidae	26.3	39	13.6	8.7	13.6	3.8	7.4	17.4	0			
Ostracoda	5.5	11.2	0	18.8	28.5	9.1	10.5	17	0.4			
Lge ostracoda	1	38.9	0				6.8	17.6	0			
Sml chironomid	7.5	44.6	0	2.3	-	-	2.7	-	-	4.1	-	-
Med chironomid	41.3	49.8	32.8	55.2	66.6	43.8	48.3	61	35.6	53.4	73.3	33.5
Lge chironomid				1.1	14.2	0	1	20	0	5.5	24.6	0
Snail	0.7	-	-	0.8	-	-	3	23.9	0			
Oligochaetae	1.7	43.4	0	0.8	-	-	5.1	13.6	3.4			
Sml asellus				0.4	-	-						
Lge asellus				0.2	-	-	0.3	-	-	2.7	-	-
Sml gammarus							0.3	-	-			
Lge gammarus				0.2			2	16.1	0	4.1	-	-
Baetidae	1	38.9	0	4.7	14.8	0	5.4	10.8	0	28.8	30.2	27.4
Hemiptera							0.7	-	-	1.4	10.4	0
Coleoptera				0.2	-	-						
Zygoptera				0.2	-	-	0.7	-	-			
Trichoptera	0.7	-	-				0.7	-	-			

Table 26: Results of Cochran's analysis indicating percentage abundance with confidence interval of individual invertebrate taxa identified from the stomach contents of allopatric threespine stickleback populations.

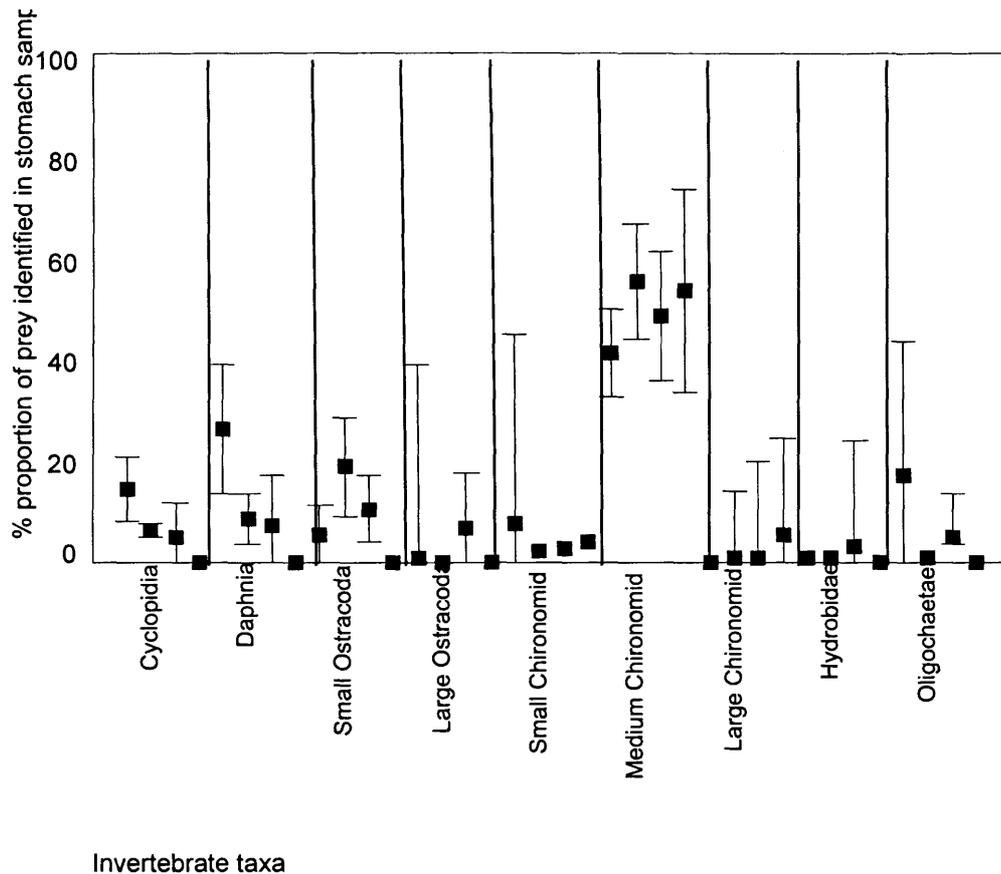


Fig 16: Error bar chart representing composition of invertebrate prey species identified from stomach samples of the different size classes of allopatric threespine fish. For each prey category the marker closest to the y-axis represent size class 0, size classes' increase in ascending order from left to right.

In all size classes of allopatric threespines medium sized chironomids comprise approximately half of the total invertebrate intake (between 41-55%). Although these fish showed a preference for chironomids (which was not evident amongst the sympatric threespines) the remainder of their diet was more variable than that of the sympatric threespine fish. The remainder of the stomach contents comprised of zooplankton (*Daphnia* and *Cyclops*) in the smallest fish, ostracods in the middle size classes (classes 1 & 2) and Baetid in the largest fish. With the exception of the smallest fish who consume zooplankton, the allopatric threespines diet predominantly includes invertebrate taxa found in the benthic habitat.

5.3.2: Statistical analysis of the most abundant invertebrate prey identified from stomach samples.

In order to determine whether the differences observed in the stomach contents of stickleback populations were significantly different the raw data were analysed using the non-parametric Kruskal Wallis test. The null hypothesis to be tested in this case was that there are no differences in the abundance of invertebrate species consumed by sticklebacks from different populations. Kruskal Wallis analysis was performed on all three stickleback populations combined, for each

individual prey type for each size class of fish. By analysing each prey type individually more detail is given about consumption patterns both within size classes of fish and between populations. The results of the Kruskal Wallis analysis are given in table 27.

Invertebrate Prey	Statistic	Size 0	Size 1	Size 2	Size 3
Cyclopodia	K	2.928	5.054	16.68	6.652
	df	2	2	2	2
	P	0.231	0.080	0.000	0.036
Daphnia	K	1.626	3.703	2.435	0.000
	df	2	2	2	2
	P	0.443	0.157	0.296	1.0
Small Ostracoda	K	0.009	0.512	6.293	11.895
	df	2	2	2	2
	P	0.995	0.774	0.043	0.003
Large Ostracoda	K	5.517	9.433	2.417	6.694
	df	2	2	2	2
	P	0.063	0.009	0.299	0.035
Medium Chironomid	K	3.755	8.580	9.080	2.801
	df	2	2	2	2
	P	0.153	0.014	0.011	0.246
Large Chironomid	K	3.409	6.423	4.778	1.580
	df	2	2	2	2
	P	0.182	0.04	0.092	0.454
Hydróbidae	K	0.540	1.123	7.372	5.000
	df	2	2	2	2
	P	0.763	0.570	0.025	0.082
Oligochaetae	K	1.132	9.154	3.722	7.0
	df	2	2	2	2
	P	0.568	0.01	0.156	0.03

Table 27: Results of Kruskal Wallis analysis comparing content of individual invertebrate taxa in the stomach contents of different size classes of stickleback populations.

The abundance of Cycloids in the stomach contents showed a significant result for class size 2 (df= 2; K= 16.680; P<0.001) and 3 (df=2; K= 6.652; P=0.036), with an almost significant result being obtained for the fish in class size 1(df=2; K= 5.054; P=0.08). The majority of Cyclops are consumed by sticklebacks in sympatric sites (both ninespine and threespine), mainly by larger sympatric threespines (from Cochran's analysis (table 24) class 2: 31.8% \pm 30.3; class 3: 48.9% \pm 27.3) however the error margin (Cochran's) is high indicating that whilst some fish may consume a number of Cyclops others may take none. The high proportion of Cyclops in the sympatric fish's diets is not reflected in that of the allopatric fish, where only the smallest fish consume Cyclops and then only in moderate numbers.

Both size class 2 (df=2; K=6.293; P=0.043) and 3 (df=2; K= 11.895; P= 0.003) show a significant difference of predation upon smaller Ostracods between the fish populations. Examination of the data indicates that in size class 2 the majority of Ostracods are consumed by the sympatric threespine, with lower but more similar amounts consumed by the ninespine and allopatric threespine sticklebacks. This situation is reversed in larger fish (class 3) where now the

ninespine fish consume significantly more Ostracods. In addition the ninespines are the only population of fish to consume larger Ostracods in any significant number.

With regards to consumption of medium sized chironomids significant results are obtained between size classes 1 ($df= 2$; $K=4.834$; $P=0.028$) and 2 ($df= 2$; $K=6.608$; $P=0.010$). Here the majority of medium sized chironomids were found in the stomach contents of the allopatric fish whilst lower but similar proportions of medium sized chironomids were found in the stomach samples of both sympatric threespine and ninespine fish. In the larger fish (class 2) the proportion of medium size chironomids is doubled in the stomach content of the ninespine fish compared with that of the sympatric threespine fish. Significant results were found for class size 1 ($df=2$; $K=6.423$; $P=0.040$) and 2 ($df=2$; $K= 4.778$; $P= 0.092$) for numbers of large Chironomid found in the stomach contents. In both size classes the ninespines and allopatric threespines consume similar amounts of the largest chironomids whilst the same size classes of sympatric fish virtually ignore this prey.

Both the ninespine and allopatric threespine ate similar proportions of Hydrobidae although the proportions are generally low ($>6\%$). The same size class of sympatric threespines significantly ignore this prey source ($df= 2$; $K= 7.372$; $P=0.025$)

Only sympatric threespines, and of them only fish in class size 1, consumed a significant amount of oligochaetes ($df= 2$; $K=9.154$; $P=0.010$), but in this case the oligochaetes constituted 50% of the total invertebrate prey ingested in this group. In the largest fish only the ninespine fish foraged on oligochaetes and in this instance in very small amounts (5.5%)

5.3.3: DFA prey in stomach samples

The analysis so far has concentrated on how individual invertebrate taxa are represented in the diets of the three stickleback populations. A stronger indicator of divergent diet is to see if the sticklebacks can be separated into their appropriate populations on the strength of diet characteristics alone. DFA is used to investigate dietary differences between populations. The raw data of frequency occurrence of invertebrate taxa in the stomach samples were \log_{10} transformed and used in subsequent analysis. As in previous analysis (chapter 3) the sticklebacks are grouped according to their original site and stickleback species. The results of the analysis are given in tables 28 through to 31, and figure 17.

Function	Eigenvalue	% of variance	Wilks' Lambda
1	1.130	36.4	0.000
2	0.491	15.8	0.000

Table 28: Discriminant Function Analysis between invertebrate preys identified from stickleback stomach samples

Dependent Variable	DF1	DF2
Small Asellus	-0.079	0.239
Large Asellus	-0.006	0.221
Baetid	0.770	0.481
Cyclops	-0.224	0.542
Coleoptera	0.034	-0.222
Small chironomid	0.616	-0.244
Large Chironomid	-0.075	-0.172
Daphnia	0.258	-0.207
Small Gammarus	-0.056	0.116
Large Gammarus	0.022	-0.106
Hemiptera	-0.098	0.117
Hydracarina	0.099	-0.002
Oligochaetes	-0.143	0.449
Small Ostracod	-0.094	-0.234
Large Ostracod	-0.076	0.049

Table 29: Standardized Canonical Discriminant Function Coefficients Analysis between invertebrate preys identified from stickleback stomach samples

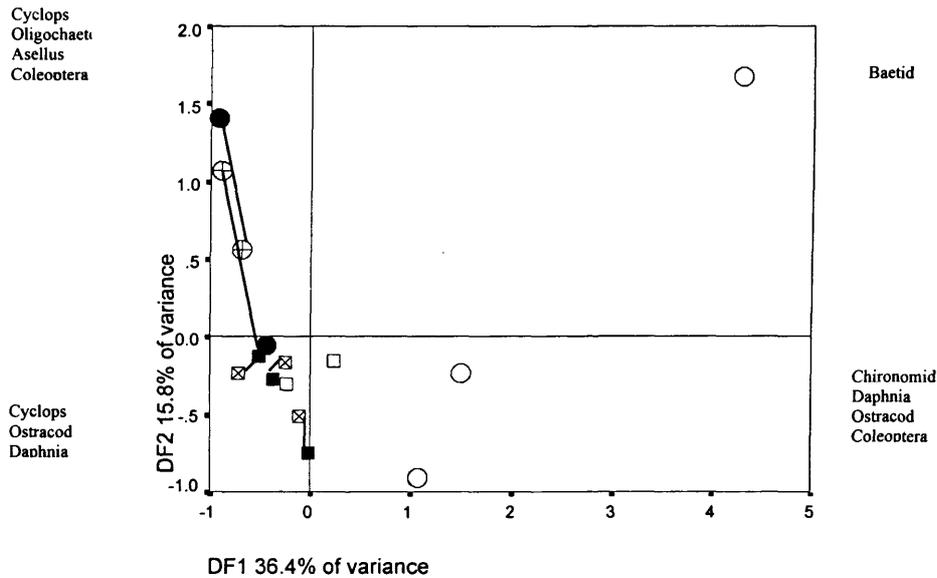


Figure 17: DFA of stomach contents of stickleback populations. Circular markers indicate fish collected in Leicestershire; square markers indicate fish collected from Yorkshire. Filled markers denote sympatric threespine fish; empty markers indicate allopatric threespine fish. Partially filled markers indicate sympatric ninespine fish. Sticklebacks from individual sympatric populations are joined by solid line.

Site & population	% correctly classified to original site	% misclassified within sympatric	% misclassified within allopatric	% misclassified within ninespine	% correctly classified to population
1 sympatric	154	65.3	15.3	3.	80.7
3 sympatric	24.2	48.5	3.0	24.2	72.7
6 sympatric	6.7	6.4	9.7	16.1	74.1
7 sympatric	39.	39.3	10.8	10.7	78.6
10 sympatric	27.6	41.3	27.6	3.4	68.9
2 allopatric	16.2	54.0	16.2	13.5	32.4
4 allopatric	25.9	44.4	18.5	11.1	44.4
5 allopatric	57.1	0	35.7	7.1	92.8
8 allopatric	24.1	24.0	48.2	3.4	72.3
9 allopatric	50.0	23.3	23.4	3.3	73.4
11 ninespine	27.3	36.3	18.1	18.1	45.4
12 ninespine	38.9	44.4	11.1	5.6	44.5
13 ninespine	27.6	48.2	3.4	20.7	48.3
14 ninespine	25.0	42.9	10.8	21.4	46.4
15 ninespine	8.8	61.7	23.5	5.9	14.7

Table 30: Summary of classification results, invertebrate taxa identified from stomach samples indicating correct classification to original site, misclassification to alternative sites within the original population, misclassification to alternative populations and correctly classified to original population (i.e. allopatric, sympatric or ninespine stickleback). 30.4% of original grouped cases correctly classified

Site, population & location	% correctly classified to original site	% misclassified within Leicestershire	% misclassified within Yorkshire	% correctly classified to location
1 sympatric, Yorks	15.4	69.1	15.3	30.7
3 sympatric, Yorks	24.2	54.2	21.2	45.4
6 sympatric, Leics	67.7	12.9	1.3	80.6
7 sympatric, Leics	39.3	35.7	25.1	75.0
10 sympatric, Yorks	27.6	51.7	20.6	48.2
2 allopatric, Yorks	16.2	51.3	32.4	48.6
4 allopatric, Yorks,	25.9	55.5	18.5	44.4
5 allopatric, Leics	57.1	35.7	7.1	92.8
8 allopatric, Leics	24.1	65.4	10.2	89.5
9 allopatric, Leics	50.0	26.7	23.3	76.7
11 ninespine, Yorks	27.3	40.8	31.7	59.0
12 ninespine, Yorks	38.9	38.9	22.2	61.1
13 ninespine, Leics	27.6	65.5	6.8	93.1
14 ninespine, Leics	25.0	35.8	39.3	60.8
15 ninespine, Yorks	8.8	52.9	38.2	47.0

Table 31: Summary of classification results, invertebrate taxa identified from stomach samples indicating correct classification to original site, misclassification to alternative sites within the original geographic location, misclassification to alternative geographic location and correctly classified to original geographic location (i.e. Leicestershire or Yorkshire). 30.4% of original grouped cases correctly classified

The first discriminant function most successfully segregates the allopatric fish from Leicestershire from the remaining populations. The second function separates the sympatric ninespine and threespine fish also from Leicestershire. This has resulted in a conspicuous cluster of sticklebacks from Yorkshire in the lower left quartile of the plot of centroids (Figure 17). These groupings clearly denote that the geographic location has an influence on the diet of the stickleback fish. Moreover, the close grouping of the sympatric fish from the same location

particularly those fish from Yorkshire, suggests that these fish have diets more similar to their counterpart than they do to any of the other populations. The outlying population is a group of fish from Leicestershire who were the only fish which ingested large numbers of *Baetid* in their diet.

5.4: Conclusions

The rationale for this chapter was to examine the stomach contents of the stickleback fish in order to investigate any potential differences in diet between populations. The literature indicates that dietary preferences of the ninespine and threespine sticklebacks overlap (Hynes, 1950; see Wootton, 1976) as such it was proposed that the sympatric threespine fish might choose food that was different to the ninespine in order to reduce competition in the sympatric habitat. Morphological divergence between sympatric and allopatric threespine fish (chapter 3) indicated that the sympatric threespine fish are better adapted to forage on zooplankton, consequently there should be a higher proportion of these prey in the sympatric fish's diet. Previous analysis (chapter 2) found that the invertebrate fauna did not significantly differ between allopatric and sympatric sites but indicated that invertebrate abundance differed according to geographical location. *Gammarus* were significantly more abundant in sites in North Yorkshire, particularly allopatric sites, whilst *Asellus*, *Glossiphonidae* and *Oligochaetes* were more abundant in Leicestershire. If diet choice is solely related to abundance of prey items these same patterns between habitat and geographic location should be mirrored in the stomach contents.

5.4.1: Similarities and differences between stickleback diets

The zooplankton *Cyclops* was amongst the most abundant prey choice for the sympatric threespines, the proportion of which varied between the different size classes of these fish. Generally as the sympatric threespines increased in size so did the proportion of *Cyclops*. This finding agrees with the assumption that the morphology of the sympatric fish should include a predominantly zooplanktivorous diet. *Cyclops* also constituted a large proportion of the ninespines diet, but in this case the proportion of prey decreased as the fish got larger. As the ninespine stickleback is reported to forage predominantly in the benthic habitat (see Wootton, 1976) the proportion of *Cyclops* in the stomach contents (up to 1/3 of the total stomach contents) is regarded as surprising. Only the very smallest allopatric threespines consumed zooplankton (*Daphnia* and *Cyclops*) in any large number; this follows more usual patterns of foraging behaviour in fish where generally only juveniles consume zooplankton in any large quantities. Unfortunately *Cyclops* were not uncovered in the invertebrate analysis (chapter 2) so it is unclear what the status of these zooplankton is in the sympatric or allopatric environment. It is likely that

conditions in the sympatric environment are more conducive to growth of zooplankton as the water is slower running in this habitat which encourages larger quantities of phytoplankton, the zooplankton's food source, to grow. At the time of the year when the fish were sampled (late summer) levels of zooplankton are at their highest. It is possible that the larger numbers of Cyclops found in the sympatric sticklebacks stomach contents (both threespine and ninespine) is as a consequence of a higher abundance of these invertebrates in the sympatric habitat making them an easily available food source for the sympatric fish. Because Cyclops were found in large quantities in both sympatric ninespine and threespine stomach contents it is possible that these fish will face some level of competition for this food source, but this should be tested when levels of Cyclops are limited in the environment. It is also possible that the morphological divergence observed in the sympatric fish is a consequence of adaptation to a higher abundance of the zooplankton in the sympatric environment, rather than as a result of these fish having to find an alternative food source due to character displacement. However these ideas are purely speculative and require further analysis of the abundance of Cyclops at each site. What has emerged from these observations of zooplankton in the stickleback's diet is that the sympatric fish rely more heavily on this prey for food than do the allopatric threespine fish.

The allopatric fish consumed significantly more chironomids than the fish in the sympatric habitat (both ninespine and threespine). Chironomids are a particularly important prey source for the allopatric threespine fish as this prey constituted half of the total amount of invertebrates eaten. This result is considered surprising as levels of chironomid were found to be the same in both allopatric and sympatric habitats (chapter 2). Chironomids were eaten in relatively similar proportions by the sympatric fish and were only particularly abundant in the stomach contents of the smallest ninespine and sympatric threespine fish. As the sympatric threespine fish increased in size oligochaetes became an important food source although these were the only population to consume this particular prey type. Both chironomids and oligochaetes are soft bodied and require minimal handling, but as they are found on or just beneath the surface of the substrate in areas of open water and vegetation require a different foraging strategy to that of zooplankton. Because the sympatric threespines ate significantly less chironomids than the allopatric fish, despite there being similar levels of this prey available in the sympatric environment, it is possible that somehow foraging on chironomids is restricted in the sympatric threespines: Either the morphology of the sympatric threespine whilst not precluding chironomids from the diet, may make the fish less effective at hunting and handling this benthic prey. Or, alternatively, the sympatric threespines may eat less chironomids as a result of direct competition from the ninespine fish. The presence of the ninespine may deter the sympatric threespine fish from foraging amongst the vegetated region of the habitat.

Ostracods were commonly found in the stomach contents of the larger allopatric fish and particularly the ninespine fish. However with the exception of the larger sympatric threespines (class size 2) this prey was largely absent in the stomach contents of this population. These small prey are commonly found on the substrate in vegetated region of the habitat. Whilst Ostracods were found in both the allopatric and sympatric habitat their numbers were limited, this may encourage competition between the sympatric fish for this food resource. It is possible that although this prey is small enough for the sympatric fish to handle they may be overlooked as a prey item by these fish because of their affinity with the vegetated region of the habitat where the ninespine fish is possibly the stronger competitor.

The sympatric threespine fish continue to forage on both benthic (Chironomids and Oligochaetes) and limnetic (Cyclops) prey as they increase in size. This inclination to forage in the open water does not persist in either the ninespine or allopatric threespine fish where only the smallest fish (class size 0) ate zooplankton in any large quantity. In the larger ninespine and allopatric threespine fish the diet is comprised of invertebrates found exclusively on the substrate. The results indicate that a large number of *Baetid* were consumed by the largest (class size 3) allopatric fish but this prey was only consumed by one population of allopatric fish in Leicester (site 5). The ninespine fish have the most diverse diet of all the stickleback populations and were the only fish that foraged on larger invertebrates to any great extent. In particular *Asellus* made up a significant portion of the ninespines diet, with the exception of the smallest fish (size class 0). As the ninespine fish have the largest gape width of all the sticklebacks (chapter 3) they are able to handle this larger prey more effectively. It is assumed that the smaller gape width of the threespine fish restricts handling capability, as such larger invertebrates (*Gammarus* and *Asellus*) were found in the stomach contents of only the largest threespine fish, but even then, only in a limited number of cases.

When DFA was performed on fish from individual sites the results showed conspicuous clusters of fish which were grouped according to geographical location. The most obvious clustering was a division between sites in Leicestershire and North Yorkshire. The strongest association was that between the stomach contents of sticklebacks in North Yorkshire, in this case there was no obvious distinction between fish from allopatric and sympatric sites. The stickleback fish from Leicestershire however did segregate into fish from allopatric sites and fish from sympatric ones. Most notable was the strong association between sympatric fish from the same site; this clearly indicates that these fish are not partitioning resources within a site as was previously assumed. These results show a clear association between geographic location and stomach contents,

therefore the stickleback's diet is more heavily influenced by prey availability in the individual site rather than competitive pressure from a heterospecific.

There was some difficulty in deciphering some of the prey choices made by the sticklebacks as previous analysis of the invertebrate fauna (chapter 2) failed to detect any zooplankton. Without any information regarding relative abundance of zooplankton in the habitat it was not possible to interpret their presence in the stomach contents, in particular their persistence in the sympatric threespines diet. As there is evidence of a different dietary regime between allopatric and sympatric sites in Leicestershire it would be beneficial to examine these habitats more closely to try to determine potential differences in ecological conditions (both biotic and abiotic) at these sites that may have been overlooked in previous analysis (chapter 2). It is appreciated that the results of the stomach contents analysis only presents a 'snap-shot' of the sticklebacks diet for the time of year they were caught, with this in mind it might be interesting to observe the stomach contents of fish caught at different times of the year. The fish in the analysis were captured in September where there would still have been a plentiful supply of food thus foraging conditions in the environment will have been relaxed. It would be useful to examine the stomach contents of fish captured in the winter when conditions would be harsh and the fish would most likely have to compete for limited food resources.

5.4.2: Food for thought.

Analysis of the fauna uncovered significant differences in the relative abundance of invertebrates between allopatric and sympatric sites rather than differences in taxa present (chapter 2), as such the same invertebrate taxa were available as prey to sticklebacks in both habitats. However the sticklebacks did not always elect to forage on the most abundant food source available to them. Threespine fish in sympatric sites ate significantly fewer chironomids than did their counterparts in allopatric sites, despite there being similar levels of chironomids in sympatric sites. Both the sympatric threespine and ninespine fish consumed approximately the same proportion of chironomids neither one of them being an obviously stronger competitor. It is unclear why the sympatric threespines consumed less of an abundant and favoured food source, clearly some other undetected factor must be influencing this prey choice.

Morphological divergence between sympatric and allopatric threespine fish (chapter 3) indicated that the sympatric threespine fish are better adapted to forage on zooplankton, and it was correctly predicted that there should be a higher proportion of these prey in the sympatric fish's diet. Analysis of the stomach contents identified significant differentiation between the diet of

the sympatric and allopatric stickleback populations. Most significantly the sympatric threespine continue to forage on zooplankton as they increase in size, whereas only the smallest allopatric fish consume zooplankton in any large quantity. At this stage it is unclear whether the high proportion of zooplankton in the sympatric fish's diet is an artefact of a greater persistence of these invertebrates in the sympatric habitat or if the fish are showing a preference for this prey type. Further examination of zooplankton abundance at allopatric and sympatric sites would give further insight.

As the stomach contents of sympatric fish from the same site were the most similar it is concluded that the strongest influence on prey choice are environmental conditions at individual sites. This conclusion is reinforced from evidence that stomach contents of fish in the same geographic region are more similar than they are to fish in the alternative location. There appears to be little competition between sympatric ninespine and threespine fish as analysis indicates a degree of similarity between their food preferences. However at the time of the year when the fish were caught food sources would be abundant and competition would be relaxed. It would be beneficial to examine stomach contents at a time of year when food sources are limited in the environment such as winter. Further analysis of fish in a limited environment would determine whether competition exists between the sympatric stickleback species.

I am not very sceptical... a good deal of scepticism in a scientific man is advisable to avoid much loss of time, but I have met not a few men, who... have often thus been deterred from experiments or observations which would have proven serviceable.

- Charles Darwin (1809-1882)

Chapter 6: Foraging behaviour

6.1: Introduction

In this final experimental chapter morphological divergence is examined within a behavioural context. One of the criteria of the character displacement hypothesis states that it should be evident that divergence between the focal trait(s) contributes to the fitness of the animal. It should therefore be shown that the trait(s) consistently improves the performance of the individual in the environment. In order to ascertain whether the morphological adaptations observed in the sympatric threespines' form have enhanced foraging ability this chapter observes representatives of all three stickleback populations in a series of different foraging situations.

Evidence from the analysis of the stomach contents (chapter 5) indicates a degree of segregation between the diets of the allopatric and sympatric threespines and the ninespine fish. As investigations found there to be little difference between sites (chapter 3) this segregation of invertebrate taxa included in the fish's diets is not likely to be an artefact of inherent differences between invertebrate distributions at allopatric and sympatric sites. Results from the morphological analysis indicate that the sympatric threespine fish should be most efficient foraging on small zooplankton in the open-water. If character displacement is to be implicated as the process responsible for causing morphological divergence between allopatric and sympatric threespine fish it is essential that the divergent morphology of the sympatric threespine be shown to enhance the fish's foraging performance on zooplanktivorous prey.

6.1.1: Foraging on different prey requires different foraging abilities.

Sticklebacks foraging upon different prey types will require different morphological adaptations. Zooplankton is generally found in the water column. As all the fish in the study are physically able to eat zooplankton, the fish's competitive ability is enhanced by the individual's ability to reach the prey first. By their nature zooplankton are erratic swimmers and require a predator with the ability to rapidly strike at them. Once the fish catches the prey negligible handling is needed to consume it. A study by Gill & Hart (1996) found that the chance of a stickleback winning a meal of *Daphnia* over that of a competing conspecific is significantly increased by the fish's ability to reach the prey first. A degree of familiarity with the intended prey species will also increase the fish's chances of successfully capturing itself a meal. *Daphnia* are generally found free-swimming in the water column rather than close to the benthic substrate. A fish that is most

familiar with foraging for zooplankton may be more likely to hang in the water column, rather than near the substrate whilst it attempts to find a potential meal. If the fish are actively hunting in the open water they will increase their chances of becoming the first to encounter zooplanktivorous prey.

Prey found in the benthic region is generally larger and less motile than free-swimming zooplankton. In the behavioural analysis, Chironomids are used as a typical benthic species. Sticklebacks have to forage in the sediment to find Chironomids which are typically found on or buried just below the sediment surface. Capturing such benthic prey will require a different foraging strategy to that required for foraging on zooplankton. As Chironomids are less mobile than the zooplankton the stickleback does not need the ability to rapidly lunge at this prey. However, as the Chironomid is larger than zooplankton it will require a greater degree of handling by the stickleback. After initial capture of the Chironomid the stickleback fish may need to orientate its prey in order to place it in the easiest position to consume (see Gill & Hart, 1996). Orientation of the Chironomid may involve the fish 'spitting' the prey out and re-capturing it when the prey is at a different angle. This behaviour may be repeated a number of times until the stickleback finds the Chironomid in the easiest position in which to swallow it. As the fish re-orientates its prey it faces the danger of inadvertently losing its prey entirely to another fish. A larger mouth, which increases gape width, will serve to reduce the amount of handling time required to consume a Chironomid.

It is proposed that when preying on zooplankton the morphology of the sympatric threespine stickleback should allow these fish to outperform their counterparts from the other populations. However, the sympatric fish's specialisation will come at a cost of a subsequent reduction of its abilities in the benthic and vegetated regions of the habitat. The smaller mouth of the sympatric fish will place a restriction on the largest size of prey they can handle. The allopatric and ninespine sticklebacks with their wider mouths will be able to take more varied invertebrate taxa from a larger size spectrum.

6.1.2: Influence of other fish in the environment

A degree of morphological homogeneity was observed between the sympatric threespine fish (chapter 3). It was speculated that this consistency in the sympatric threespine's form would benefit these fish if they formed shoals. Foraging groups of fish have been found to show a more homogenous phenotypic composition than those groups that were randomly formed by investigators (Lindström & Ranta, 1993). It is thought that fish living in open-waters are more

likely to shoal because of the increased dangers of being picked out by predators (safety in numbers) and the increased benefit of finding food (Krause *et. al.* 1998; Peuhkiri, 1998a; 1998b). Stickleback fish are certainly influenced by the behaviour of other conspecifics. When offered food patches of variable profitability, groups of six fish divided themselves up in proportion to the profitability of the food patches, thus following the ideal free distribution (Milinski, 1994). However, in a study by Gotceitas & Colgan (1991), when an isolated individual is allowed to observe conspecifics foraging in tanks on either side of it a different foraging strategy is revealed. When offered food patches of different profitability, individual fish indicate a preference for patches of food that are surrounded by the greatest number of conspecifics, regardless of the amount of food available at the patch. When more food is made available to the fish, phenotypic heterogeneity as well as group size has been observed to increase significantly (Lindström & Ranta, 1993). Relative hunger levels of the stickleback will influence shoaling behaviour (Barber *et. al.*, 1995). A fully satiated fish will spend a greater amount of time within one body length of the shoal, and spend less time out of visual contact with the shoal than it does after a period of food deprivation.

6.1.3: How are morphological adaptations expected to improve foraging ability of the sympatric threespine stickleback?

Some of the earlier studies on the sympatric threespine pairs in B.C., Canada investigated dietary composition between the limnetic and benthic fish (Bentzen & McPhail, 1984). These studies found that the limnetic and benthic fish avoided competing with each other by foraging in different environments. The limnetic fish are specialist in the open-water and their diets consists mainly of smaller zooplankton. Meanwhile the benthic fish forage on the larger invertebrate taxa found in the vegetated and benthic region of the lakes. Transplant experiments were performed whereby each fish was monitored as to its efficiency at foraging in its preferred environment and in the alternative environment of the corresponding fish. The results indicate that the fish performed best in its own preferred habitat and least well in the habitat of its counterpart.

The sympatric threespine is shown to be divergent from the allopatric fish (Chapter 3) in that they possess a small, conical shaped mouth and a deep mid-body depth which should enhance the sympatric threespines ability to rapidly strike at its prey (see Walker, 1997). It follows then that the divergent morphology of the sympatric threespine should allow them to be most efficient at foraging on zooplankton (see Walker, 1997). The sympatric threespine's greater ability to accelerate should improve their chances of capturing the evasive zooplanktivorous prey. As the sympatric fish draws nearer to its intended prey it opens its mouth to 'suck' the zooplankton out

of the water. The more conical shaped mouth increases this suction force and in doing so increases the chances of a successful capture (Walker, 1997).

6.1.4: Chapter Rationale

In order to assess trait utility sticklebacks from the three different populations were observed in a series of foraging trials whereby they were offered either limnetic *Daphnia* or benthic Chironomids, whilst either alone or in the presence of a con- or heterospecific. In the chapter I propose to test the overarching hypothesis that there are differences in the foraging abilities of the three stickleback populations. The following subsidiary null-hypotheses will be tested; that there is no difference in foraging behaviour when a stickleback forages on both limnetic or benthic prey; and that there is no difference in the stickleback's behaviour when foraging either alone or in the presence of a con- or heterospecific.

6.2: Methods

Each trial involved releasing a stickleback into the main observation tank whereupon the fish were observed foraging. The fish used in the observational studies came from sites 4 (allopatric) and 7 (sympatric). (Ultimately this was decided by the restrictions imposed by the recent foot and mouth crisis as neither of these sites is on agricultural land). The fish were collected in the autumn of 2001. Each population (allopatric, sympatric threespines and ninespines) was represented by 12 individuals of similar size (30-35mm). When the trial involved observations on the presence of a conspecific in the habitat 6 pairs of fish were used in a trial. The next day the same fish were used in the same type of trial but this time each paired with a different individual from that of the previous day. Therefore no pairs of fish were ever the same, which avoided the effects of pseudo-replication in the statistical analysis. Conditions were altered by providing different prey species of either *Daphnia* or Chironomid, and altering the foraging pairs so that fish were either on their own, with a conspecific or with a heterospecific. All trials were recorded on video-camera to be analysed later in the laboratory, and behavioural observations were made with the 'FIT-system' software on a hand held 'Palm-Pilot'. Data were downloaded onto PC for further analysis in EXCEL.

6.2.1: Observation conditions

The behavioural experiments were conducted in a 'tank arena' that had been constructed for previous behavioural analysis. A full description of the equipment is given in Hart & Ison (1991), but essentially the tank arena comprised of six separate tanks each with a trap door that lead into the main observational tank. In this arrangement, human handling of the fish is kept to a

minimum, which in turn should minimise their stress levels. The fish were introduced to the tank arena two weeks prior to the beginning of observations. This two week period allowed the fish to familiarise themselves with the tank environment, and to be trained to travel through a trap door into the main observation tank where they would be fed. The food provided was live *Daphnia* and Chironomid larvae. Both these prey would be used in the trial conditions so feeding them with the prey beforehand ensured that all fish were familiar with both types of prey. Prey was obtained from an aquarium supplier, meaning that that both the *Daphnia* and the Chironomids were of uniform size.

Previous experiments have shown that threespine fish recognise and are less aggressive to shoal mates. If familiar fish are separated for more than two weeks, they will forget their previous association and aggressive encounters will increase (Utne-Palm & Hart, 2000). In order to minimise aggression between the fish between trials the sticklebacks were housed in tanks with conspecifics from their original population. Satiation levels of the fish will affect their decision of whether to eat or not (Gill & Hart, 1994). To increase the chance of observing the fish foraging all sticklebacks were starved for at least 24 hours prior to the beginning of observations. The 24 hour starvation period ensures that the gut is fully evacuated and that all fish in the experiment had similar hunger levels. No fish was used in more than one trial in any day. After each sequence of trials had ended all the fish were fed to satiation with *Daphnia* and Chironomid.

6.2.2: Are foraging and handling times different between sticklebacks from different populations for different prey species?

In order to establish whether morphology might influence prey handling times, individual stickleback fish were offered either *Daphnia* or Chironomid. The presentation of the prey was controlled by the investigator. The time that prey (either *Daphnia* or Chironomid) was introduced to the observation tank was recorded on the Palm-Pilot along with the time the fish first responded to the food, the handling time and finally whether the food was eventually consumed or rejected by the stickleback. The conditions for each series of trials are summarised in table 32.

Foraging behaviour was broken down into a series of individual behavioural states as described in Gill & Hart (1996). The fish was considered to have 'located' the food if it orientated itself toward the food and then moved towards it. Sometimes the fish would stop swimming towards the prey and 'hang' in the water whilst still being fixated on the prey. This behaviour was

included within the category 'locating' behaviour. 'Handling' included any physical contact with the prey, which may have resulted in either the consumption or rejection of the prey item. When the stickleback is eating its prey there is noticeable exaggerated movement of the operculum and opening and closing of the mouth. When these actions had ceased the fish was considered to have finished eating. Handling behaviour might also include 'spitting' where the fish expels the prey from its mouth. The trial ended either when the stickleback had consumed the prey or after 5 minutes if no prey was taken.

Stickleback Population	Prey offered	
Solitary sympatric threespine	Trial 1: Single daphnia	Trial 4: Single Chironomid
Solitary allopatric threespine	Trial 2: Single daphnia	Trial 5: Single Chironomid
Solitary ninespine	Trial 3: Single daphnia	Trial 6: Single Chironomid

Table 32: Summary of trial conditions for observing pursuit and handling times between solitary fish.

6.2.3: Is foraging behaviour altered in the presence of a conspecific?

In order to observe potential influence of conspecific foraging behaviour on a stickleback fish the basic trial format was the same as previously described where the different populations were observed foraging on benthic and limnetic prey, except this time pairs of conspecific fish were placed in the observational tank. A summary of trial conditions is provided in table 33. This trial monitored the effect of another conspecific being present in the environment. As such the same behaviours as previously described (section 6.2.4) were observed, though in addition the time between one fish foraging and the second one joining it was also noted.

Stickleback Population	Prey offered	
Sympatric threespine pair	Trial 7: Single daphnia	Trial 10: Single Chironomid
Allopatric threespine pair	Trial 8: Single daphnia	Trial 11: Single Chironomid
Ninespine pair	Trial 9: Single daphnia	Trial 12: Single Chironomid

Table 33: Summary of trial conditions for observing pursuit and handling times between conspecific pairs of fish.

6.2.4: Which fish will win in a heterospecific food contest?

The divergent threespine's form dictates that it should be better at foraging on small evasive limnetic prey, though this increased efficiency in the limnetic zone should theoretically come with a subsequent loss of ability when foraging on larger benthic invertebrates. In order to test this assumption the sympatric fish were placed in a series of head-to-head trials with heterospecific fish (either an allopatric threespine or a ninespine) during which the fish were offered either a *Daphnia* or a Chironomid prey. The fish that consumed the prey was declared the victor. To determine whether the ninespine fish face stronger competition with the non-divergent form of the allopatric fish, allopatric threespine fish were placed in direct competition

with the ninespine fish to see which would win either the limnetic or benthic prey. A summary of trial situations is provided in table 34.

Population	Sympatric threespine	Allopatric threespine	Ninespine
Sympatric threespine		Chironomid	Chironomid
Allopatric threespine	<i>Daphnia</i>		Chironomid
Ninespine	<i>Daphnia</i>	<i>Daphnia</i>	

Table 34: Summary of who competes with whom in a head-on contest for either *Daphnia* or Chironomid prey.

6.3: Results

The objective of this chapter is to assess whether the morphological adaptations observed in the sympatric threespine fish serve to increase their foraging efficiency in the limnetic regions of the habitat. The primary null-hypothesis to be tested is that there will be no difference in the foraging ability of sticklebacks from the three populations, whether they are foraging on limnetic or benthic prey, or when in direct competition with either a conspecific or a heterospecific.

6.3.1: Are foraging and handling times different between sticklebacks from different populations for different prey species?

The location and handling times of individual fish foraging on *Daphnia* are summarised in table 35, none of the individual ninespines actively foraged for *Daphnia*. A t-test indicates that the times for allopatric and sympatric fish to detect a limnetic prey was non-significant ($df=13$; $t=0.240$; $P=0.823$). A second t-test analysed pursuit time and reveals a non-significant difference between the two threespine populations ($df=12$; $t=-0.574$; $P=0.578$). These results indicate that there is little difference between individual sympatric and allopatric threespines ability to pursue and handle *Daphnia*.

Population	Time to locate <i>Daphnia</i> (s)	SE	Time to pursue <i>Daphnia</i> (s)	SE
Sympatric threespine	20.6	± 14.95	7.0	± 1.5
Allopatric threespine	21.45	± 5.089	11.09	± 6.919
Ninespine	n/a		n/a	

Table 35: Location and pursuit times of individual stickleback fish foraging on *Daphnia*.

Individual fish all showed some reluctance to forage on their own when they were offered Chironomids. Once again the individual ninespine fish did not forage on the prey offered, 60% of the sympatric fish chose to ignore the prey and a third of the allopatric fish (38%) did not eat. The results of the fish that did choose to eat are summarised in table 36. T-test analysis between the allopatric and the sympatric threespine fish showed a non-significant time difference between the two fish's ability to spot a Chironomid prey ($df=41$; $t=1.407$; $P=0.256$), a non-significant time difference for pursuing the prey ($df=6$; $t=1.329$; $P=0.310$) and a non-significant time difference for finally handling the prey ($df=6$; $t=1.329$; $P=0.310$). Numbers of individual fish

included in the analysis of handling behaviour were much reduced because of the large numbers of individuals that abstained from eating.

Population	Time to locate Chironomid (s)	SE	Time to pursue Chironomid (s)	SE	Time to handle Chironomid	SE
Sympatric threespine	50.6	±23.365	8.67	±3.215	12.0	±4.77
Allopatric threespine	80.6	±26.758	10.6	±7.871	4.6	±0.814
Ninespine	n/a		n/a		n/a	

Table 36: Location, pursuit and handling times of individual stickleback fish foraging on Chironomid

6.3.2: Is foraging behaviour altered in the presence of a conspecific?

A summary of the time taken for pairs of fish to locate and pursue *Daphnia* is provided in table 37. In this trial the ninespine fish foraged on *Daphnia*. The time taken for pairs of allopatric and sympatric threespine fish to locate *Daphnia* is not significantly different from the time taken by solitary fish from either threespine population (times of individual fish compared with times of individuals when paired, allopatric threespine fish: $df=31$; $t=0.97$; $P=0.924$; times of individual fish compared with times of individuals when paired, sympatric threespine fish $df=7$; $t=0.24$; $P=0.981$). A one-way ANOVA showed there to be no significant difference between how long it took the fish from the different stickleback populations to spot a potential prey after it had been introduced ($df=2$, 41; $F=1.407$; $P=0.256$). The sympatric fish were the fastest fish to react to a sighting of the limnetic prey and only took 3.25s (± 2.63) to reach and consume the *Daphnia*. The ninespine fish took by far the longest time to feed on the *Daphnia* after it has been spotted and were twice as slow as the allopatric fish and 9 times slower than the sympatric threespines. 1-way ANOVA reveals that these differences in time from the fish first spotting the *Daphnia* to finally consuming it are significantly different ($df=2$, 41; $F=4.306$; $P=0.020$). *Post hoc* analysis (Games Howell) reveals that the sympatric fish are significantly faster at pursuing and consuming *Daphnia* than both the allopatric ($P=0.049$) and the ninespine fish ($P=0.004$). These results reveal that although there is little difference in the time taken to spot a limnetic prey between the three stickleback populations there is a significant likelihood that the sympatric threespine fish will be the fastest to pursue and consume this prey. The average time taken for either solitary or pairs of conspecific fish to locate and pursue *Daphnia* are summarised in figures 18 and 19. Solitary ninespine fish are omitted because no data was recorded for these specific foraging behaviours.

Population	Time to locate <i>Daphnia</i> (s)	SE	Time to pursue <i>Daphnia</i> (s)	SE
Sympatric threespine	21.0	±10.53	3.25	±1.315
Allopatric threespine	20.82	±4.147	10.45	±2.539
Ninespine	34.47	±7.412	26.32	±6.05

Table 37: Location and pursuit times of pairs of stickleback fish foraging on *Daphnia*.

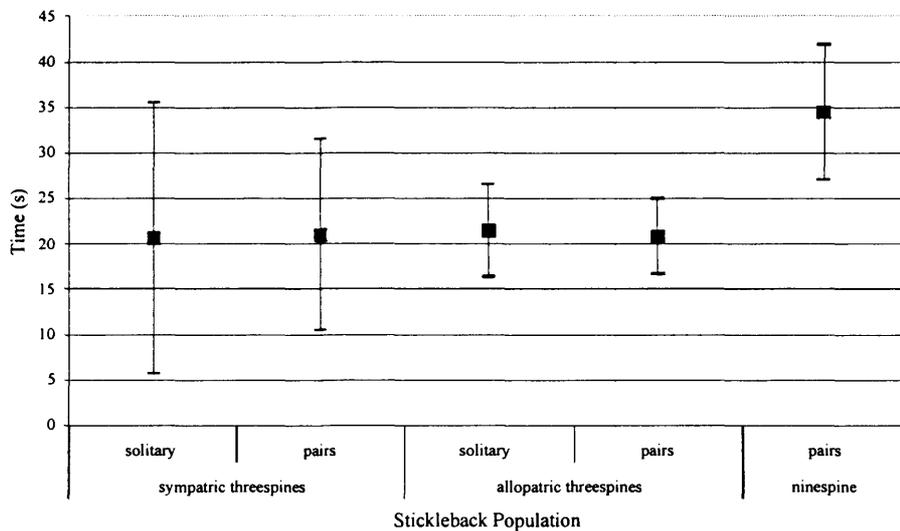


Figure 18: Average time plus and minus SD taken to locate a *Daphnia* between solitary fish and pairs of conspecifics from all three stickleback populations

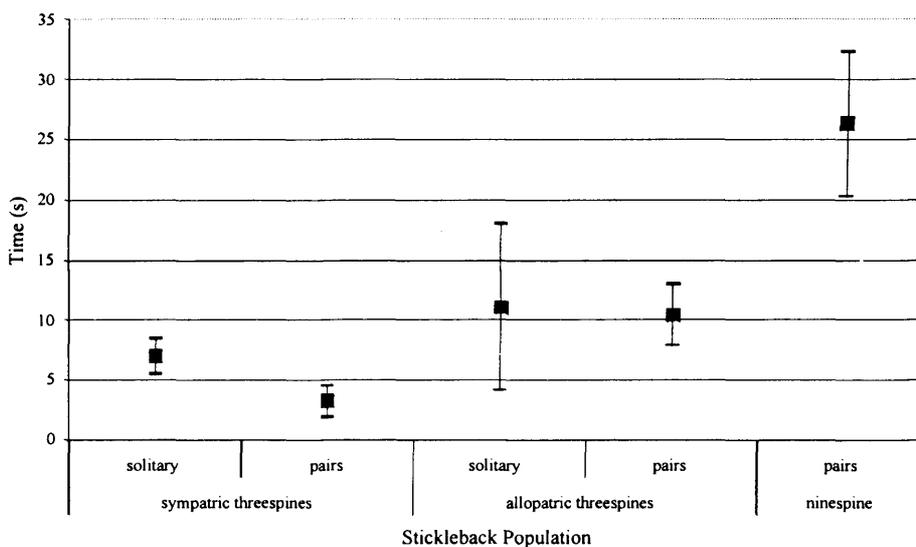


Figure 19: Average time plus and minus SD taken to pursue *Daphnia* between solitary fish and pairs of conspecifics from all three stickleback populations

When conspecific pairs of sticklebacks were offered chironomids all populations of fish ate the prey. Results are summarised in table 38. The sympatric threespine fish were the fastest fish to initially detect the prey, and pairs of fish were significantly faster to locate their prey than solitary sympatric threespine fish ($df=12$; $t=-2.602$; $P=0.026$). There was no significant difference between the time taken for solitary allopatric threespine fish and pairs of allopatric fish to locate the Chironomid prey ($df=16$; $t=-1.647$; $P=0.159$). 1-way ANOVA shows no significant differences between the abilities of fish in the three different populations to spot chironomids when with a conspecific ($df=2, 33$; $F=1.545$; $P=0.228$).

After having located the Chironomid the sympatric threespine fish were the slowest to pursue and capture their prey. 1-way ANOVA analysis between the pursuit rates of the three stickleback populations reveals a non-significant result, (df=2, 30; F=2.094; P=0.141). Handling times between the allopatric and sympatric threespines were comparable, and the ninespines were the slowest. 1-way ANOVA of results for handling times reveals a non-significant difference, (df=2, 30; F=2.092; P=0.141). The average time taken for either solitary or pairs of conspecific fish to locate, pursue and handle Chironomid are summarised in figures 20 to 22. Again solitary ninespine fish are omitted because no data was recorded for these specific foraging behaviours.

Population	Time to locate Chironomid (s)	SE	Time to pursue Chironomid (s)	SE	Time to handle Chironomid	SE
Sympatric threespine	11.75	±3.495	20.5	±16.565	6.75	±1.7
Allopatric threespine	33.54	±10.05	4.86	±0.824	6.15	±0.688
Ninespine	54.0	±11.398	7.82	±2.229	9.53	±1.484

Table 38: Location and pursuit times of pairs of stickleback fish foraging on Chironomid

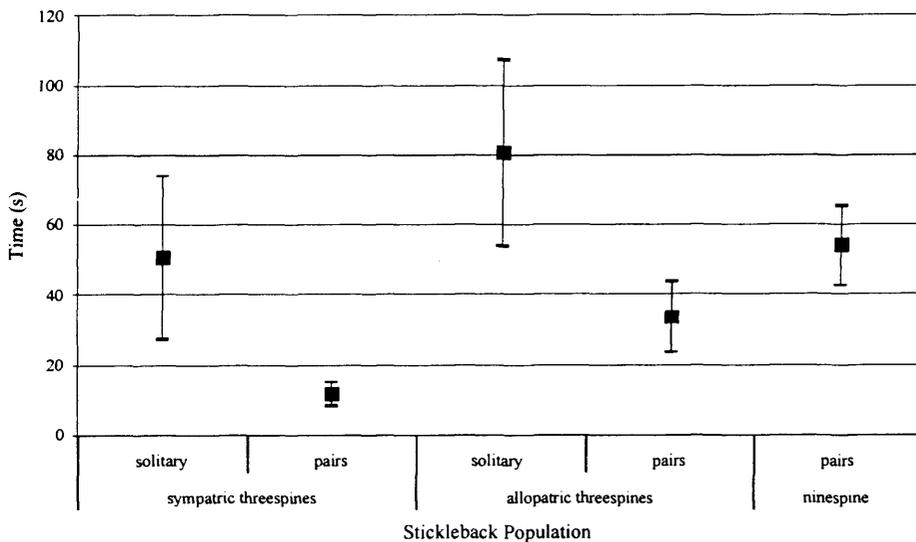


Figure 20: Average time plus and minus SD taken to locate Chironomid between solitary fish and pairs of conspecifics from all three stickleback populations

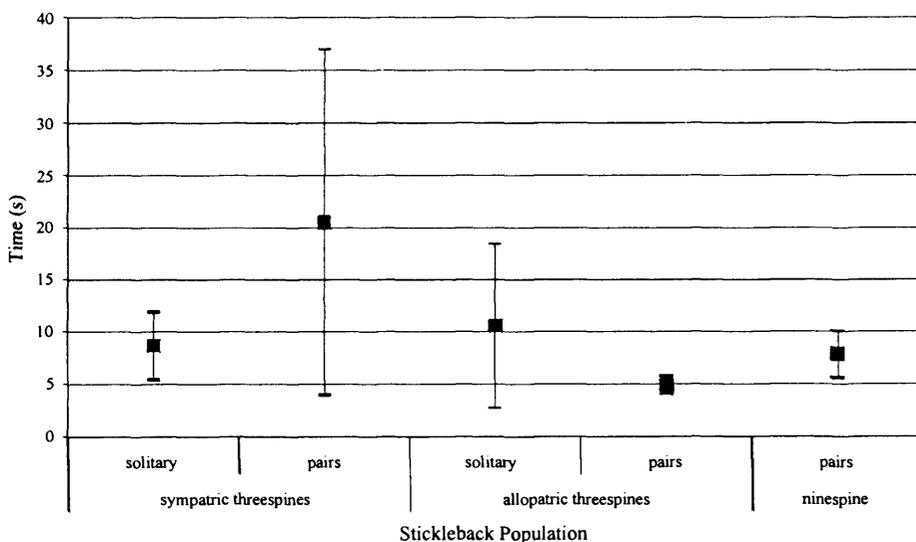


Figure 21: Average time taken plus and minus SD to pursue Chironomid between solitary fish and pairs of conspecifics from all three stickleback populations

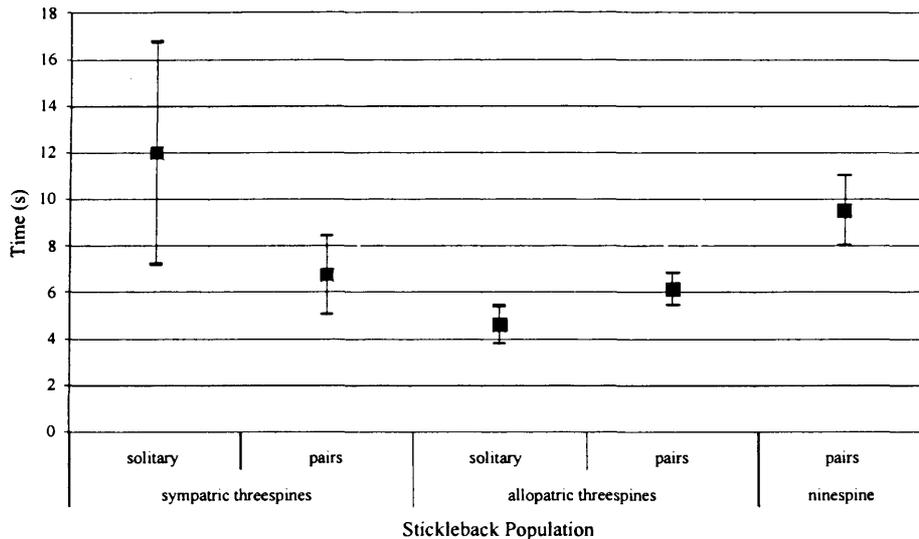


Figure 22: Average time plus and minus SD taken to handle Chironomid between solitary fish and pairs of conspecifics from all three stickleback populations

In order to assess the influence of the presence of a conspecific on the foraging behaviour of an individual the time between one fish beginning to forage and then the second reacting and joining in with foraging was noted. It took an average 10.89s (± 10.67) for an allopatric fish to react to a conspecific that had just begun foraging; the sympatric fish took a comparable 10s (± 11.36) on average to join its shoal mate. The ninespine fish reacted far more slowly to a conspecific foraging in its presence taking on average 37.74s (± 50.88) to join a conspecific. 1-way ANOVA indicates that this result is not significant ($df=1, 2; F=2.395; P=0.105$).

6.3.3: Which will win in a heterospecific food contest?

The results of the assessment of the competitive ability of a stickleback in the presence of a heterospecific competitor are summarised in table 39. In a direct contest between an allopatric threespine and a ninespine stickleback, competing for *Daphnia*, the allopatric fish won 45% of the time, chi² analysis (on the raw data) indicated that this was non-significant ($df=1; \chi^2=0.091; P=0.763$). When the same pairs of fish compete for Chironomid prey the allopatric fish was the victor 43% of the time, this result is also non-significant ($df=1; \chi^2=0.143; P=0.705$). These results indicate when competing for either *Daphnia* or Chironomid prey neither the allopatric threespine or the ninespine fish has a competitive advantage.

When allopatric threespine fish are placed in a competitive situation with a sympatric threespine fish for *Daphnia* the allopatric threespines' chances of victory are much reduced in comparison to their odds against the ninespine fish in the same situation. The allopatric fish only succeeded in reaching the *Daphnia* before the sympatric threespine fish 14% of the time, this result is almost significant ($df=1; \chi^2=3.571; P=0.059$). However the odds for the allopatric fish are reversed when competing for Chironomid. In a contest between allopatric threespines and

sympatric threespines competing for chironomids the allopatric fish won 86% of the bouts, this result is almost significant ($df=1$; $\chi^2=3.571$; $P=0.059$). Although results are not significant they show some indication that in a competitive situation the allopatric fish fare better when the prey offered is a benthic Chironomid, though the situation is reversed when allopatric and sympatric threespine fish are competing for limnetic *Daphnia* where the sympatric threespine will most likely be the victor. These observations warrant further analysis.

When Chironomid prey was offered to ninespines and sympatric threespine fish the ninespine fish won the prey 67% of the time. This result was not significant ($df=1$; $\chi^2= 1.286$; $P=0.257$). However when the same fish are competing for *Daphnia* the ninespine only won 21% of the bouts, this result was significant ($df=1$; $\chi^2=4.571$; $P=0.033$). These results indicate that there is no forgone conclusion as to which fish of either sympatric threespine or ninespine will win a Chironomid prey when competing against each other, but that the sympatric threespine fish has a significantly better chance of successfully consuming *Daphnia* when in competition with a ninespine.

Population	Competitor	Competing for <i>Daphnia</i>			Competing for Chironomid		
		χ^2	P	Victor	χ^2	P	Victor
Sympatric threespine	Allopatric threespine	3.571	0.059	Sympatric threespine	3.571	0.059	Sympatric threespine
	Ninespine	4.571	0.033	Sympatric threespine	1.286	0.257	neither
Allopatric threespine	Sympatric threespine	3.571	0.059	Sympatric threespine	3.571	0.059	Sympatric threespine
	Ninespine	0.091	0.736	neither	0.143	0.705	neither
Ninespine	Sympatric threespine	4.571	0.033	Sympatric threespine	1.286	0.257	neither
	Allopatric threespine	0.091	0.736	neither	0.143	0.705	neither

Table 39: Results of χ^2 comparing pairs of heterospecific fish directly competing for prey. In all cases $df=1$. Significant results in bold type.

The majority of the results for all the analyses proved non-significant by the 5% chance margin. The fact that some of the results are *almost* significant still gives some indication of behavioural foraging differences between the stickleback populations, though few concrete conclusions can be made it certainly highlights potential differences in some of the foraging behaviours and most definitely warrants further behavioural analysis in this area with a greater sample size of fish.

6.4: Conclusions

The rationale for this chapter was to assess whether the morphological divergence observed amongst the sympatric threespine population has modified foraging ability. The experiments in

this chapter aimed to test whether morphological adaptation amongst divergent fish has contributed to the fitness of the animal in its respective environment as stated in the 'character displacement' hypothesis. It was proposed that the divergent morphology of the sympatric threespine fish should allow these fish to become more effective zooplanktivores but that there would be a subsequent reduction in efficiency when these same fish are made to forage on benthic prey.

6.4.1: Are there differences in foraging ability?

Whether individually, or together with a conspecific, both the allopatric and sympatric threespines spent a similar amount of time locating *Daphnia*. Solitary allopatric and sympatric threespines also took a similar amount of time to pursue and capture *Daphnia*. When the allopatric fish were paired with a conspecific, pursuit and handling times for *Daphnia* were similar to those of individual allopatric fish. This indicated that the presence of a conspecific fish in the allopatric's habitat has little effect on either pursuit or handling behaviours. However, when the sympatric threespines were placed with a conspecific their pursuit time after *Daphnia* was significantly increased. Solitary ninespine fish were reluctant to forage on *Daphnia* and although more inclined to forage when in the presence of a conspecific their reaction and pursuit times were significantly slower than either of the threespine fish; in particular the pursuit time of the sympatric threespines. In the presence of a conspecific, individual sympatric threespines significantly increase pursuit times. This observation is reinforced from the results of the direct contests between the allopatric and sympatric threespine fish when competing for *Daphnia*, where the sympatric fish had a significantly higher chance of winning the prey over the allopatric fish (section 6.3.3). The original analysis of the sympatric threespines morphology (chapter 3) predicted that these fish should be able to accelerate more efficiently than either of the other sticklebacks and increase the fish's ability to rapidly lunge at its prey. The results of the behavioural analysis confirm that of the fish tested the sympatric threespine are the most effective zooplanktivores. The sympatric fish's enhanced ability to catch limnetic prey has come at an apparent reduction of ability when it comes to capturing Chironomids. The sympatric threespine fish are as quick to spot a Chironomid as their allopatric counterparts but are slower at handling the benthic prey. Morphological analysis indicated that the mouth of the sympatric threespine is smaller than the mouths of either the allopatric or the ninespine fish, and it was correctly predicted that a smaller gape width would inhibit handling ability in the sympatric threespine fish. Hunt and pursuit times for Chironomids were comparable between the three stickleback populations but the sympatric threespines took the longest time to handle this prey. These results were shown to be not significant but were close enough to the significance level as

to warrant further investigation with a larger sample size. As they stand behavioural observations indicate that the sympatric threespine is less efficient at handling larger Chironomid prey than either of its counterparts from other stickleback populations. It was suggested that the smaller mouth of the sympatric threespines would make manipulating larger prey more difficult, which would in turn increase handling time. Presumably the smaller gape width of the sympatric threespine fish whilst not precluding larger Chironomid prey from the diet has made the fish less effective at handling the larger prey.

6.4.2: Does being in the presence of another fish influence foraging behaviour?

When foraging on *Daphnia*, the presence of a conspecific did not affect the time taken for an individual fish to locate its prey for either population of the threespine fish in any significant way. Neither did being in the presence of a competitor affect the pursuit time of the allopatric threespine. However, the sympatric threespines did show a significant reaction to a competitor in the environment. The time taken for a sympatric threespine to pursue its prey was significantly increased when it was in the presence of another sympatric threespine. The strongest impact a conspecific had was on the foraging behaviour of the ninespine fish. Solitary ninespines refused to eat either of the prey offered. The solitary ninespine fish spent most of the time either motionless in a corner of the tank or attempting to swim back through the trapdoor to their own holding tank. The ninespine fish were more likely to forage on the prey offered to them when in the presence of a conspecific, though the ninespines took more time to initially locate the prey and then to consume it than fish from either of the threespine populations. It is possible that conditions in the observation tank were more inhospitable to the ninespine fish than they were for either of the threespines. It is documented that ninespine fish prefer to spend the majority of their time in vegetation (see Wootton, 1976; Copp, 1992; 1998). A more recent study observed that whilst ninespine fish will forage for food both amongst vegetation and in open-water, they prefer to be in or near vegetation more than do the threespine sticklebacks (Hart, 2003). As the observation tank contained no vegetation it is likely that the surroundings did not meet the requirements of the conditions ninespines prefer for foraging, which may explain their reluctance to forage. It would be of benefit to the study to repeat the behavioural trials but in this case with vegetation included in order to make the tank environment more appropriate for ninespine sticklebacks.

6.4.3: Summary

As predicted from morphological analysis the sympatric threespine fish are faster than the allopatric fish when it comes to pursuing and capturing zooplankton in the presence of a

competitor. As was also expected, the sympatric threespine's enhanced ability at pursuing limnetic prey has come at the cost of a loss of efficiency when foraging on larger benthic invertebrates. The sympatric threespines took three times longer to handle the Chironomid prey compared to the allopatric fish, which may be a result of the sympatric threespine's smaller gape width. The allopatric threespine and ninespine's jaw and body depth morphologies were found to be more comparable to each other than either was to the sympatric threespine, and behavioural analysis indicates that if these fish were in the same habitat competition between them could be high. Either allopatric threespine or ninespine fish had an equal chance of winning either benthic or limnetic prey in a direct contest. It is supposed that the divergent form of the sympatric threespine fish has reduced competition between the ninespine fish, particularly so in the limnetic region where the faster sympatric threespine will be the first to capture its intended zooplanktivorous prey. Individual sympatric threespine fish were more reluctant to pursue and capture a Chironomid prey. It is suggested that a longer handling time will occupy the fish's attention and could leave the fish more vulnerable to predation. This increased vulnerability of the sympatric threespines might be a reason why a number of the solitary fish chose to ignore this prey. The results from the behavioural analysis concur with the presupposition that the divergent morphology of the sympatric threespine has increased its efficiency for foraging in the limnetic zone but in doing so has incurred a cost in the form of a reduction in the fish's ability to forage on larger invertebrate prey.

Science is always wrong. It never solves a problem without creating ten more.

— George Bernard Shaw (1856-1950)

Chapter 7: Conclusions

7.1: Introduction

The work presented in this thesis has begun to investigate the ecological processes permitting the co-existence of threespine and ninespine sticklebacks. These investigations have produced some exciting results, which lead to some interesting hypotheses explaining this co-existence. The study found some evidence of morphological divergence between allopatric and sympatric threespine sticklebacks and investigated whether the cause of this divergence might be a result of competition driven character displacement in threespines sticklebacks when living together with ninespine sticklebacks.

7.2: Why the stickleback is a good model for investigating morphological adaptation.

In Britain the threespine and the ninespine sticklebacks are often found living side-by-side in freshwater streams. Threespine and ninespine sticklebacks are closely related, share similar niches and are often found living in sympatry, and as such they are ideal candidates for investigating competitive interactions. The phenomenon of two such ecologically similar species being able to co-exist without the high levels of competition between them removing one or the other from the habitat begs us to question exactly what ecological process is allowing this situation to occur.

The threespine stickleback species complex (*Gasterosteus aculeatus*) has emerged as an excellent model organism for studying evolutionary and ecological processes. Reviews of recent stickleback literature reveal a number of morphologically divergent threespines (see Bell 1994). The divergent sticklebacks are characterised according to the habitat they are found in such as stream type, lacustrine, riverine and marine. In each of these cases ecological conditions in each environment has determined the divergent morphology.

One of the more exciting developments is the discovery of populations of threespine sticklebacks, which are reproductively isolated, co-existing trophic morphs in British Columbia, Canada (Thomson *et. al.* 1997; Taylor & McPhail, 2000; Rundle *et. al.* 2000; Reusch *et. al.* 2001; Hatfield & Ptolemy, 2001). The limnetic and benthic morphs (species?) are believed to have diverged as a result of character displacement and remain one of the most widely cited works on the ecological controls of speciation.

The basic premise of the character displacement theory (Brown & Wilson, 1956) is that divergence occurs between two closely related species whose ecological niches overlap; interspecific competition will cause these species to become more phenotypically different when they are found in sympatry. Over the years this hypothesis has met with much scepticism from ecologists since very few examples are apparent in nature (see Schluter, 2000a). The reason for this may be twofold. Either character displacement does not commonly occur in nature, or, if it does, it is difficult to demonstrate convincingly.

7.3: The role of competition in adaptation

Interspecific competition is deemed to be one of the major ecological processes driving speciation and the origin of biodiversity. The competitive exclusion principle states that complete competitors cannot co-exist (Gause, 1935) and this places a limit on how ecologically similar the niches of coexisting species can be before one or the other of them will be excluded from the habitat. Whilst this concept is intuitively clear it does not account for the many examples of ecologically similar species that co-exist in natural environments. In light of the situation found in natural communities a more appropriate question to ask is ‘how different do two species have to be in order to co-exist in the same environment?’ (Mac Arthur, 1972). Animals most frequently interact over shared food resources, and are deemed competitors when this interaction causes a reduction in foraging success to one or the other; thus the success of a given individual will depend on its ability to compete. Species must increase their competitive ability by adapting and partitioning resources in order to co-exist.

Our problem is that competition is a dynamic process; we are only ever able to observe the results of species interactions and infer competition retrospectively. In the past many studies have taken this route but descriptive studies of species already co-existing are not always useful for understanding the role of competition in natural populations. The work in this study has begun to deduce how the two stickleback species can co-exist but has not been able to confidently determine the ecological processes that facilitated this co-existence. Two species differ as a by-product of speciation. Observing differences between two species already co-existing does not necessarily mean competition has caused these differences. The threespine sticklebacks in this study were found in both allopatric and sympatric populations. As it is not possible to turn back the evolutionary clock we can use such occurrences to our advantage. The allopatric threespine population serves as a useful control with which to compare the sympatric threespine population.

7.4: What might morphological divergence be telling us?

Results from morphological analysis indicate that the sympatric fish are in general characterised by a deeper mid-body depth which gives these fish a 'diamond' shaped profile. They also have a deeper caudal peduncle and longer snouts with smaller mouths, creating a long narrow cone shape and more gill rakers. In comparison the allopatric fish tend to have a more constant body depth along the body, a narrow caudal peduncle and short snouts with wide mouths and fewer gill rakers. Like the allopatric threespines the ninespine fish have a constant body-depth but possess the shortest snouts and the widest and largest mouths of all the populations tested.

If character displacement is the cause of the morphological changes observed then any phenotypic change must confer a fitness advantage to the species. If sympatric populations of fish are different from allopatric populations, the differences should mean that the fish are better able to compete with ninespines for resources. The direction morphological divergence took in sympatric threespines was not predictable from previous cases of divergence as the sympatric threespines did not show the predicted response of adopting a limnetic morphology. A series of hypotheses are offered which speculate on how morphological adaptation might have increased the sympatric threespines competitive ability.

The first of these hypotheses is that the deep mid-body depth and enlarged caudal peduncle of the sympatric threespines will improve c-start response and manoeuvrability (Eaton et al. 1977). A second hypothesis observes that the sympatric threespines jaw morphology and higher gill raker count should dictate that these fish are more effective at capturing small prey items. The most effective method of capturing zooplankton whose locomotion is generally erratic and directionless is through the 'ram' method (Norton & Brainerd, 1993). The ram method requires the ability to rapidly accelerate and 'strike' at the prey and this behaviour should be enhanced by the sympatric fish's deeper caudal region and greater ability to turn around its vertical axis. The narrower cone shape of the mouth should increase the fish's ability to 'suck' the small prey item from the water. There is some evidence that the sympatric fish are more zooplanktivorous than allopatric and ninespine sticklebacks as the results of stomach contents analysis indicated that the sympatric fish feed on a higher proportion of zooplankton than either of their counterparts, and that this preference is maintained in the adult fish. Behavioural analysis also substantiates the claim that the sympatric threespine is an effective zooplanktivore as these fish, when in the presence of a competitor, were significantly faster at pursuing *Daphnia* than either the allopatric or ninespine fish. At this stage it is unclear what ecological process has raised the sympatric fish's competitive ability for pursuing zooplankton. Further detailed analysis of zooplankton in

the allopatric and sympatric habitat should indicate whether this food source is abundant in one or the other. If the sympatric habitat contains disproportionate levels of zooplankton it is possible that morphological divergence in the sympatric threespine is a reaction to these conditions, rather than a consequence of competition with the ninespine stickleback.

The third hypothesis states that the constant depth along the allopatric threespines body will incur less frictional drag thereby enhancing constant straight line swimming. These fish will be better fitted to using faster flowing reaches of the stream. This together with their relatively wider mouths will allow them to exploit bottom invertebrates in the middle reaches of the stream. In comparison to the sympatric fish the mouth of the allopatric fish is wider, and this is the basis for the fourth hypothesis stating that the allopatric fish are able to handle larger prey more efficiently than their sympatric counterparts. Larger prey items are more typically found in vegetation and either partially or completely buried in the substrate, and will require a different foraging strategy than that necessary for foraging on planktivores. Unlike the sympatric threespines the allopatric fish are less restricted about the size of prey they eat, and as such can consume a wider variety of prey types. This claim is reinforced by the significantly higher incidence of Chironomid prey and other benthic invertebrates found in the allopatric fish's stomach contents. The allopatric fish were also significantly quicker at handling larger benthic prey than the sympatric threespines, but were more equally matched in their handling ability to that of the ninespine fish.

Previous behavioural observations have established the differential use of habitat space by the two species of stickleback (Hart, 2003). The ninespine fish positioned themselves in the upper part of the water column amongst vegetation, whilst the threespine fish stayed closer to the substrate but in the open water. Threespine fish only entered the vegetation when it contained food, whereas the ninespine fish used vegetation even when food was absent. The fish used in this particular behavioural analysis were from allopatric populations so had had no previous contact with the heterospecific stickleback. In the stream habitats studied in this work the open water habitat is reduced compared to lake or large river habitats. As a result the threespines may have to forage in areas also used by ninespines which may intensify competition between the two species. The findings of Hart (2003) coupled with evidence of adaptive divergence in this study predict that differential habitat use could limit competition amongst populations of sympatric nine and threespine fish.

Further observations reveal that allopatric threespines from different geographical locations are readily distinguished from each other. Whilst all the populations have essentially the same shape, fish in North Yorkshire have a relatively deeper body than do those fish in Leicestershire. The distinction is less obvious between geographically separated ninespine populations living with threespines, this is particularly apparent in the spring sampled fish. The sympatric threespine fish sampled in the spring form a more homogenous group indicating that these populations are more similar in shape to each other regardless of location, than they are to the allopatric threespines. It is suggested that divergence is stronger in these populations as these fish would have overwintered in the stream and endured harsh conditions in a limited environment, which presumably would have raised competition levels between the sympatric fish. These results indicate that whilst geographic location does have an influence on the morphology of threespine sticklebacks, its effects may be over-ridden possibly by the presence of a competitor in the form of the ninespine stickleback.

The results from morphological analysis indicate that the sympatric fish have undergone a transition from a generalised form to one that exhibits a more stringently determined morphology; what is not clear from this study is what the cause of this divergence might be. Investigations of allopatric threespines indicates that the geographic location of the population will have an effect on the final morphology of the fish, however the homogeneity of the shape amongst sympatric fish populations suggests that the effect of geographic location can be over-ridden by internal factors. The fact that threespine sticklebacks are found in such a diversity of aquatic environments might be symptomatic of an innate plasticity, and that what we are observing in the sympatric threespine fish is in fact an expression of this plastic reaction to its environment. It is possible that the plastic nature of the threespine's morphology is flexible enough that each generation of fish will respond to competitive conditions it experiences during development thus eliciting a temporary plastic response to conditions in the environment. A second possibility is that morphological traits are heritable over generations, therefore only a threespine morphology determined by the genotype will be able to co-exist with the ninespine fish. Several studies have shown (Houston, 2001; Reusch *et. al.*, 2001; Taylor & McPhail, 1999; Thompson *et. al.*, 1997; Taylor *et. al.*, 1997) that there is a high degree of trait heritability in threespines.

An increasing number of studies report cases of species pairs formed through trophic divergence amongst northern temperate fish species (Taylor, 1999). In each of these cases divergence has followed a predictable path and differentiation has resulted in species pairs of benthic and limnetic/pelagic morphs. In general the benthic morph in the pair will have a deeper body and

wider mouth specifically adapted for foraging in the benthic region, and, conversely the limnetic/pelagic morph of the species pair will have a narrower, more streamlined body and smaller mouth specifically adapted for foraging on planktivorous prey. It was originally proposed that the dichotomy between the Canadian limnetic and benthic threespines might be mirrored in the dichotomy between the sympatric threespine and ninespine fish and the sympatric threespine fish would present similar morphological adaptation as that seen in the limnetic eco-morph. However, the patterns of morphological divergence amongst sympatric threespines in this study have not followed this route; they have developed a deeper body and deeper caudal peduncle but also have a narrower mouth than their allopatric counterparts. Most of the cases of character divergence described in the literature are for fish in lakes and it would appear that life in a small stream has imposed different selective pressures on trophic morphology leading to the unique combinations shown in this study.

Whether morphological divergence between the allopatric and sympatric threespine sticklebacks is caused by competition driven character displacement requires further investigation. Nevertheless the results from this study have provided a useful insight into adaptation in species. Despite the age of the hypothesis (Brown & Wilson, 1956) evidence of character displacement still remains elusive and is restricted to only a handful of examples in the literature, such as the Canadian threespine sticklebacks and most commonly birds on island archipelagos (see Grant, 2001 for an overview). A number of studies have uncovered evidence of reproductive character displacement (see Ptacek, 2000 for overview) but reproductive isolation is considered a consequence of previous ecological character displacement. What we still do not fully appreciate is the effect environmental conditions have on initiating adaptation, that is our “substantial challenge”, and “until that challenge is met we cannot claim that the problem of explaining adaptive radiation is solved” (Grant, 2001).

7.5: In reflection

The work in this thesis examined how two species of ecologically similar sticklebacks are able to co-exist in sympatry. This study has been important because to date there have been few studies that have examined the implications of inter-specific competition from a member of the same genus. Investigations have uncovered evidence of adaptive divergence between allopatric and sympatric threespine fish. Morphological adaptation in the sympatric threespine fish dictates that these fish should be effective zooplanktivores. Diet analysis and behavioural observations support this proposal and found that the competitive ability of the sympatric threespines is increased when these fish forage on zooplankton. As the ninespine fish indicate a preference for

benthic invertebrates morphological adaptation in the sympatric threespine fish may serve to reduce competition between them and the sympatric ninespine fish. At this stage it is unclear whether environmental conditions or competition based character displacement might be the ecological mechanism behind the observed divergence, but the nature of the divergence which suggests that the sympatric fish are more effective zooplanktivores should keep character displacement as a plausible mechanism. The findings in this thesis should certainly ignite interest for further investigation.

The opening statement of the thesis asked ‘why are there so many species?’ a question that has been deliberated by evolutionary scientists for the past 150 years. There are still more questions than answers to this query, but I believe the work contained in this thesis has contributed in part an answer.

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Then there's my ever wonderful family, ma, pa, wee sis Donna and her Nigel who have put up with me and put me up throughout. Not only have they been on hand with bucketfuls of love and security, (both financial and emotional) they have been known to come and help me with the field work; and yes true to form Nigel *did* fall in the water. So, huge thanks to them, if it hadn't been for their constant support and in some cases persistent nagging (mother and wee sis...) I would never have got this far. Drinks all round for them....erm, pops....you couldn't loan us a tenner?

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If there's anyone who I have forgotten then I truly am sorry, but yer more than welcome to join us at the bar!

The secret to creativity is knowing how to hide your sources.

—Albert Einstein. (1879-1955)

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Invertebrate Taxa	Common name
Ancylidae	Freshwater limpet
Asellidae	Freshwater hog louse
Baetidae	Swimming mayfly nymph
Caenidae	Mayfly larvae
Chironomidae	Non-biting midge larvae
Coleoptera	Water beetle larvae
Diptera larvae	True fly larvae
Dryopidae	Water beetle
Dytiscidae	Water beetle
Ecydionuridae	Flattened mayfly nymph
Elminthidae	Riffle beetle
Ephemeridae	Burrowing mayfly nymph
Erpobdellidae	Leech
Gammaridae	Freshwater shrimp
Glossiphonidae	Leech
Haliplidae	Water beetle
Heteroceridae	Water beetle
Hirudinidae	Leech
Hydracarina	Water mite
Hydrobidae	Spire shell snail
Leptoceridae	Caddis fly larvae (smooth quill case)
Leptophlebiidae	Mayfly nymph
Limnephilidae	Caddis fly larvae (rough case)
Lymnaeidae	Pond snail
Notonectidae	Greater water boatman
Oligochaetae	True worm
Ostracod	Ostracod
Planaridae	Flatworm
Planorbiiidae	Rams-horn snail
Silidae	Alderfly larvae
Sphaeridae	Pea mussel
Tipulidae	Crane fly larvae
Trichoptera	Caddis fly larvae
Tricladia	Flatworm
Veliidae	Water cricket
Zygoptera	Damselfly nymph

Table 40: List of all invertebrate fauna identified from kick samples. Where possible genus names are supplied, in all other cases family names are given.

Invertebrate Taxa	Sympatric sites					Allopatric sites				
	3 Y	6 L	7 L	10 Y	Total	4 Y	5 L	8 L	9 L	Total
Ancylidae	0	0	0	0	0	1	0	0	1	2
Asellidae	1	6	31	0	38	1	54	22	2	79
Baetidae	0	0	0	0	0	21	0	0	0	21
Caenidae	0	0	0	0	0	0	0	177	10	187
Chironomidae	33	11	3	1	48	7	14	0	69	90
Coleoptera	0	0	0	0	0	1	0	0	0	1
Diptera larvae	0	0	0	0	0	3	0	0	0	3
Dryopidae	0	0	0	0	0	0	0	0	0	0
Dytiscidae	0	0	0	0	0	0	0	0	0	0
Ecydionuridae	0	0	0	0	0	7	0	0	0	7
Elminthidae	0	0	0	0	0	13	0	0	0	13
Ephemeridae	0	0	0	1	1	0	0	0	0	0
Erpobdellidae	0	3	0	0	3	0	4	1	7	12
Gammaridae	1	0	3	1	5	25	101	1	2	129
Glossiphonidae	0	1	3	0	4	0	0	2	4	6
Haliplidae	0	1	0	0	1	0	0	1	0	1
Heteroceridae	0	0	0	0	0	0	0	6	3	9
Hirudinidae	0	0	0	0	0	0	1	0	1	2
Hydracarina	0	0	1	0	0	1	0	18	0	19
Hydrobidae	4	111	0	2	117	2	0	12	32	46
Leptoceridae	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	2	0	0	1	3	4	0	0	0	4
Limnephilidae	0	0	0	0	0	3	0	0	0	3
Lymnaeidae	1	0	0	0	1	0	0	0	2	2
Notonectidae	0	0	0	0	0	0	0	0	0	0
Oligochaetae	97	424	185	7	713	66	295	173	304	838
Ostracod	34	0	0	0	34	1	0	0	0	1
Planaridae	0	7	7	0	14	0	5	0	0	5
Planorbiidae	0	0	0	0	0	0	0	13	8	21
Silidae	4	0	0	0	4	0	0	1	0	1
Sphaeridae	0	5	1	0	6	2	0	5	13	20
Tipulidae	0	0	0	0	0	0	0	0	0	0
Trichoptera	1	0	0	14	15	0	0	0	0	0
Tricladia	0	0	0	0	0	0	0	0	0	0
Veliidae	0	0	0	0	0	0	0	1	0	1
Zygoptera	0	1	0	0	1	0	0	2	0	2
BMWP Score	34.1	30.9	24.7	33.8		48	20.8	42	45.8	
ASPT	4.26	3.43	3.53	5.63		4.8	3.47	3.82	3.82	

Table 41: Summary of invertebrates identified and counted from the spring collection grouped according to site. L= sites in Leicestershire; Y= sites in North Yorkshire. Invertebrates included in statistical analysis indicated in bold type.

Invertebrate Taxa	Sympatric sites						Allopatric sites					
	1 Y	3 Y	6 L	7 L	10 Y	Total	2 Y	4 Y	5 L	8 L	9 L	Total
Ancylidae	0	0	0	0	0	0	0	0	0	0	0	0
Asellidae	2	0	25	15	0	45	0	0	28	25	27	80
Baetidae	0	0	0	0	0	0	0	0	0	25	0	0
Caenidae	0	0	0	0	0	0	0	0	0	0	36	36
Chironomidae	20	38	2	6	6	72	0	0	7	0	2	9
Coleoptera	0	0	0	0	0	0	0	3	0	0	0	3
Diptera larvae	0	0	0	0	0	0	0	4	0	0	0	4
Dryopidae	0	0	0	0	8	8	0	0	0	0	0	0
Dytiscidae	1	0	0	0	3	4	0	0	0	2	2	4
Ecydonuridae	0	0	0	0	0	0	0	0	0	0	0	0
Elminthidae	0	0	0	0	0	0	0	0	0	0	0	0
Ephemerae	0	0	0	0	0	0	0	9	0	0	0	9
Erpobdellidae	1	0	3	0	0	4	0	0	20	6	6	32
Gammaridae	10	0	0	0	0	10	132	40	7	0	0	189
Glossiphoniidae	0	1	1	4	1	7	0	1	1	2	2	6
Halipidae	1	0	0	0	1	2	0	0	0	0	0	0
Heteroceridae	0	0	0	0	0	0	0	0	0	0	0	0
Hirudinidae	0	0	0	0	0	0	0	0	0	0	0	0
Hydracarina	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	1	0	3	49	65	118	0	8	3	42	45	98
Leptoceridae	1	0	0	0	0	1	0	0	0	0	0	0
Leptophlebiidae	0	0	0	0	0	0	0	0	4	0	0	4
Limnephilidae	1	1	0	0	1	3	0	0	0	0	0	0
Lymnaeidae	0	0	0	0	1	1	0	0	0	0	0	0
Notonectidae	0	0	0	0	1	1	0	0	0	0	0	0
Oligochaetae	0	10	1	94	6	111	43	0	35	0	2	82
Ostracod	0	0	0	0	0	0	0	0	0	0	0	0
Planariidae	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	3	0	0	3	0	0	0	3	2	5
Silidae	10	1	0	0	0	11	0	0	0	0	0	0
Sphaeriidae	0	0	0	6	0	6	0	2	0	2	2	6
Tipulidae	1	1	0	0	1	3	0	0	0	0	0	0
Trichoptera	0	0	0	0	0	0	0	0	0	0	0	0
Tricladia	0	0	37	0	0	37	0	0	0	0	0	0
Veliidae	0	0	0	0	0	0	0	0	0	0	0	0
Zygoptera	0	0	0	0	0	0	0	0	0	0	0	0
BMWP Score	50.5	27.2	22	19.9	48.7		8	24.4	32.5	28.5	37.5	
ASPT	4.6	4.53	3.14	3.32	4.43		4	4.88	4.06	3.56	3.75	

Table 42: Summary of invertebrates identified and counted from the autumn collection grouped according to site. L= sites in Leicestershire; Y= sites in North Yorkshire. Invertebrates included in statistical analysis indicated in bold type.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	9.150	2.450	0.149
Location (Leicester/Yorkshire)	1	1.426	0.382	0.550
Season (Spring/ Autumn)	1	1.910	0.512	0.491
Population * Location	1	29.332	7.855	0.019
Population * Season	1	3.338	0.894	0.367
Location * Season	1	8.673	2.323	0.158
Population * Location * Season	1	6.580	1.762	0.214
Residual (error)	10	3.734		
Corrected Total	17			

Table 43: Results of univariate ANOVA comparing the abundance of Asellidae. Square root data, significant results indicated in bold type.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	1.181	0.225	0.646
Location (Leicester/Yorkshire)	1	3.691	0.703	0.422
Season (Spring/ Autumn)	1	5.426	1.033	0.333
Population * Location	1	1.733	0.330	0.578
Population * Season	1	7.208	1.372	0.269
Location * Season	1	1.358	0.259	0.622
Population * Location * Season	1	0.103	0.020	0.891
Residual (error)	10	5.253		
Corrected Total	17			

Table 44: Results of univariate ANOVA comparing the abundance of Chironomidae. Square root data.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	4.134	3.103	0.109
Location (Leicester/Yorkshire)	1	<0.002	0.012	0.914
Season (Spring/ Autumn)	1	0.274	0.206	0.660
Population * Location	1	1.252	0.940	0.355
Population * Season	1	0.123	0.092	0.767
Location * Season	1	<0.002	0.016	0.902
Population * Location * Season	1	<0.001	0.001	0.981
Residual (error)	10	1.332		
Corrected Total	17			

Table 45: Results of univariate ANOVA comparing the abundance of Erpobdellidae. Square root data.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	77.778	10.554	0.009
Location (Leicester/Yorkshire)	1	40.758	5.531	0.041
Season (Spring/ Autumn)	1	2.610	0.354	0.565
Population * Location	1	31.405	4.261	0.066
Population * Season	1	1.851	0.251	0.627
Location * Season	1	1.016	0.138	0.718
Population * Location * Season	1	3.673	0.498	0.496
Residual (error)	10	7.369		
Corrected Total	17			

Table 46: Results of univariate ANOVA comparing the abundance of Gammaridae. Square root data, significant results indicated in bold type.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	<0.004	0.173	0.686
Location (Leicester/Yorkshire)	1	1.851	9.132	0.013
Season (Spring/ Autumn)	1	0.130	0.642	0.441
Population * Location	1	<0.001	0.051	0.827
Population * Season	1	<0.001	0.059	0.813
Location * Season	1	<0.006	0.292	0.601
Population * Location * Season	1	<0.001	0.003	0.960
Residual (error)	10	0.203		
Corrected Total	17			

Table 47: Results of univariate ANOVA comparing the abundance of Glossiphonidae. Square root data, significant results indicated in bold type.

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	4.588	0.401	0.541
Location (Leicester/Yorkshire)	1	35.383	3.092	0.109
Season (Spring/ Autumn)	1	1.795	0.157	0.700
Population * Location	1	1.113	0.097	0.762
Population * Season	1	1.060	0.093	0.767
Location * Season	1	0.536	0.047	0.833
Population * Location * Season	1	3.756	0.328	0.579
Residual (error)	10	11.442		
Corrected Total	17			

Table 48: Results of univariate ANOVA comparing the abundance of Hydrobidae. Square root data

Source of Variation	df	MS	F	P
Population (Sympatric/Allopatric)	1	10.031	0.752	0.406
Location (Leicester/Yorkshire)	1	5.220	0.391	0.546
Season (Spring/ Autumn)	1	372.873	27.958	0.000
Population * Location	1	139.895	10.489	0.009
Population * Season	1	12.464	0.935	0.356
Location * Season	1	18.112	1.358	0.271
Population * Location * Season	1	10.511	0.788	0.396
Residual (error)	10	13.337		
Corrected Total	17			

Table 49: Results of univariate ANOVA comparing the abundance of Oligochaetae. Square root data, significant results indicated in bold type.

Population	Site	Morphological Feature												
		Statistic	Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Sympatric threespine	1	min	30.3	4.7	6.4	7	7.5	6.4	1.5	2.5	1.8	2	1.6	2.2
		max	47	9.3	11.5	11.9	12	11	3	5.6	3.9	4.4	3.7	4.4
		stdev	3.76	0.94	1.12	1.14	1.13	1.12	0.32	0.74	0.47	0.43	0.46	0.51
		mean	39.50	6.68	8.77	9.46	9.94	8.64	2.11	3.87	2.68	2.88	2.46	3.23
Allopatric threespine	2	min	36.2	5.9	7.7	8	8.5	7.2	1.9	2.9	2.6	2.7	1.7	2.9
		max	60	9.6	12.9	14.3	15.4	14.5	3.7	5.6	4.2	4.4	3.3	4.8
		stdev	5.54	0.93	1.22	1.48	1.63	1.81	0.35	0.68	0.39	0.36	0.40	0.50
		mean	44.41	7.19	9.38	10.47	11.13	9.96	2.49	4.00	3.15	3.31	2.54	3.69
Sympatric threespine	3	min	21.5	3.5	4.6	5.1	5	4.5	1	1.7	1.3	1.8	1.2	1.5
		max	50.4	8.2	11.7	13.3	13.1	11.5	2.5	4.7	3.6	3.6	3.2	3.8
		stdev	7.48	1.28	1.85	2.08	2.11	2.02	0.45	0.81	0.67	0.53	0.57	0.73
		mean	33.47	5.47	7.21	7.9	8.14	7.27	1.67	2.85	2.31	2.58	1.90	2.59
Allopatric threespine	4	min	27.7	4.4	5.8	6.3	6.5	5.6	1.3	2.1	1.8	1.9	1.2	1.8
		max	57.4	8.4	12.1	14.5	14.9	13.4	3.2	4.6	4.7	3.6	3.2	4.4
		stdev	6.26	1.01	1.46	1.75	1.85	1.83	0.43	0.62	0.66	0.41	0.47	0.67
		mean	40.75	6.35	8.62	9.83	10.21	9.36	2.20	3.40	2.93	2.80	2.26	3.35
Allopatric threespine	5	min	26.2	4.3	5.2	5.8	6.1	5.1	1.2	2.1	1.7	1.8	1.4	1.8
		max	54.1	9.8	12.2	12.9	14	12.3	12.4	5.7	4	3.4	3.5	5.2
		stdev	6.20	1.12	1.47	1.51	1.57	1.51	1.83	0.74	0.53	0.38	0.51	0.72
		mean	37.94	6.21	7.91	8.69	9.03	7.67	2.11	3.27	2.53	2.66	2.24	3.13
Sympatric threespine	6	min	0.23	3.7	4.9	0.8	5.5	4.8	1	1.7	1.3	1.9	1.1	1.7
		max	2.08	8.1	11.1	12.6	12.4	11.2	2.8	4.5	3.7	3.5	2.9	4.1
		stdev	0.33	0.85	1.23	1.72	1.32	1.22	0.34	0.53	0.47	0.31	0.34	0.49
		mean	33.74	5.36	6.93	7.57	8.02	6.81	1.55	2.64	2.14	2.51	1.74	2.62
Sympatric threespine	7	min	26.9	4.7	5.7	6.4	6.5	5.3	1.1	1.9	1.5	2.2	1.4	2
		max	52.2	9	11.7	13.7	13.7	12.7	2.7	5.2	3.6	3.8	3.5	4.8
		stdev	7.48	1.14	1.55	1.87	1.86	1.83	0.43	0.84	0.62	0.42	0.54	0.71
		mean	39	6.21	8.03	8.91	9.08	7.90	1.91	3.17	2.62	2.81	2.13	3.17

Table 50: Descriptive statistics for morphological features measured on sticklebacks collected in the spring. Continued over

Population	Site	Morphological Feature												
		Statistic	Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Allopatric threespine	8	min	30.3	4.6	6.1	6.5	6.8	5.6	1.1	2.4	1.6	2.2	1.6	1.9
		max	40.3	7.2	8.7	9.3	10	8.5	2.1	4.1	2.8	3	2.5	3.6
		stdev	2.798	0.62	0.80	0.83	0.95	0.82	0.24	0.43	0.29	0.21	0.26	0.41
		mean	35.37	5.84	7.33	7.92	8.43	7.01	1.64	3.19	2.19	2.56	2.03	2.85
Allopatric threespine	9	min	20.7	3	3.8	4.3	4.6	3.6	0.8	1.5	1.1	1.8	0.9	1.3
		max	44.4	6.6	8.6	9.6	10.2	8.5	2.2	3.3	3.2	3.5	2.5	3.5
		stdev	6.03	0.94	1.22	1.38	1.43	1.22	0.34	0.48	0.48	0.41	0.41	0.53
		mean	26.51	3.97	5.06	5.77	6.03	4.90	1.28	1.99	1.72	2.37	1.34	1.89
Sympatric threespine	10	min	21.1	3.1	4	4.5	4.9	4.1	0.9	1.6	1.1	1.7	0.9	1.3
		max	40.2	6.9	9.2	9.9	10.6	10.8	2.8	3.4	4.8	3.1	2.2	3.3
		stdev	5.18	0.85	1.27	1.43	1.46	1.56	0.37	0.48	0.63	0.33	0.36	0.49
		mean	29.43	4.65	6.18	6.86	7.17	6.17	1.58	2.41	2.03	2.19	1.51	2.11
Sympatric ninespine	11	min	24.7	3.9	4.5	4.7	4.8	3.9	0.8	2	1.5	1.9	1.3	1.6
		max	49.5	7.2	8.3	9.8	10.1	6.8	1.5	5	3.2	2.9	2.8	3.6
		stdev	4.28	0.56	0.73	0.92	1.01	0.62	0.18	0.56	0.30	0.23	0.26	0.33
		mean	32.40	4.76	5.85	6.18	6.26	5.15	1.14	2.88	2.07	2.23	1.65	2.25
Sympatric ninespine	12	min	23.9	3.1	4.3	4.2	4.5	3.8	0.8	1.6	1.5	1.9	1	1.5
		max	49.8	6.3	8.2	9.2	9.4	7.3	1.8	4.1	3.4	2.8	2.7	3
		stdev	6.85	0.85	1.11	1.25	1.24	0.98	0.28	0.64	0.51	0.26	0.44	0.43
		mean	34.44	4.85	6.28	6.60	6.75	5.48	1.19	2.85	2.41	2.42	1.66	2.23
Sympatric ninespine	13	min	27.7	4	5.1	5.6	5.6	4.4	0.9	2.3	1.8	2.1	1.5	1.8
		max	50.9	8.1	9.1	9.6	9.5	8.2	1.8	5.5	3.5	3.2	2.7	3.5
		stdev	5.62	0.81	0.93	0.96	1.03	0.91	0.22	0.65	0.40	0.25	0.28	0.41
		mean	41.47	5.8	7.26	7.81	7.78	6.36	1.28	3.57	2.69	2.74	2.13	2.80
Sympatric ninespine	14	min	33.9	4.7	6	6.1	6.4	5.2	1	2.4	1.8	1.8	1.17	1.53
		max	49.5	7.2	9.2	9.9	10.2	8.9	1.9	4.3	3.1	3.2	2	2.7
		stdev	4.01	0.62	0.86	0.90	0.95	0.93	0.22	0.44	0.31	0.35	0.20	0.28
		mean	38.05	5.50	7.07	7.72	7.81	6.48	1.22	3.06	2.33	2.49	1.56	2.14
Sympatric ninespine	15	min	20.1	3	3.8	4	4	3.2	0.7	1.6	1.5	1.9	0.8	1.3
		max	39.5	5.9	7.7	8.7	8.8	6.7	1.6	3.6	3.1	2.9	2	2.8
		stdev	5.70	0.76	0.98	1.12	1.18	0.91	0.22	0.52	0.42	0.29	0.32	0.41
		mean	29.42	4.24	5.55	5.95	6.18	4.90	1.05	2.37	2.18	2.38	1.31	1.89

Table 50: Descriptive statistics for morphological features measured on sticklebacks collected in the spring.

Population	Site	Morphological feature											
		Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Sympatric threespine	1	39.51	6.681	8.771	9.461	9.935	8.642	2.113	3.871	2.681	2.884	2.465	3.232
	3	33.47	5.47	7.21	7.9	8.143	7.27	1.67	2.85	2.31	2.577	1.897	2.593
	6	33.746	5.363	6.931	7.571	8.023	6.806	1.554	2.64	2.14	2.514	1.743	2.62
	7	39	6.21	8.031	8.907	9.076	7.897	1.914	3.172	2.621	2.817	2.131	3.172
	10	29.434	4.647	6.176	6.863	7.166	6.171	1.576	2.408	2.029	2.195	1.516	2.113
Allopatric threespine	2	44.419	7.194	9.384	10.471	11.129	9.965	2.494	4.003	3.152	3.31	2.535	3.69
	4	40.755	6.348	8.621	9.831	10.21	9.362	2.197	3.397	2.928	2.797	2.255	3.352
	5	37.949	6.209	7.911	8.686	9.034	7.669	2.109	3.271	2.529	2.663	2.237	3.126
	8	35.371	5.843	7.325	7.918	8.432	7.007	1.639	3.186	2.189	2.564	2.029	2.85
	9	26.519	3.972	5.063	5.769	6.025	4.894	1.281	1.991	1.725	2.369	1.344	1.894
Ninespine	11	32.402	4.759	5.848	6.178	6.259	5.15	1.137	2.885	2.067	2.226	1.646	2.249
	12	34.44	4.853	6.277	6.603	6.747	5.48	1.187	2.85	2.407	2.42	1.663	2.227
	13	41.47	5.8	7.263	7.81	7.783	6.363	1.283	3.57	2.693	2.74	2.133	2.803
	14	38.05	5.503	7.067	7.72	7.807	6.48	1.223	3.057	2.334	2.494	1.559	2.143
	15	29.421	4.241	5.554	5.951	6.179	4.892	1.046	2.372	2.179	2.377	1.308	1.892

Table 51: Descriptive statistics for morphological features measured on sticklebacks collected in the spring

Population	Site	Morphological Feature												
		Statistic	Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Sympatric threespine	1	min	14.7	1.9	2.4	3.2	3.1	2.7	0.5	0.9	1.92	3.36	1.12	2.08
		max	46.4	8	10.1	11.8	12.6	10.6	2.7	4.4	10.24	10.4	6.08	10.72
		stdev	8.304	1.548	1.924	2.226	2.346	2.105	0.538	0.881	1.914	1.778	1.081	1.944
		mean	23.325	3.513	4.6	5.628	5.984	4.722	1.084	1.534	4.13	5.31	2.23	4.095
Allopatric threespine	2	min	16.2	2.2	2.8	3	3.5	2.9	0.8	1	2.56	3.68	1.76	2.72
		max	42.9	7.2	8.9	10.1	10.5	8.8	2.6	4	9.28	53.28	6.4	10.72
		stdev	4.572	0.816	0.97	1.120	1.09	0.992	0.324	0.49	1.083	7.379	0.827	1.253
		mean	25.786	3.895	4.871	5.707	6.11	4.821	1.29	2.002	4.16	6.937	2.789	4.693
Sympatric threespine	3	min	14.1	1.8	2.4	2.7	2.7	2.4	0.6	1	2.88	3.2	1.28	2.4
		max	41	6.2	8.4	9.9	10.3	9.3	2.1	3	8.16	8	5.76	7.2
		stdev	6.498	1.165	1.451	1.626	1.682	1.495	0.381	0.548	1.401	1.234	0.993	1.302
		mean	23.868	3.724	4.868	5.649	5.949	4.77	1.149	1.686	4.432	5.492	2.837	4.182
Allopatric threespine	4	min	16.5	2.2	2.9	1	3.6	3.1	0.8	0.9	2.24	3.84	1.44	2.4
		max	53.7	8	10.3	11.4	12.1	9.8	2.6	4.2	10.4	10.56	6.72	11.2
		stdev	9.164	1.558	1.963	2.365	2.252	1.99	0.562	0.854	2.167	1.765	1.343	2.363
		mean	30.356	4.567	5.841	6.603	7.272	5.99	1.446	2.115	5.559	6.593	3.245	5.842
Allopatric threespine	5	min	14.6	2	2.4	2.5	2.5	1.1	0.4	0.7	2.4	2.88	0.96	2.4
		max	49.2	8.5	10.1	12	12.5	10.7	2.8	4.1	9.12	8.8	6.56	11.2
		stdev	10.227	1.871	2.22	2.656	2.767	2.588	0.681	1.024	2.134	1.689	1.5	2.612
		mean	32.045	4.981	6.1	7.339	7.781	6.445	1.597	2.374	5.29	6.379	3.37	6.245
Sympatric threespine	6	min	17.6	2.6	3	3.2	3.3	2.9	0.7	1	2.08	3.84	1.12	2.72
		max	35.2	5.5	6.8	7.8	8.1	7.1	1.8	2.9	5.6	6.88	4.16	6.56
		stdev	3.696	0.659	0.784	0.902	0.962	0.833	0.212	0.39	0.753	0.684	0.656	0.835
		mean	23.062	3.59	4.29	4.993	5.162	4.252	0.986	1.662	3.1	5.07	1.937	3.686
Sympatric threespine	7	min	15.9	2.3	2.8	3.1	3.2	2.5	0.6	1.1	1.92	3.68	1.12	2.24
		max	48.1	8.8	10.5	11.8	11.7	10.6	2.7	4.8	8.16	9.28	7.2	11.36
		stdev	6.623	1.236	1.498	1.729	1.69	1.56	0.415	0.719	1.185	1.235	1.142	1.658
		mean	24.447	3.894	4.659	5.225	5.331	4.359	1.103	1.969	3.31	5.31	2.515	4.145

Table S2: Descriptive statistics for morphological features measured on sticklebacks collected in the autumn. Continued over.

Population	Site	Morphological Feature												
		Statistic	Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Allopatric threespine	8	min	14.4	2.2	2.6	3	2.4	2.3	0.4	0.9	2.08	3.04	1.28	2.08
		max	32.9	5.4	6.6	7.5	8.2	7.1	1.6	2.9	5.92	6.88	6.08	6.72
		stdev	4.908	0.900	1.109	1.266	1.406	1.231	0.271	0.626	1.091	0.971	1.028	1.293
		mean	21.383	3.447	4.077	4.67	4.967	4.02	0.847	1.71	3.275	4.757	2.363	3.904
Allopatric threespine	9	min	16.7	2.5	3	3.4	3.8	3.3	0.7	0.6	1.92	3.52	1.44	2.72
		max	28.8	4.8	5.7	6.6	7	6	1.6	2.4	4.64	6.56	3.36	5.92
		stdev	3.258	0.507	0.665	0.796	0.828	0.708	0.224	0.382	0.619	0.64	0.424	0.75
		mean	21.55	3.316667	3.85	4.55	4.893	4.083	0.99	1.587	2.997	4.592	2.101	3.909
Sympatric threespine	10	min	14.3	2.3	2.8	3.1	3.4	2.5	0.7	0.9	2.24	3.52	1.12	2.56
		max	29.1	4.7	6.2	7.3	7.8	6.6	1.6	2.3	5.28	6.88	3.04	5.28
		stdev	2.916	0.54	0.696	0.818	0.891	0.767	0.213	0.285	0.746	0.689	0.395	0.68
		mean	20.133	3.183	3.99	4.67	5.023	3.95	1	1.307	3.419	4.987	1.712	3.803
Sympatric ninespine	11	min	16.2	2.4	2.7	3.1	3.1	2.7	0.5	1.1	2.72	3.36	1.28	2.72
		max	42.5	5.9	7.1	8.8	9.2	6.9	1.7	3.1	7.36	7.68	5.28	7.52
		stdev	7.351	0.882	1.121	1.403	1.501	1.149	0.275	0.557	1.285	1.036	0.944	1.139
		mean	30.486	4.059	4.962	5.779	5.986	4.859	1.093	2.055	5.401	5.86	3.062	5.131
Sympatric ninespine	12	min	21.4	3.2	4	4.2	4.1	4	0.7	1.5	4.42	4.68	2.34	3.38
		max	42.9	6.1	7.4	7.8	8.7	8.2	2	3.2	7.8	8.32	5.2	7.28
		stdev	5.319	0.646	0.851	0.889	1.029	1.017	0.255	0.422	0.995	0.946	0.665	1.093
		mean	34.084	4.714	5.851	6.414	6.643	5.941	1.227	2.419	6.282	6.851	3.612	5.439
Sympatric ninespine	13	min	14.8	2.1	2.7	2.8	2.5	2.1	0.5	1.1	1.76	3.2	1.12	2.24
		max	35.9	5.5	6.3	7.2	7.2	5.7	1.1	3.2	5.76	6.88	3.68	6.24
		stdev	5.524	0.899	0.908	1.042	1.104	0.911	0.14	0.542	0.978	0.856	0.677	1.064
		mean	25.193	3.77	4.55	4.783	4.733	3.677	0.79	2.087	3.936	5.163	2.197	4
Sympatric ninespine	14	min	19	3.2	3.7	4	4	3.1	0.5	1.3	2.56	4.32	1.92	2.56
		max	40.2	6	7	7.6	7.5	6	1.1	3.7	5.6	7.36	5.6	5.76
		stdev	6.007	0.85	0.956	1.047	1.062	0.9	0.167	0.683	0.921	0.902	0.852	0.88
		mean	27.745	4.334	5.052	5.207	5.231	4.297	0.807	2.441	3.785	5.55	3.244	3.95
Sympatric ninespine	15	min	15	2.1	2.6	3.3	3.2	2.5	0.5	1	3.04	3.52	1.28	2.56
		max	40.6	5.8	7.4	8.6	9	7.2	2.1	3.7	6.88	8	4.48	6.4
		stdev	6.829	0.973	1.227	1.328	1.417	1.222	0.344	0.615	1.192	1.075	0.846	1.171

	mean	25.995	3.722	4.565	5.157	5.408	4.216	0.954	1.93	4.718	5.548	2.573	4.29
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Table 52: Descriptive statistics for morphological features measured on sticklebacks collected in the autumn.

Population	Site	Morphological feature											
		Standard length	Body depth over eye	Depth over operculum	Body depth over spine 1	Body depth over spine 2	Body depth over spine 3	Depth over caudal peduncle	Mouth width	Mouth length	Eye orbit	Chin depth	Snout length
Sympatric threespine	1	23.32	3.51	4.6	5.628	5.984	4.722	1.084	1.534	4.13	5.31	2.23	4.095
	3	23.86	3.724	4.868	5.649	5.949	4.77	1.149	1.686	4.432	5.492	2.837	4.182
	6	23.06	3.590	4.29	4.993	5.162	4.251	0.986	1.662	3.101	5.07	1.937	3.686
	7	24.44	3.894	4.659	5.225	5.331	4.359	1.103	1.969	3.31	5.31	2.515	4.145
	10	20.13	3.183	3.99	4.67	5.023	3.95	1	1.307	3.419	4.987	1.712	3.803
Allopatric threespine	2	25.78	3.895	4.871	5.707	6.11	4.821	1.29	2.002	4.16	6.937	2.789	4.693
	4	30.35	4.567	5.841	6.603	7.273	5.99	1.446	2.115	5.559	6.593	3.245	5.842
	5	32.04	4.981	6.1	7.339	7.781	6.445	1.597	2.374	5.29	6.379	3.370	6.245
	8	21.38	3.447	4.077	4.67	4.967	4.02	0.847	1.71	3.275	4.757	2.363	3.904
	9	21.55	3.317	3.85	4.55	4.893	4.083	0.99	1.587	2.997	4.592	2.101	3.909
Ninespine	11	30.48	4.059	4.962	5.779	5.986	4.859	1.093	2.055	5.401	5.859	3.062	5.131
	12	34.08	4.714	5.851	6.414	6.643	5.941	1.227	2.419	6.282	6.851	3.612	5.439
	13	25.19	3.77	4.55	4.783	4.733	3.677	0.79	2.087	3.936	5.163	2.197	4
	14	27.74	4.334	5.052	5.207	5.231	4.297	0.807	2.441	3.785	5.55	3.244	3.95
	15	25.99	3.722	4.565	5.157	5.408	4.216	0.954	1.93	4.718	5.548	2.573	4.289

Table 53: Descriptive statistics for morphological features measured on sticklebacks collected in the autumn.

Dependent Variable	df	F	P
Standard length	2, 490	3.877	0.021
Body depth over eye	2, 490	22.471	0.000
Body depth over operculum	2, 490	27.476	0.000
Body depth over spine 1	2, 490	33.725	0.000
Body depth over spine 2	2, 490	54.897	0.000
Body depth over spine 3	2, 490	68.598	0.000
Body depth at caudal peduncle	2, 490	149.603	0.000
Mouth width	2, 490	2.630	0.073
Mouth length	2, 490	25.030	0.000
Eye orbit	2, 490	65.305	0.000
Chin depth	2, 490	63.614	0.000
Snout length	2, 490	94.481	0.000

Table 54: ANOVA; Tests of between subjects effects between all stickleback populations, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 316	5.257	0.023
Body depth over eye	1, 316	2.329	0.128
Body depth over operculum	1, 316	1.070	0.302
Body depth over spine 1	1, 316	2.876	0.091
Body depth over spine 2	1, 316	3.466	0.064
Body depth over spine 3	1, 316	1.942	0.164
Body depth at caudal peduncle	1, 316	4.305	0.039
Mouth length	1, 316	32.869	0.000
Eye orbit	1, 316	55.652	0.000
Chin depth	1, 316	34.405	0.000
Snout length	1, 316	42.237	0.000

Table 55: *post-hoc* ANOVA; Tests of between subjects effects between allopatric and sympatric threespine stickleback populations, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 336	0.004	0.951
Body depth over eye	1, 336	27.937	0.000
Body depth over operculum	1, 336	41.635	0.000
Body depth over spine 1	1, 336	38.396	0.000
Body depth over spine 2	1, 336	76.800	0.000
Body depth over spine 3	1, 336	115.346	0.000
Body depth at caudal peduncle	1, 336	236.565	0.000
Mouth length	1, 336	0.032	0.859
Eye orbit	1, 336	8.613	0.000
Chin depth	1, 336	25.528	0.000
Snout length	1, 336	44.969	0.000

Table 56: *post-hoc* ANOVA; Tests of between subjects effects between sympatric threespine and ninespine stickleback populations, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 326	5.954	0.015
Body depth over eye	1, 326	40.522	0.000
Body depth over operculum	1, 326	46.634	0.000
Body depth over spine 1	1, 326	71.287	0.000
Body depth over spine 2	1, 326	93.523	0.000
Body depth over spine 3	1, 326	110.922	0.000
Body depth at caudal peduncle	1, 326	237.746	0.000
Mouth length	1, 326	45.150	0.000
Eye orbit	1, 326	129.909	0.000
Chin depth	1, 326	148.118	0.000
Snout length	1, 326	225.533	0.000

Table 57: *post-hoc* ANOVA; Tests of between subjects effects between allopatric threespine and sympatric ninespine stickleback populations, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	2, 494	29.575	0.000
Body depth over eye	2, 494	13.215	0.000
Body depth over operculum	2, 494	7.913	0.000
Body depth over spine 1	2, 494	3.174	0.043
Body depth over spine 2	2, 494	6.865	0.001
Body depth over spine 3	2, 494	6.436	0.002
Body depth at caudal peduncle	2, 494	15.726	0.000
Mouth width	2, 494	34.559	0.000
Mouth length	2, 494	5.080	0.007
Eye orbit	2, 494	17.428	0.000
Chin depth	2, 494	11.656	0.000
Snout length	2, 494	23.806	0.000

Table 58: ANOVA; Tests of between subjects effects between all stickleback populations, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 322	18.607	0.000
Body depth over eye	1, 322	12.179	0.001
Body depth over operculum	1, 322	7.115	0.008
Body depth over spine 1	1, 322	5.301	0.022
Body depth over spine 2	1, 322	11.385	0.001
Body depth over spine 3	1, 322	11.769	0.001
Body depth at caudal peduncle	1, 322	10.462	0.001
Mouth width	1, 322	19.628	0.000
Mouth length	1, 322	10.219	0.002
Eye orbit	1, 322	8.373	0.004
Chin depth	1, 322	23.064	0.000
Snout length	1, 322	26.216	0.000

Table 59: *post-hoc* ANOVA; Tests of between subjects effects between allopatric and sympatric threespine stickleback populations, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 334	66.758	0.000
Body depth over eye	1, 334	28.854	0.000
Body depth over operculum	1, 334	18.198	0.000
Body depth over spine 1	1, 334	3.650	0.057
Body depth over spine 2	1, 334	0.871	0.351
Body depth over spine 3	1, 334	2.262	0.134
Body depth at caudal peduncle	1, 334	5.501	0.020
Mouth width	1, 334	81.117	0.000
Mouth length	1, 334	3.030	0.083
Eye orbit	1, 334	10.922	0.001
Chin depth	1, 334	1.945	0.164
Snout length	1, 334	3.281	0.071

Table 60: *post-hoc* ANOVA; Tests of between subjects effects between sympatric threespine and ninespine stickleback populations, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 331	10.739	0.001
Body depth over eye	1, 331	1.680	0.196
Body depth over operculum	1, 331	1.041	0.308
Body depth over spine 1	1, 331	0.597	0.440
Body depth over spine 2	1, 331	6.776	0.010
Body depth over spine 3	1, 331	4.320	0.038
Body depth at caudal peduncle	1, 331	28.191	0.000
Mouth width	1, 331	13.504	0.000
Mouth length	1, 331	2.105	0.148
Eye orbit	1, 331	28.082	0.000
Chin depth	1, 331	10.271	0.001
Snout length	1, 331	38.010	0.000

Table 61: *post-hoc* ANOVA; Tests of between subjects effects between allopatric threespine and ninespine stickleback populations, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 158	4.202	0.042
Body depth over eye	1, 158	1.168	0.281
Body depth over operculum	1, 158	0.227	0.635
Body depth over spine 1	1, 158	0.371	0.544
Body depth over spine 2	1, 158	0.330	0.567
Body depth over spine 3	1, 158	0.006	0.938
Body depth at caudal peduncle	1, 158	0.632	0.428
Mouth width	1, 158	0.766	0.383
Mouth length	1, 158	0.154	0.695
Eye orbit	1, 158	2.764	0.098
Chin depth	1, 158	0.011	0.916
Snout length	1, 158	5.309	0.022

Table 62: ANOVA; Results of between subjects effects (Leicester V Scarborough) for Sympatric threespines, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 160	74.653	0.000
Body depth over eye	1, 160	73.461	0.000
Body depth over operculum	1, 160	73.889	0.000
Body depth over spine 1	1, 160	84.111	0.000
Body depth over spine 2	1, 160	66.706	0.000
Body depth over spine 3	1, 160	79.168	0.000
Body depth at caudal peduncle	1, 160	15.971	0.000
Mouth width	1, 160	40.486	0.000
Mouth length	1, 160	24.747	0.000
Eye orbit	1, 160	35.538	0.000
Chin depth	1, 160	27.014	0.000
Snout length	1, 160	24.260	0.000

Table 63: ANOVA; Results of between subjects effects (Leicester V Scarborough) for Sympatric ninespines, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 147	63.520	0.000
Body depth over eye	1, 147	46.409	0.000
Body depth over operculum	1, 147	64.876	0.000
Body depth over spine 1	1, 147	79.026	0.000
Body depth over spine 2	1, 147	77.781	0.000
Body depth over spine 3	1, 147	102.579	0.000
Body depth at caudal peduncle	1, 147	70.176	0.000
Mouth width	1, 147	49.460	0.000
Mouth length	1, 147	34.731	0.000
Eye orbit	1, 147	0.906	0.343
Chin depth	1, 147	12.293	0.000
Snout length	1, 147	23.625	0.000

Table 64: ANOVA; Results of between subjects effects (Leicester V Scarborough) for Allopatric threespines, spring sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 159	2.847	0.094
Body depth over eye	1, 159	3.964	0.048
Body depth over operculum	1, 159	0.030	0.864
Body depth over spine 1	1, 159	0.578	0.448
Body depth over spine 2	1, 159	2.394	0.124
Body depth over spine 3	1, 159	0.432	0.512
Body depth at caudal peduncle	1, 159	0.101	0.751
Mouth width	1, 159	14.664	0.000
Mouth length	1, 159	18.432	0.000
Eye orbit	1, 159	0.030	0.863
Chin depth	1, 159	0.121	0.728
Snout length	1, 159	0.195	0.659

Table 65: ANOVA; Results of between subjects effects (Leicester V Scarborough) for sympatric threespines, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 162	9.429	0.003
Body depth over eye	1, 162	0.586	0.445
Body depth over operculum	1, 162	2.331	0.129
Body depth over spine 1	1, 162	13.988	0.000
Body depth over spine 2	1, 162	22.081	0.000
Body depth over spine 3	1, 162	25.189	0.000
Body depth at caudal peduncle	1, 162	44.713	0.000
Mouth width	1, 162	1.367	0.244
Mouth length	1, 162	0.001	0.975
Eye orbit	1, 162	15.687	0.000
Chin depth	1, 162	9.904	0.002
Snout length	1, 162	4.403	0.037

Table 66: ANOVA; Results of between subjects effects (Leicester V Scarborough) for sympatric ninespines, autumn sample. Significant results given in bold type.

Dependent Variable	df	F	P
Standard length	1, 171	8.761	0.004
Body depth over eye	1, 171	3.497	0.063
Body depth over operculum	1, 171	9.792	0.002
Body depth over spine 1	1, 171	5.093	0.025
Body depth over spine 2	1, 171	10.101	0.002
Body depth over spine 3	1, 171	6.473	0.012
Body depth at caudal peduncle	1, 171	14.031	0.000
Mouth width	1, 171	3.733	0.055
Mouth length	1, 171	18.408	0.000
Eye orbit	1, 171	19.714	0.000
Chin depth	1, 171	7.973	0.005
Snout length	1, 171	5.237	0.023

Table 67: ANOVA; Results of between subjects effects (Leicester V Scarborough) for allopatric threespines, autumn sample. Significant results given in bold type.

Population	Site	Statistic	Gill Raker number	5 th Gill raker	8 th Gill Raker	Standard Length
Sympatric threespine	1	Min	17	0.31	0.50	30.30
		Max	22	0.75	1.06	47
		Mean	19.29	0.49	0.78	39.51
		St dev	1.22	0.10	0.13	3.76
Allopatric threespine	2	Min	16	0.30	0.45	36.20
		Max	22	0.80	0.95	50.00
		Mean	18.55	0.44	0.70	43.48
		St dev	1.43	0.11	0.14	4.28
Sympatric threespine	3	Min	16	0.15	0.25	21.50
		Max	21	0.80	0.85	50.40
		Mean	18.79	0.38	0.56	33.63
		St dev	1.10	0.13	0.13	7.41
Allopatric threespine	4	Min	16	0.15	0.33	27.70
		Max	19.00	0.70	0.95	48.70
		Mean	17.54	0.36	0.61	40.16
		St dev	1.04	0.11	0.17	5.48
Allopatric threespine	5	Min	15	0.29	0.38	26.20
		Max	20.00	0.86	0.90	48.10
		Mean	17.33	0.46	0.60	37.46
		St dev	1.39	0.15	0.16	5.38
Sympatric threespine	6	Min	16	0.238	0.4	26.9
		Max	21	0.5	0.762	48.6
		Mean	18.17	0.36	0.57	36.22
		St dev	1.24	0.07	0.10	5.99
Sympatric threespine	7	Min	14.00	0.19	0.29	32.10
		Max	21.00	0.38	0.62	37.50
		Mean	18.33	0.29	0.50	34.47
		St dev	1.72	0.07	0.09	1.66
Allopatric threespine	8	Min	16.00	0.14	0.38	28.90
		Max	21.00	1.20	2.20	40.30
		Mean	18.25	0.54	0.87	35.01
		St dev	1.58	0.26	0.46	2.96
Allopatric threespine	9	Min	16.00	0.10	0.25	20.70
		Max	20.00	0.55	1.00	44.40
		Mean	17.64	0.23	0.41	25.91
		St dev	1.42	0.11	0.15	5.62
Sympatric threespine	10	Min	17.00	0.15	0.30	21.50
		Max	22.00	0.40	0.70	40.20
		Mean	19.61	0.28	0.46	30.53
		St dev	1.07	0.08	0.13	5.14

Table 68: Descriptive statistics for gill features measured on threespine stickleback populations collected in the spring.

Population	Site	Statistic	Gill Raker number	5 th Gill raker	8 th Gill Raker	Standard Length
Sympatric threespine	1	Min	16.00	0.13	0.19	19.10
		Max	22.00	0.44	0.94	46.40
		Mean	19.32	0.23	0.41	27.49
		St dev	1.73	0.09	0.21	8.49
Allopatric threespine	2	Min	15.00	0.10	0.10	17.40
		Max	20.00	0.55	0.65	42.90
		Mean	17.67	0.24	0.38	25.95
		St dev	1.21	0.10	0.12	4.74
Sympatric threespine	3	Min	17.00	0.05	0.10	17.00
		Max	24.00	0.35	0.65	41.00
		Mean	19.38	0.20	0.40	24.74
		St dev	1.78	0.08	0.15	6.85
Allopatric threespine	4	Min	14.00	0.10	0.10	18.5
		Max	21.00	0.65	1.00	53.7
		Mean	16.94	0.34	0.50	31.11
		St dev	1.56	0.14	0.21	8.89
Allopatric threespine	5	Min	16.00	0.10	0.25	17.20
		Max	20.00	0.90	0.95	49.20
		Mean	18.30	0.39	0.58	34.12
		St dev	1.27	0.19	0.22	9.22
Sympatric threespine	6	Min	16.00	0.10	0.20	17.30
		Max	21.00	0.30	0.60	33.10
		Mean	18.22	0.20	0.39	25.44
		St dev	1.17	0.07	0.12	4.65
Sympatric threespine	7	Min	17.00	0.10	0.20	18.60
		Max	21.00	0.30	0.45	35.20
		Mean	19.00	0.17	0.33	23.56
		St dev	1.33	0.06	0.06	3.54
Allopatric threespine	8	Min	15.00	0.10	0.25	16.20
		Max	21.00	0.40	0.70	32.90
		Mean	17.95	0.20	0.37	23.68
		St dev	1.32	0.08	0.11	4.02
Allopatric threespine	9	Min	16.00	0.10	0.15	16.70
		Max	20.00	0.30	0.50	28.80
		Mean	17.72	0.17	0.31	21.65
		St dev	1.46	0.06	0.08	3.27
Sympatric threespine	10	Min	16.00	0.05	0.15	1.24
		Max	22.00	0.35	0.60	1.46
		Mean	19.75	0.18	0.32	1.31
		St dev	1.14	0.07	0.10	0.06

Table 69: Descriptive statistics for gill features measured on threespine stickleback populations collected in the autumn.