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Evolution, Functional Morphology, and Asymmetry of Predator Defense in Stickleback

by

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B.Sc., University of Arizona, 1995

A Dissertation Submitted in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

We accept this dissertation as conforming
to the required standard

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Abstract

The relative importance of stochasticity and adaptation to biodiversity has long been of interest to evolutionary biologists. Multiple, closely related insular populations provide ideal natural experiments with which to determine the relative strength of these two factors. An example of one such system is the threespine stickleback, *Gasterosteus aculeatus*.

Threespine stickleback have predator defenses comprised of heritable lateral bony plates and large spines. Morphologically invariant marine stickleback have colonized freshwater habitats across the northern hemisphere, resulting in multiple independently derived freshwater forms highly variable in predator defenses. The islands of Haida Gwaii (the Queen Charlotte Islands), British Columbia, contain populations of freshwater stickleback that exhibit defensive variability comparable to the entire species, and vary in defensive asymmetry. Previous studies showed that numbers of defensive lateral plates and plate symmetry are positively correlated with the presence of predatory trout on these islands, but the effect of avian predators, another predator of stickleback, on plate number evolution remains unexplored. The purpose of this thesis is to determine whether plate number reduction is a defensive adaptation to avian predation, and to study functional implications of asymmetry in structural defenses from 115 natural populations.

Experiments showed that plate number reduction in threespine stickleback enhanced fast-start velocity; a possible advantage to fish being pursued by diving birds with similar swimming speeds. Avian injury frequencies increased in populations as plate numbers increased at low plate numbers, but did not increase in populations with plate numbers greater than ten. Trout injury frequencies decreased as plate numbers increased among populations, probably due to methodological problems. There were no correlations between avian or trout induced injuries and plate number within populations. Experiments indicated that selective predation of lateral plate numbers in stickleback by captive Hooded Mergansers was weak but insignificant, resulting in a very slight reduction in plate numbers after predation.

The degree of fluctuating asymmetry (FA) of defensive structures showed a geographical cline across the archipelago, being elevated in lowland stained ponds, and reduced in clear large lakes. FA of juveniles was not correlated with pH, conductivity, light transmittance, or lake size among populations. Asymmetric individuals did not have reduced survivorship, contrary to predictions from previous studies of FA. However, asymmetry of lateral plates was negatively correlated with plate number, and asymmetry of plates that provide structural integrity to the defensive spines was greatly reduced relative to 'non-structural' plates, supporting the hypothesis that biomechanically important traits have greater symmetry. Structural plate asymmetry decreased as water clarity, and the chances of capture by predators, increased, and when the degree of overlap between plates and spine supports increased. Plate asymmetry was weakly associated with susceptibility to parasitism, but only where overall plate numbers were low. This supports the hypothesis that FA/fitness correlations are trait and habitat specific, and that sensitivity of asymmetry to developmental instability can be reduced in biomechanically important traits.

In conclusion, reduction in armour in stickleback may be adaptive, but there is only weak evidence of selection by avian predators on lateral plates in the wild. Whether armour reduction is a direct adaptation to avoid capture or a cost-minimization strategy is not clear, but repeatability of reduced armour in habitats with diving birds, and the hydrodynamic benefit it provides, suggest the former. The associations between asymmetry and function suggest that asymmetry should be included in comparisons of divergent populations, as it lends insight into the functional implications of morphological diversity. Lastly, because multiple independent lineages have evolved similar phenotypes in similar habitats in stickleback, this research has reinforced the idea that local adaptation to unique habitats is the driving force of diversification.

Keywords: threespine stickleback, *Gasterosteus aculeatus*, adaptation, adaptive radiation, selective predation, allopatry, geographical isolation, island biogeography, functional morphology, hydrodynamic performance, fast-start, avian piscivore, fluctuating asymmetry, developmental stability, canalization, lateral plate reduction, Queen Charlotte Islands, parasitism, survivorship, environmental stress, homozygosity.

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Dedication

To my folks, all 4 of them, for encouraging me on my chosen path with love and support and wisdom.

Chapter 1: General Introduction

Island biogeography and adaptive radiation

The evolution of divergent forms in nature is frequently the result of geographical isolation. Since the times of Wallace and Darwin, the study of the origin of new species and the process of diversification has implicated geographical isolation as a crucial ingredient (Mayr 1963), although recent developments indicate that speciation in sympatry may be relatively common (Rice and Hostert 1993; Johannesson 2001). Geographical isolation prevents gene flow between closely related populations. This allows for the slow accumulation of genetic differences in response to selective factors that are specific to each habitat. Isolation has been widely accepted as an important component of divergence, and its power as a generative force has been exemplified by the study of island biogeography.

Islands provide a natural experiment with which to investigate the evolutionary outcome of populations that are isolated and exposed to unique selection regimes (Cox and Moore 1985). Compared to mainland populations, island populations are more subject to local conditions because of extrinsic barriers to dispersal. Local conditions on islands almost invariably differ from those on the mainland in a number of ways, primarily involving new combinations or types of predators, prey, and competitors (MacArthur and Wilson 1967). Colonizing populations are suddenly exposed to a unique set of selective pressures that can cause differential survivorship and reproduction among its individuals. As a result, evolution among island populations can be quite rapid, frequently resulting in adaptive radiation (Carlquist 1974).

Adaptive radiation is defined as the 'evolution of ecological and phenotypic diversity within a rapidly multiplying lineage' (Schluter 2000). Although there are exceptions, the colonizing lineage is typically a generalist that subsequently diverges into more specialist forms, each suitably adapted to its particular niche (Simpson 1953). The field of functional morphology has contributed vastly to our understanding of the diversity of forms we see in adaptive radiations, for it provides a mechanistic description of the relationship between phenotype and fitness that is applicable to ecological contexts (Wainwright 1994; Galis 1996; Irschick and Garland 2001).

There are several classic examples of adaptive radiation that have occurred on archipelagos. One of the best-known examples is the work by David Lack (1947) and later by Peter Grant (1986) on the finches of the Galapagos Islands. Ancestral finches colonized the islands approximately 3 million years ago and began the process of diversification among islands and habitats (Grant and Grant 2002). Present day variation among the finch species is morphologically and ecologically associated with the exploitation of different food resources that are specific to different habitats across the archipelago, resulting in differences in beak shape and width (Grant 1986).

A second example is the adaptive radiation that resulted in 500 species of haplochromine cichlids among and within lakes of central Africa in the last ~12, 500 years (Liem 1973; Meyer *et al.* 1990; Bouton *et al.* 1999). As in the Galapagos finches, African cichlid diversity is primarily a function of the utilization of different food types, resulting in the evolution of a wide array of feeding structures and behaviours.

A third example of adaptive radiation demonstrates diversification in locomotor capacity. Mainland ancestors of the genus *Anolis* have colonized islands of the

Caribbean and diverged into about 140 species (Williams 1969; Losos 1990; Jackman *et al.* 1997). These perching lizards have evolved into several 'ecomorphs' on the islands, each morphologically adapted to perching and locomotion on different vegetation types and shapes. Species of the same ecomorph that reside on separate islands share many ecological and morphological characteristics yet have evolved independently of each other (Losos *et al.* 1998). However, the exact mechanism of selection that has driven the divergence, whether it is competition (Losos *et al.* 1994) or predation (Losos and Irschick 1996) is unclear.

Most examples of adaptive radiation implicate variation in resource acquisition as the primary selective agent driving the divergence. There are relatively few examples of adaptive radiation that are the result of variation in predation regimes, presumably because island habitats are usually depauperate in predator species. This releases the colonizers from the pressures of selective predation, allowing them to expand morphological variability in ways that previously conflicted with effective predator avoidance (MacArthur and Wilson 1967). However, if the newly colonized habitat is relatively diverse in its assemblage of predators, selective predation may be the primary source of divergence among the resulting prey lineages.

Predation is widely recognized for its capacity to generate and maintain diversity (Paine 1966; Sih 1987; for review see Edmunds 1974; Curio 1976), and there are numerous examples in this literature (Brodie III 1992; Forsman and Appleqvist 1998; Stoks *et al.* 1999). A classic example is the evolution of colour and courtship variation among guppy populations in small streams in northeastern South America. These guppies vary in many characters pertinent to behaviour, morphology and life history as a function of predation intensity by larger fish (Endler 1995). Damselflies of the genus *Enallagma* have evolved different swimming speeds and body sizes depending on whether they reside in lakes with fish predators or dragonfly predators (McPeek *et al.* 1996), and divergence of closely related species of freshwater amphipods is associated with a spatial gradient in predation risk (Wellborn *et al.* 1997). Colour variation and crypsis in some gastropod species, such as those in the genera *Cepaea* and *Littorina,* is primarily driven by visual predators (Cain and Sheppard 1954; Reimchen 1979).

While these examples are an indication of the ubiquity of selective predation, there are few examples of adaptive radiation on archipelagos that are primarily a function of divergence in defenses against predators. One species that provides a striking exception to this is the threespine stickleback (*Gasterosteus aculeatus*).

Adaptive radiation of the threespine stickleback

Gasterosteus aculeatus has a circumboreal distribution consisting of marine, anadromous, and freshwater populations (reviewed in Wootton 1984). The marine form is ancestral and has repeatedly colonized streams, lakes, and ponds in northern temperate coastal regions, resulting in large numbers of geographically isolated freshwater populations (Penczak 1965; Hagen and Gilbertson 1972; Bell 1976; Moodie and Reimchen 1976). The marine form of *G. aculeatus* is homogeneous for several structural traits that protect the stickleback against predators, including a series of bony lateral plates running along the entire length of both sides of the fish, three dorsal spines, two pelvic spines and a small anal spine. The expression of spines and the number of plates is genetically determined (Hagen 1973; Hagen and Gilbertson 1973a; Avise 1976; Peichel *et al.* 2001). The highly conserved maximal expression of structural defenses in marine stickleback is in sharp contrast to the immense variation of these traits both among and within freshwater populations

Variation among freshwater populations of stickleback has stimulated an immense body of work on the evolutionary implications of adaptive radiation (see review in Bell and Foster 1994). Allopatry is a consistent geographical component of stickleback divergence, as most variation occurs between isolated populations. There are a few exceptions to this rule. In several southern coastal lakes of British Columbia, pairs of species reside in sympatry, and have exploited either benthic or limnetic resources due to character displacement (McPhail 1992; Schluter and McPhail 1992; McPhail 1993). Species pairs demonstrate similar patterns of divergence in morphology and ecology among lakes, indicating that common selection forces among habitats have resulted in convergent phenotypes. Stickleback also demonstrate parallel parapatric divergence between lakes populations and stream populations (Bell 1982; Reimchen *et al.* 1985; Thompson *et al.* 1997), with lake stickleback having slimmer bodies and greater numbers of more slender gill rakers than stream stickleback (Hagen and Gilbertson 1972; Gross and Anderson 1984). The existence of similar patterns of divergence in inherited traits across a wide geographical range is indicative that correlations between morphology and habitat are the result of natural selection (McPhail 1994; Johannesson 2001).

The majority of morphological divergence within this species is among isolated freshwater habitats, each serving as an 'island' of unique habitat parameters and selection forces. One system of freshwater habitats that exemplifies the morphological diversity found in this species exists on Haida Gwaii (the Queen Charlotte Islands), an archipelago on the coast of British Columbia (Figure 1). Common marine stickleback ancestors colonized streams, lakes, and ponds when glaciers receded at the end of the last ice age approximately 12,000 years ago (Moodie and Reimchen 1976; O'Reilly *et al.* 1993). Populations that utilize freshwater habitats of this relatively small archipelago demonstrate morphological variation in defensive structures and body size comparable to that found throughout the rest of North America and Europe (Reimchen 1994a). Multiple founding ancestral populations, originally similar in morphology, that colonized the archipelago rapidly and repeatedly evolved into similar morphotypes in similar habitats, indicating that selective forces are generating convergent forms (Reimchen *et al.* 1985).

There is overwhelming experimental and correlational evidence that structural defenses in stickleback respond to selective predation (Hoogland *et al.* 1957; Hagen and Gilbertson 1972; Moodie 1972; Hagen and Gilbertson 1973b; Moodie and Reimchen 1976; Gross 1977; Bell and Haglund 1978; Gross 1978; Bell and Richkind 1981; Reimchen 1988, 1992a, 1995, 2000; see review in Reimchen, 1994a). A common pattern among these studies is that the numbers of lateral plates and the length of the spines increase in habitats containing trout predators. Presumably, predation pressure from large fishes is more intense in marine than freshwater habitats, resulting in the ubiquitous expression of the complete suite of lateral plates in marine stickleback (Heuts 1947; Munzing 1963; Bell 1984; Reimchen 2000). While the overall pressure from predators may be reduced in freshwater systems, the variability in the intensity and nature

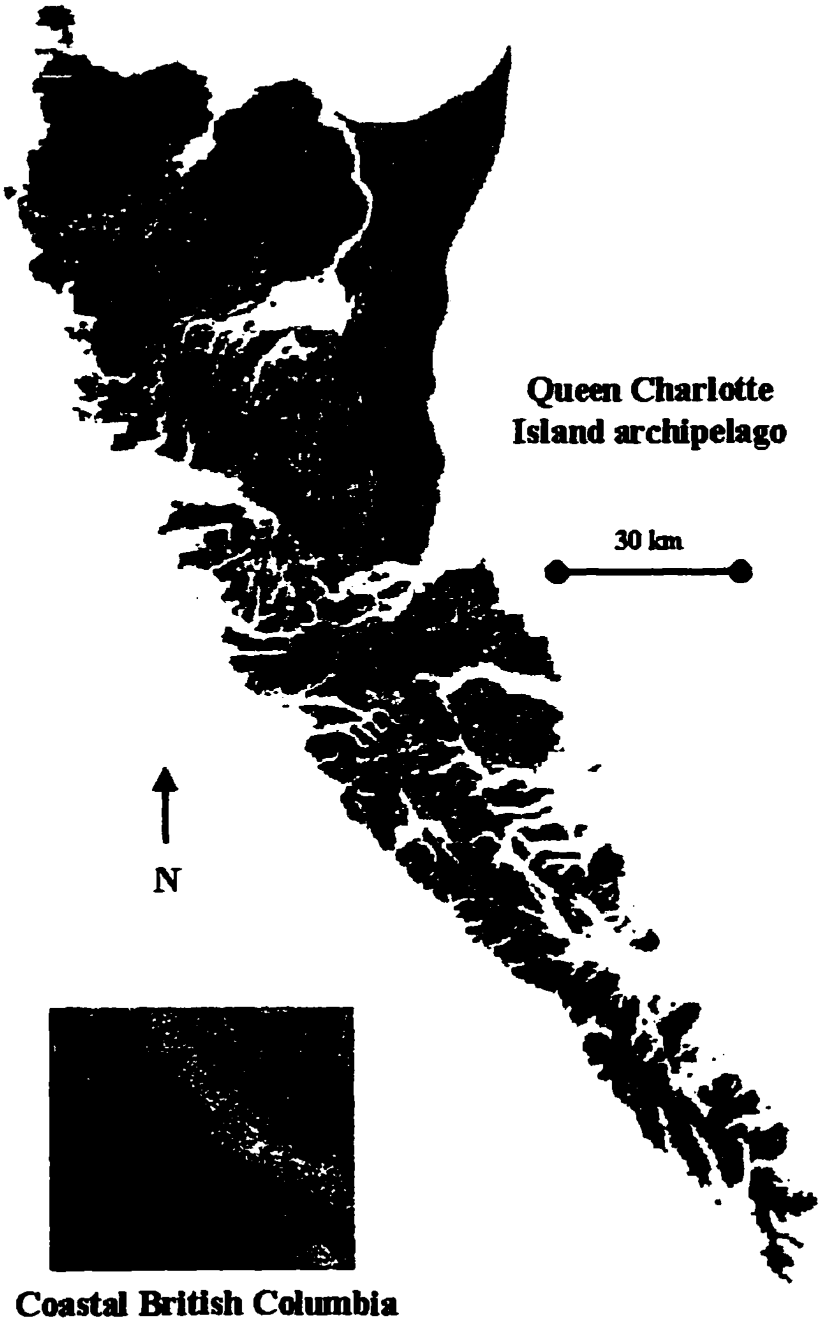

Figure 1. The Haida Gwaii (Queen Charlotte Island) archipelago.

of selection in freshwater habitats would increase due to extensive fragmentation. On Haida Gwaii, freshwater habitats encompass a large ecological range from large clear lakes that contain a high frequency of those predators commonly found in the marine environment, to small closed ponds with only a few macro-invertebrate predators on stickleback fry (Reimchen 1994a). A previous analysis of geographical variation in plate number among habitats on this archipelago found a strong association between plate number and the presence of predatory trout (Moodie and Reimchen 1976), although a considerable degree of unexplained geographical variance in armour persists. Trout are not the only predators of stickleback, and there is a large assemblage of stickleback predators associated with these water bodies (Reimchen 1994a), each potentially with their own selective characteristics. Second only to cutthroat trout, common loons, grebes and mergansers forage heavily on stickleback (Reimchen 1980; Reimchen and Douglas 1980, 1984a; Reimchen 1994a), making them a potential and relatively unstudied selective force on stickleback morphology that may account for this unexplained variance.

Body armour and its function have been described in many taxa, including but not restricted to porcupines, armadillos, gastropods, cladocerans, turtles, and several species of fishes (Edmunds 1974). These structural defenses decrease the predator's ability to ingest the prey, but present both a solution and a constraint on potential anti-predator techniques. Armour may be quite costly not only metabolically, but also to the locomotor capacity of individuals, as it is often heavy and inflexible. One would expect to find reduction in body armour in situations where the costs outweigh the benefits, for example, where the chemical constituents of the armour are missing from the habitat or where selective predation from a predator is lifted. Alternately, armour reduction may provide direct functional benefits of its own, regardless of reductions in cost. Reduction in armour may allow for the exploitation of and success in habitats that would otherwise be inaccessible. Primitive jawless fishes that lived in benthic habitats were heavily armoured, while those that lived in pelagic habitats and had active swimming life-styles had lost their body armour (Carroll 1988). Whether armour reduction in stickleback results from elevated costs associated with armour in some habitats, or because of direct advantages specific to armour reduction has not been addressed.

Reduction in the numbers of bony lateral plates and in the number and length of spines has occurred repeatedly in different populations on this archipelago (Reimchen 1994a). Studies of marine and freshwater stickleback from coastal B.C. and Haida Gwaii indicate that there are two genetic stocks of ancestral marine stickleback (O'Reilly *et al.* 1993), although they are morphologically indistinguishable. However, lateral plate reduction has occurred multiple times in both of these lineages in similar habitats on the islands (Deagle *et al.* 1996). This suggests that genetic bottlenecks and founder effects are probably not responsible for this attribute, but rather that it is an example of convergence in armour reduction.

It is my purpose, in the next two chapters of this thesis (chapters 2 and 3), to thoroughly investigate possible associations between reduction in morphological defenses of stickleback and avian predation. In chapter 2, I will conduct a geographical analysis of scars left on surviving stickleback by both diving birds and trout in order to determine if there are specific morphological attributes in stickleback that have successfully escaped following capture by these two predator types. Samples from 115 freshwater habitats in multiple watersheds on Haida Gwaii, which include the maximal range of armour expression, will be used for this analysis. In chapter 3, I will report the results of a series of experiments investigating whether hydrodynamic performance is dependant on armour expression in freshwater stickleback (Bergstrom 2002), and whether avian predators are generating selection on the numbers of lateral plates under experimental conditions. Determining if there are direct benefits to plate number reduction, and if there is selective predation on plate number by avian predators, may help to clarify the remaining unexplained variance in body armour among stickleback populations on the islands of Haida Gwaii.

Functional morphology and asymmetry

The identification of traits that have strong impacts on fitness is one of the key problems in evolutionary biology. The area of functional morphology has provided us great insight into this problem, as it serves to give biomechanical explanations for the relationship between traits and their function in ecological contexts. A relatively new area of research involving the evolutionary implications of bilateral asymmetry has shown additional promise in further clarifying the relative biomechanical importance of morphological traits.

Bilateral asymmetry can be manifested in a variety of ways. Examples of conspicuous laterality in morphology (directional or anti-symmetry; VanValen 1962; Palmer and Strobeck 1986) and behaviour are evident in claw size in decapods, sidedness in flatfish, coiling direction in gastropods, jaw structure in scale-eating cichlids, and handedness in humans. More subtle examples of asymmetry can be seen in traits that express fluctuating asymmetry (FA). FA is defined as random deviations from perfect symmetry of a bilateral trait, with the population-wide distribution of right-left differences being uni-modally distributed about a mean of zero (VanValen 1962; Palmer and Strobeck 1986). While directional and anti-symmetries are under direct genetic control and in most cases are adaptive, FA is generally thought to be associated with developmental instability (Mather 1953; VanValen 1962; Soule' 1967) and to reflect the failure of an individual to correct subtle and random departures from perfect symmetry during ontogeny (Waddington 1942; Zakharov 1992).

FA can be generated through a variety of means, including environmental stress, inbreeding depression and subsequent homozygosity, hybridization and subsequent disruption of co-adapted gene complexes, and strong directional selection (for review see Møller and Swaddle 1997). The aspect of FA that is of utility to functional morphologists is that it appears to reflect the biomechanical importance of a trait (Mather 1953; Palmer and Strobeck 1986; Balmford *et al.* 1993; Gummer and Brigham 1995; Clarke 1998); namely, it is reduced in traits whose utility is closely associated with fitness.

An earlier study of lateral plate variation in number and asymmetry among freshwater populations of threespine stickleback on Haida Gwaii indicated that plate number asymmetry varied among habitats, and was negatively correlated with the presence of predatory trout (Moodie and Reimchen 1976). Whether this correlation is the result of functional associations between predation and asymmetry or environmental and habitat associations is not clear. In chapters 4, 5, and 6 of this thesis, I will investigate the evolutionary and functional implications of asymmetry in this trait as well as several others that are associated with the predator defense apparatus in freshwater stickleback from Haida Gwaii. In order for accurate functional interpretations of asymmetry to be made, the possible effect of local environmental conditions on developmental stability and FA need to be taken into account. In chapter 4, I will do a correlational analysis of natural environmental variation in abiotic factors and the degree of FA among stickleback populations on this archipelago (Bergstrom and Reimchen in press). In chapter 5, I will investigate variation of plate asymmetry among 115 populations from these islands, and determine if high levels of asymmetry are more associated with some lateral plate positions than others (Bergstrom and Reimchen 2000). In chapter 6, I will compare the geographical distributions of asymmetries of different groups of lateral plates that may differ in their biomechanical importance. I will assess whether variation in the asymmetry of these traits among habitats on Haida Gwaii is associated with different ecological parameters that are indirect measures of the intensity of both trout and avian predators.

I predict that those characters that are crucial to the structural integrity of predator defenses will express relatively reduced asymmetry, and this reduction will be most evident in habitats where post-capture defenses are more important to the survival of the stickleback. This would indicate that trait asymmetry, in addition to trait mean, is an important character to include in the description of adaptive radiation and allopatric divergence, as it may lend functional insight into the evolutionary interpretation of trait variance among isolated habitats.

Isolated island populations are excellent systems with which to explore evolutionary questions, since their habitat characteristics tend to be relatively simple and easily defined. The isolation of large numbers of populations of freshwater stickleback on Haida Gwaii provides an ideal natural experiment with which to investigate the functional and evolutionary implications of armour reduction and asymmetry.

Chapter 2: Predator-induced injuries and morphological evolution in threespine stickleback

Introduction

Predation is an important agent of selection in wild populations (Edmunds 1974; Curio 1976; Pianka 1978). Predation is a selective agent only if predators are less than 100% efficient in their foraging efforts, thereby leaving behind selected survivors that can contribute to the next generation (Vermeij 1982; Smith and Lemly 1986). Non-lethal predator-induced injuries on prey are natural markers of individuals that have successfully escaped predation. One way of detecting selective predation at a single point in time is to compare attributes of injured individuals to a random, uninjured sample from the same population. If there are phenotypic differences between the two groups, this can implicate characters that may be directly or indirectly correlated with successful post-capture escape.

The use of predator-induced injuries to characterize predation among individuals and populations is common in the literature (Rand 1954; Shapiro 1974; Schoener 1979; Murtaugh 1981; Morin 1985; Reist *et al.* 1987; Reimchen 1988; Kowaleski and Flessa 2000), and can demonstrate large geographical variance in the presence of specific predators (Vermeij 1993). However, caution must be used in the interpretation of injury frequency variability since it is not a measure of total predation intensity, but only of successful escape after capture (Jaksic and Fuentes 1980; Vermeij 1982). Successful escape from other stages of predation, such as detection or pursuit, may not emerge from this type of analysis.

Freshwater populations of threespine stickleback on Haida Gwaii demonstrate immense variation in the expression of structural predator defenses (Moodie and Reimchen 1976; Reimchen 1994a). The numbers of lateral plates on each side of the body vary among these populations from complete absence to possession of the entire set of 30-35 (Moodie and Reimchen 1976; Reimchen *et al.* 1985; Reimchen 1994a). These lateral plates provide support for the dorsal and pelvic spines (Reimchen 1983), prevent osmotic shock resulting from tooth punctures (Reimchen 1992a), and interfere with pharyngeal handling by large predatory trout (Reimchen 2000). While previous studies of this and other stickleback communities have indicated that increased expression of structural defenses, in particular the numbers of lateral plates, is associated with the presence of large predatory fish (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1977; Bell and Richkind 1981), a considerable amount of geographical variation in these traits remains unexplained (Reimchen 1994a).

Selective pressure from other predators may be generating this variance. Avian predators occur in most aquatic habitats on this archipelago, and many species are regular foragers of threespine stickleback (Reimchen and Douglas 1984a; Reimchen 1994a). Greater plate numbers in stickleback are associated with geographical regions of these islands that contain large predatory fish (Moodie and Reimchen 1976), and plate number reduction is associated with habitats containing few trout, but many avian predators (Reimchen 1994a). Whether plate reduction is an adaptation to avian predation or simply a response to relaxed selection in the absence of predatory trout is presently unknown, but would become clearer with a comprehensive understanding of the relative selective pressures these two types of predators are placing on stickleback.

However, the relative contribution different predators are making to the cumulative selective forces on these populations is difficult to quantify. Even at one site, constant presence of an investigator throughout an entire year at the very least would be required to accomplish this task. One potential indirect way to estimate relative predation intensity at the subjugation stage by more than one predator is to assess the non-lethal injuries these predators leave on stickleback.

A study of predator-induced injuries on stickleback from Drizzle Lake, Haida Gwaii, demonstrated that naturally occurring trout and avian induced scars were present in approximately 10% of the fish, implying that a significant proportion of stickleback are escaping and surviving subjugation by both of these predators (Reimchen 1988). In particular, if predators leave distinctive marks that allow for discrimination of prey that survived capture by one specific predator type versus another, a comparison of morphology between the two prey groups may expose phenotypic differences.

The frequency of injuries in a population can be used as a conservative measure of the intensity of selection for post-capture escape mechanisms (Vermeij 1982). The first objective of this study is to correlate the frequency of trout and avian induced scars among populations of stickleback from this archipelago with the degree of structural defense expression. If trout are selecting for greater numbers of lateral plates during handling, but diving birds are not, I would expect to find a positive correlation among populations between lateral plate number and the frequency of trout scars, but not avian scars.

I will also calculate lateral plate number directional selection differentials between juvenile and adult fish in each sample. I expect to find positive plate differentials to increase in samples with greater trout scar frequencies, but not in samples with greater avian scar frequencies. Geographical distributions of selection differentials will also be examined. Post-capture defenses may be more heavily depended on in clear water and in deeper lakes where the chances of capture are greater, and therefore selection differentials may be more positive in these habitats.

In addition, I will compare injured and uninjured stickleback within each sample to determine if fish with trout and avian scars have fewer or more lateral plates than uninjured fish. This will be an indication of whether directional selection imposed by these predators is acting on lateral plate number within populations during the time range of sample collections. Geographical distributions of populations with greater or fewer plates in injured fish will also be examined.

Materials and Methods

Sample collection

T.E. Reimchen collected random samples of stickleback from lakes and streams throughout the archipelago during multiple expeditions between 1969 and 1997. Detailed habitat descriptions and collecting methods are published elsewhere (Reimchen *et al.* 1985; Reimchen 1989, 1992b, 1994b). Samples were collected between April and June using standard-mesh minnow traps placed in the littoral zones. Fish were fixed in 10% formalin and stored in 95% ethanol. One hundred and fifteen of these original samples were used for this study (Figure 2, 3). Samples were taken from sites in each of the three major geographical areas of the archipelago: the lowlands, the plateaus, and the mountains (Brown 1968; Figure 2).

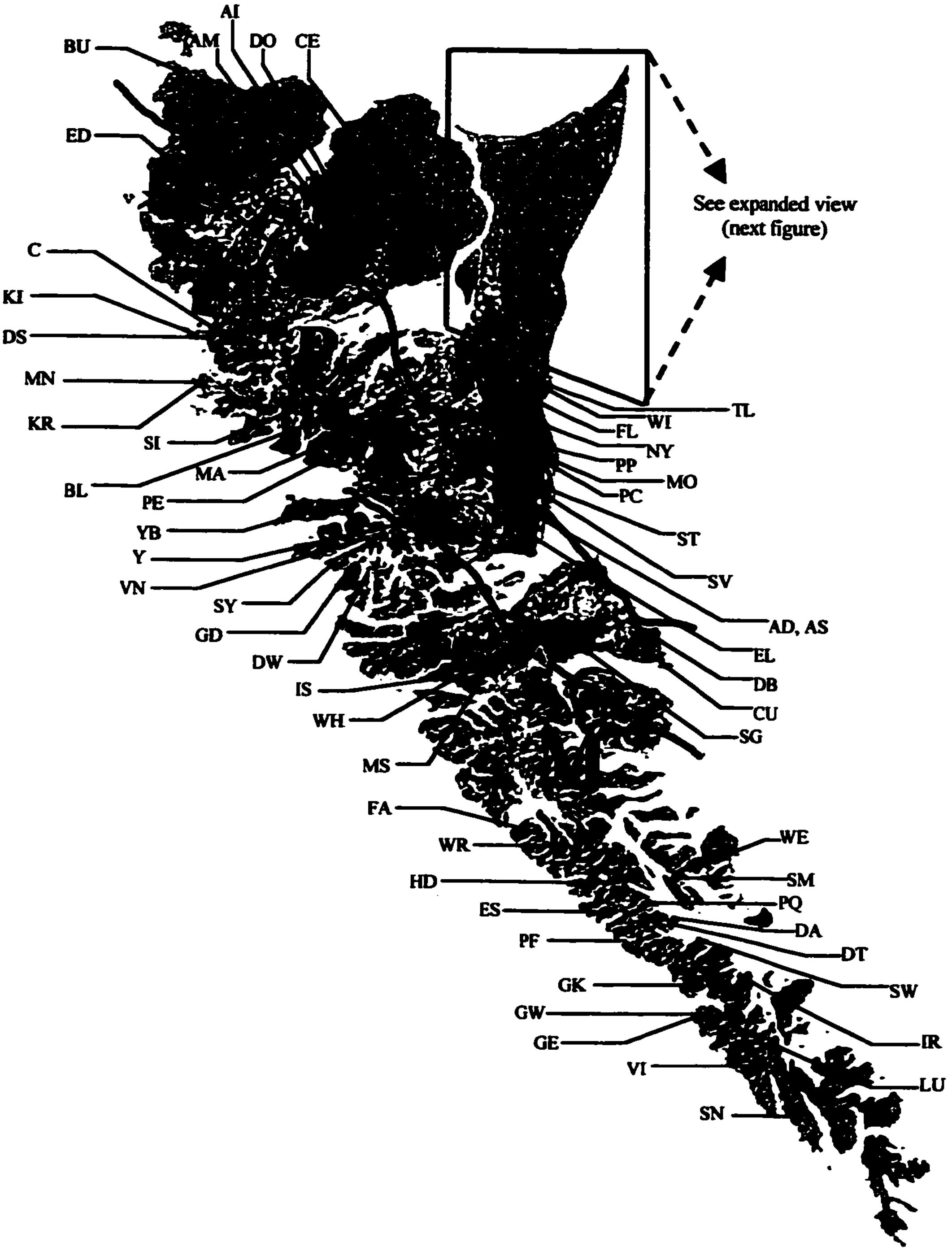
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Figure 2. Freshwater sites on Haida Gwaii where samples were collected for this study. N=115. L=lowland region, P=plateau region, M=mountain region (Brown 1968). Site names listed here and in figure 2 are abbreviated as follows: AD=Anderson North, AI=Ain, AM=Amber, AN=Anser, AS=Anderson South, B=Boulton, BD=Blue Danube, BF=Bigfish L., BL=Blackwater Cr., BR=Branta, BU=Bruin, C=Coates, CE=Cedar, CL=Clearwater, CP=Capeball, CR=Capeball R., CU=Cumshewa, C=Cygnet, D=Drizzle, DA=Darwin, DB=Debris, DI=Drizzle Inlet, DM=Dam, DO=Downtree, DS=Desolate, DT=Deadtoad, DW=Dawson, ED=Eden, EL=Elk Survey Cr., ER=Eriophorum, ES=Escarpment, FA=Fairfax, FL=Florence Cr., G2=Geikie 2 Cr., G3=Geikie 3 Cr., GC=Gold Cr., GD=Gudal, GE=Gowgaia East, GK=Goski, GO=Gosling, GR=Gross, GU=Grus, GW=Gowgaia West, HC=Hickey, HD=Hidden, HM=Heralda Middle, HR=Heralda Lower, HU=Heralda Upper, IM=Imber, IR=Irridens, IS=Inskip, JU=Juno, KI=Kiokathli, KM=Kumdis, KP=Kumdis Pond, KR=Krajina, KU=Kumara, LA=Laurel, LL=Lumme, LO=Loon, LS=Lumme Swamp, LU=Lutea, M=Mayer, MA=Marie, MC=Mica, MD=Midge, ME=Mesa, MI=Middle, MN=Menyanthes, MO=Mollitor, MS=Mosquito, NU=Nuphar, NY=New Years, ON=Otter North, OS=Otter South, OW=Oeanda R., PA=Parkes, PC=Pontoon Centre, PE=Peter, PF=Puffin, PP=Pontoon Tlell, PQ=Poque, PU=Pure, RI=Richter, RO=Rouge, S=Skonun, SA=Sangan, SB=Sangan Backwater, SE=Serendipity, SG=Skidegate, SI=Seal Inlet, SK=Skaters, SL=Slim, SM=Smith, SN=Snub, SO=Solstice, SP=Spence, SR=Spraint, ST=Stellata, SU=Stump, SV=Survey Cr., SW=Sundew, SY=Stiu L., TL=Tlell Estuary, VC=Vaccinium, VI=Victoria, VN=Van Inlet, WA=Watt, WE=Wegner, WH=White Swan, WI=Wiggins, WO=Woodpile, WR=Wright, Y=Yakoun, YB=Yakoun Backwater R.

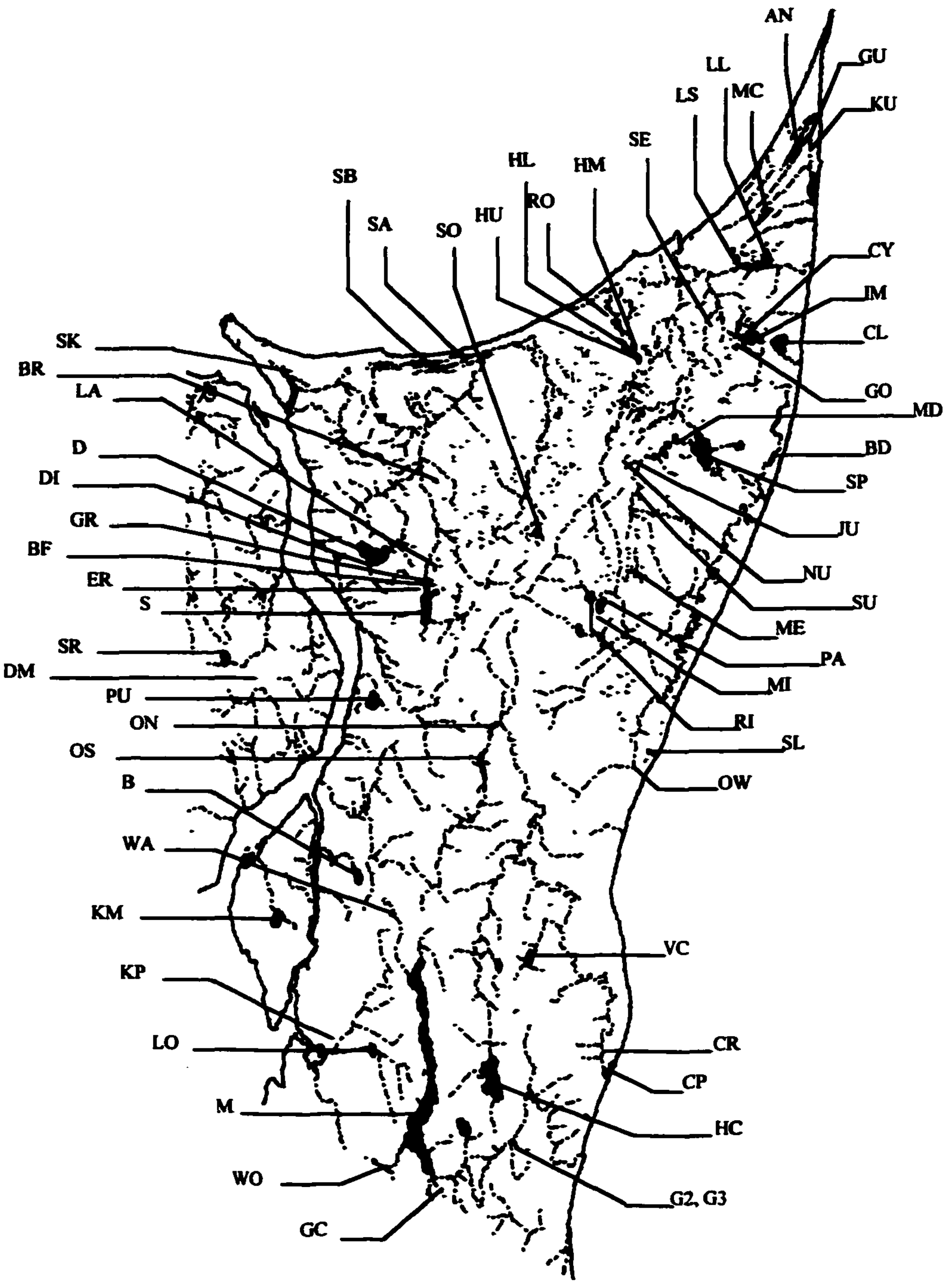

Figure 3. Expanded view of sample locations from northeastern region (cut-out from figure 2). See figure 2 legend for site names.

Water chemistry and lake data were collected from a subset of the localities at the time of collection, including percent light transmission at a wavelength of 400 nm (T400), and water depth (m). Water clarity and depth can affect reaction distance in predatory/prey reactions (Vinyard and O'Brien 1976). Structural defenses may be more important in clear, deep lakes than in stained, shallow ones. T400 values ranged from 30% to 99.6%. The range in T400 reflected the degree of water colour, or staining, due to tannins in the surrounding soil. Sites were categorized as heavily stained (T400<70%), moderately stained (70%<T400<85%), or clear (T400>85%). Wavelengths of 400 nm were used as they were the most variable between sites (Reimchen 1989).

Morphometrics

From each sample of *G. aculeatus*, up to 100 individuals comprising approximately 50 juveniles (ca. 35 - 45 mm SL) and 50 adults (> 45 mm) were measured for standard length (SL; mm) and body depth (BD; mm; Figure 4), extent of overlap between the lateral plates and the spine supports and the height and width of the ascending process (APH, APW; Figure 5), cross-sectional diameter with the spines erect (CD; mm) and the width of the first dorsal spine (WD1; Figure 6), the height and width of the ventral plate (VPH, VPW; Figure 7) total number of lateral plates per side ((R+L)/2), and sex. Sex was determined by examination of the gonads. Degree of overlap was categorized as major, partial, abutting, or no contact (Figure 8; Reimchen 1983), and an average overlap value calculated per fish. Overlap may be an indication of the effectiveness of the plates in providing lateral support to the spines (Reimchen 1983).

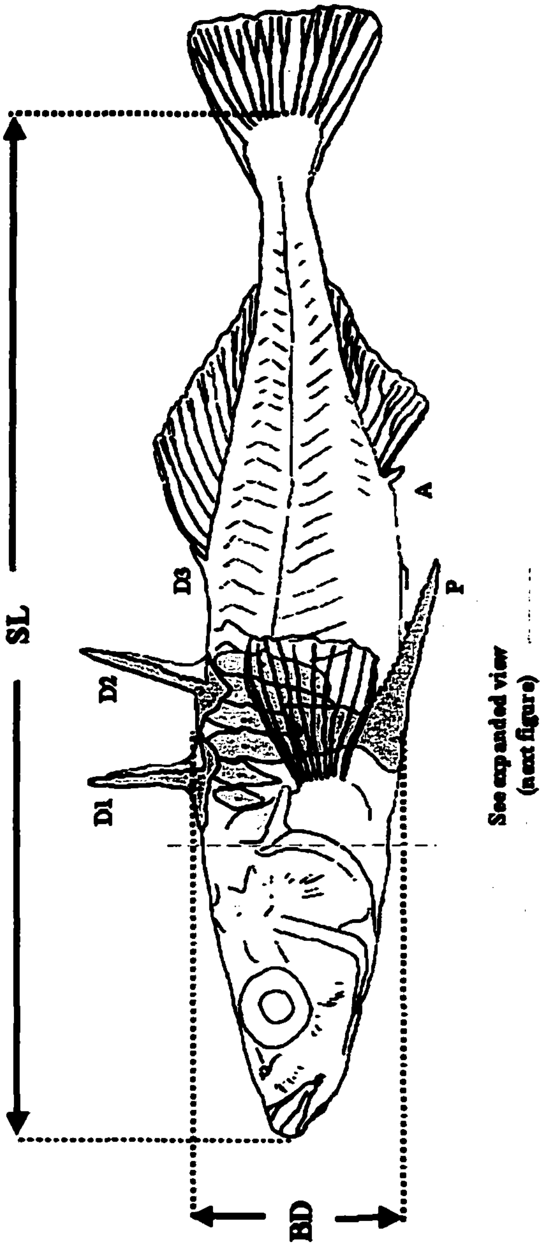

Figure 4. Sketch of stickleback showing the predator defense structures highlighted in grey. D1=1st dorsal spine; D2=2nd dorsal spine; D3=3rd dorsal spine; P=pelvic spine, left shown only; A=anal spine; SL=standard length; BD=body depth.

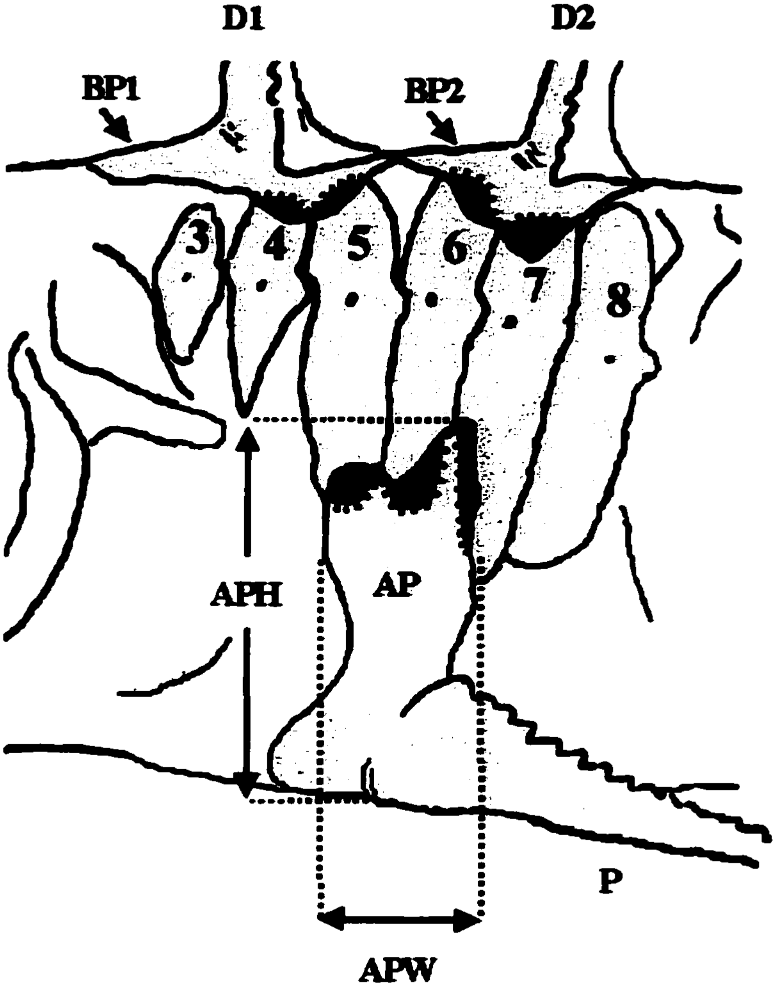

Figure 5. Close-up sketch of defensive apparatus in stickleback highlighted in light grey. D1=1st dorsal spine; D2=2nd dorsal spine; BP1=1st basal plate, BP2=2nd basal plate; P=pelvic spine, left shown only; AP=ascending process; APH=ascending process height; APW=ascending process width. Lateral plates 3 through 8 are present on the left side. Dark grey highlights show areas of overlap between the lateral plates and the basal plates, as well as the ascending process.

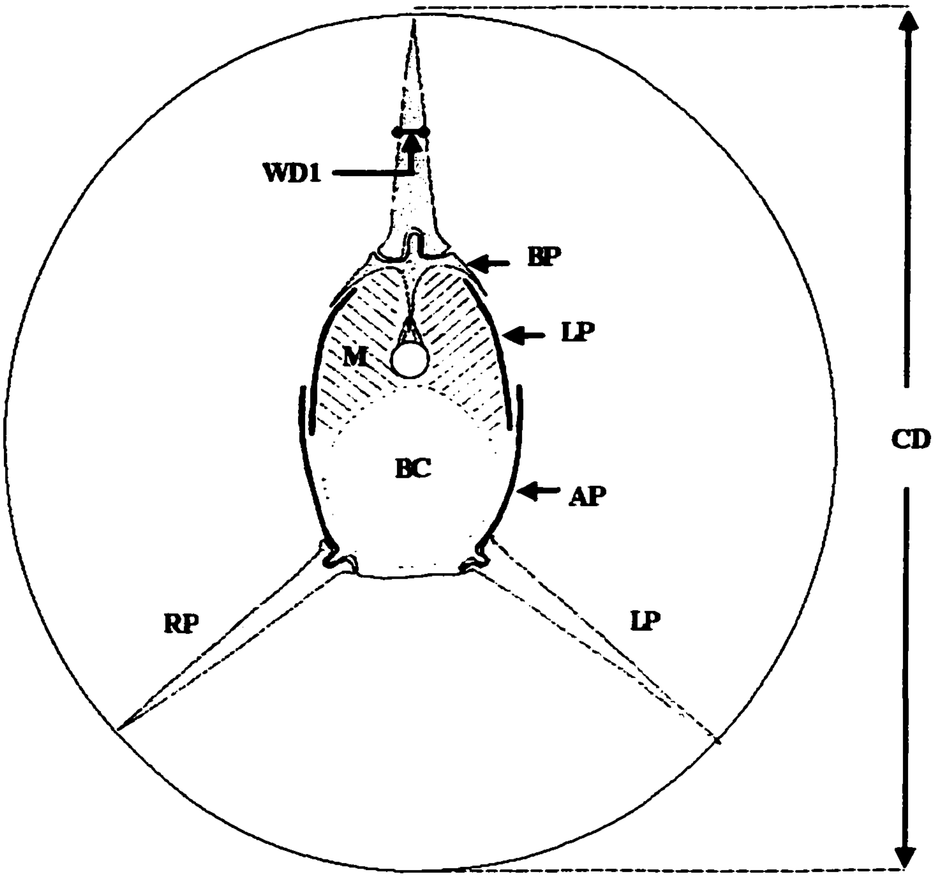

Figure 6. Cross-sectional view of stickleback, view from anterior to posterior (modified from Reimchen 1983). WD1=width of 1st dorsal spine, measured at half its height; BP=basal plate, LP=lateral plate, AP=ascending process; LP=left pelvic spine; RP=right pelvic spine; BC=body cavity; M=musculature; CD=cross-sectional diameter of fish with spines erect.

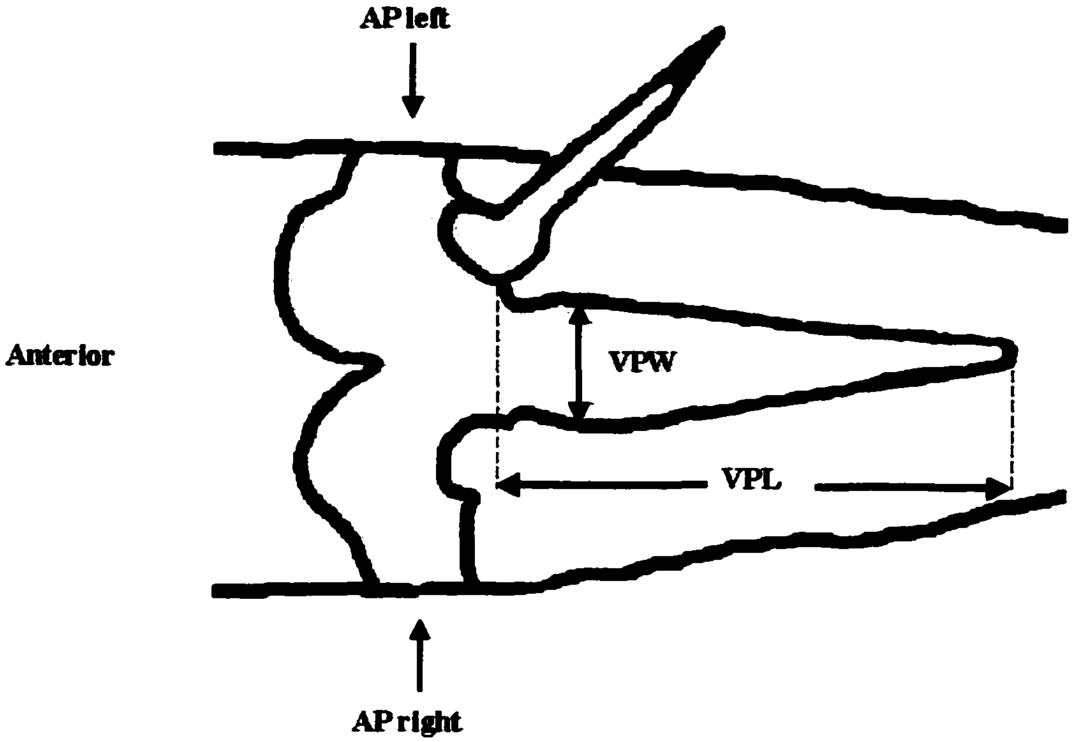

Figure 7. Ventral view of a stickleback, showing the ventral plate, left pelvic spine, and ascending processes. Right pelvic spine has been removed for easier viewing. AP=ascending process; VPL=ventral plate length; VPW=ventral plate width.

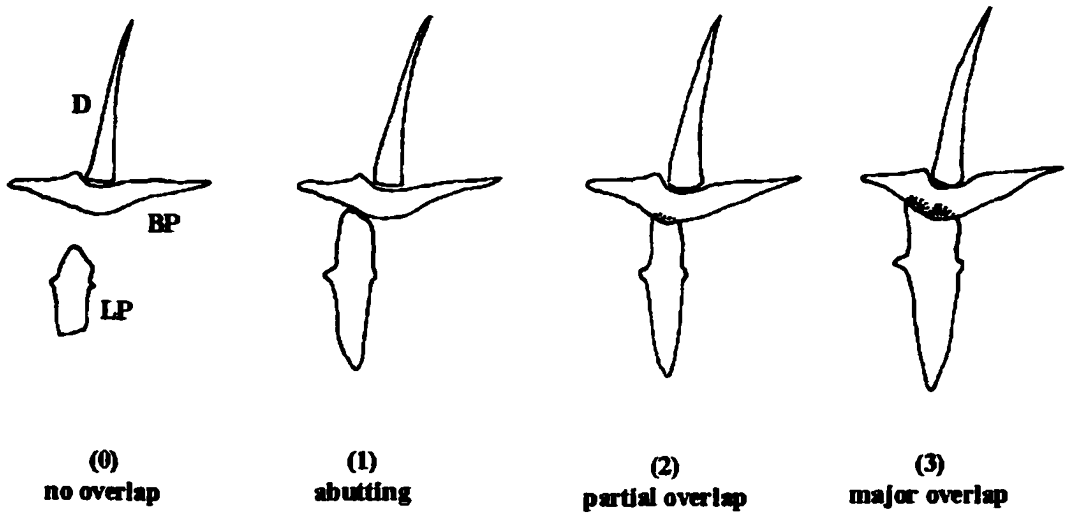

Figure 8. Different levels of overlap between a lateral plate and a dorsal spine.

LP=lateral plate; BP=basal plate; D=dorsal spine. Modified from Reimchen (1983).

Overlap was used as a categorical variable only. Lateral plate number was square-root transformed to approach normality.

After preliminary measurements were completed, 12 samples were randomly chosen for analysis of measurement error. From each sample, 20 individuals were randomly selected and re-measured. Repeatability, as measured by the intra-class correlation coefficient, was greater than 0.950 for 8 of the 10 traits, and greater than 0.830 for mean overlap and WD1 (Table 1).

I ran a principal component analysis of the correlation matrix including the variables SL, BD, APH, APW, CD, WD1, VPH, VPW, and plate number (square-root transformed). Overlap was excluded because it was a categorical variable. The analysis was run on sample means for adults only, in order to avoid confounding inter-population size variability with different age classes. The first and second components accounted for 72.4% and 16.6% of the variance among samples, respectively. The first component was a 'size' vector, of which cross-sectional diameter had the highest loading, and the second component was an 'armour' vector, of which lateral plate number had the highest loading (Table 2).

Scoring of injuries

Each stickleback was scored for the presence or absence of predator-induced injuries, as described in Reimchen (1988). Injuries were categorized as trout-induced if there were a series of narrowly spaced, curvilinear or comb-tooth shaped marks on the integument, as well as the presence of punctures (Figure 9). Injuries were categorized as

Table 1. Repeatability of morphometric characters, as measured by the intra-class correlation coefficient (r). $r=S^2A/(S^2+S^2A)$; $S^2=MS_W$; $S^2A=(MS_A-MS_W)/n_o$; $n_o=\#$ replicates (Lessells and Boag 1987). SL=standard length; BD=body depth; APH=ascending process height; APW=ascending process width; CD=cross-sectional diameter; WD1=width of 1st dorsal spine; VPL=ventral plate length; VPW=ventral plate width; PNUM=lateral plate number.

Trait	r	F	P
SL	.998	1239.82	<0.001
BD	.996	632.76	<0.001
Overlap	.904	19.81	<0.001
APH	.990	191.85	<0.001
APW	.981	55.64	<0.001
CD	.989	224.72	<0.001
WD1	.837	11.24	<0.001
VPL	.991	211.35	<0.001
VPW	.965	56.01	<0.001
PNUM	.999	17224.08	<0.001

Table 2. Principal component matrix, showing loading scores for each variable for the 'size' component as PC1, and the 'armour' component as PC2. These components explained 72.4% and 16.6% of the variation among populations, respectively. SL=standard length; BD=body depth; APH=ascending process height; APW=ascending process width; CD=cross-sectional diameter; WD1=width of 1st dorsal spine; VPL=ventral plate length; VPW=ventral plate width; PNUM=lateral plate number.

	Size (PC1)	Armour (PC2)
SL	.757	-.551
BD	.880	-.376
APH	.949	.059
APW	.844	.435
CD	.959	-.082
WD1	.930	.070
VPL	.958	.149
VPW	.895	.042
PNUM	.240	.907

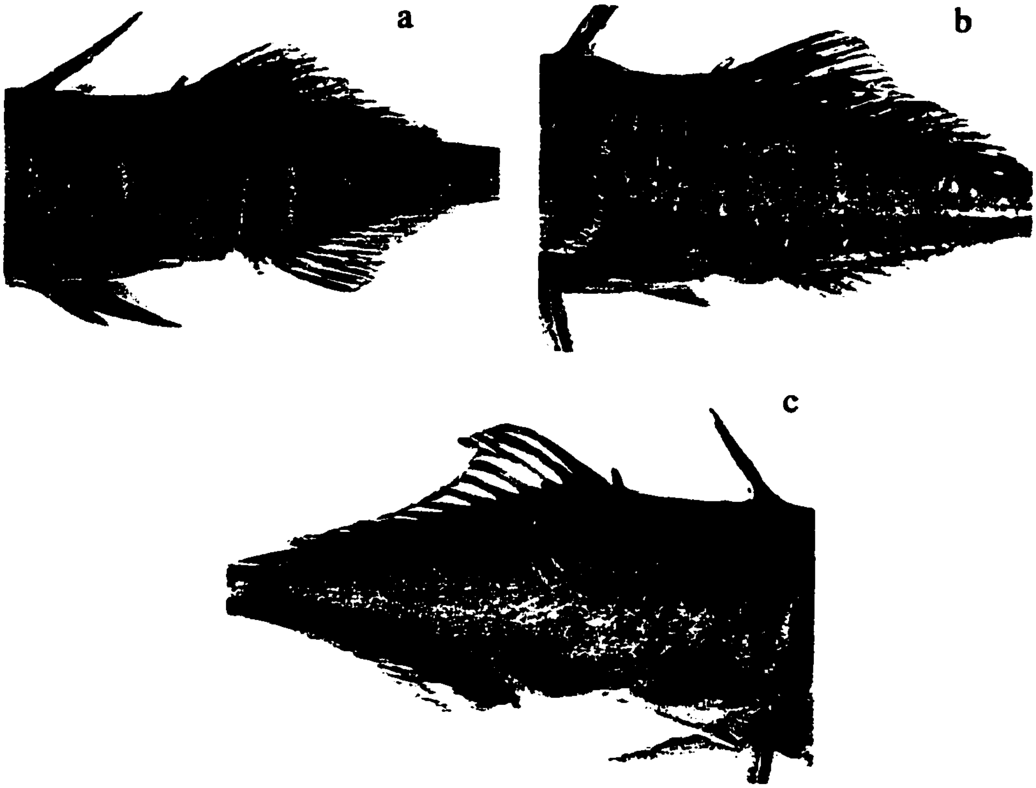

Figure 9. Typical avian scars (a,b) and curvilinear trout scars (c). Modified from Reimchen (1988).

bird-induced if there was a strong pair of parallel lines on both sides of the body, as well as broken lateral plates or ascending processes (Figure 9). Injury frequencies increase with age in stickleback (Reimchen 1988). Injuries were present in 26.5% of adult fish in all of the samples combined, but in less than 4% of the juvenile fish. Therefore, I restricted injury analyses to adults in order to avoid the confounding effects of age. Correlations between injury frequencies and degree of armour (both the 'armour' PC and lateral plate number) were assessed among populations on the archipelago.

Mean lateral plate number was compared between males and females within each sample. There were no significant differences between sexes within any sample after sequential Bonferroni corrections, although there were significantly more samples with greater plate number in males than females (62 vs. 37, respectively; X^2=6.313; df=1; P<0.025). Therefore, associations between injuries and plate number were done separately for each sex.

Lateral plate selection differentials (*i*) were calculated using juveniles and adults for both males and females within each sample (Endler 1986), and investigated for correlations with injury frequency rates among samples. I also examined the geographical distribution of the mean and direction of selection differentials as a function of region, water clarity, water depth, population lateral plate mode, and level of overlap.

Within each sample, shifts in mean lateral plate number were calculated between injured and uninjured fish for both sexes using independent sample t-tests. I examined the geographical distribution of the direction of plate shifts with injuries as a function of region, water clarity, and water depth.

Results

Geographical distribution of armour PC, plate number and injuries

Mean body armour increased significantly from the lowlands to the plateaus to the mountains (Kruskall-Wallis X^2=35.871; df=2; P<0.001; Figure 10). The armour PC was primarily a function of lateral plate number, which also increased significantly from the lowlands to the plateaus to the mountains for both males (X^2=53.022; df=2; P<0.001) and females (X^2=7.445; df=2; P<0.025).

The frequency of avian scars increased slightly with the armour PC among populations but the effect was not significant (r=0.125; P=0.200). The effect became stronger (but was still insignificant) when mean population lateral plate number was used instead of the armour PC (r=0.175; P=0.062). This effect was strongest when plate number means increased from 0 to 10 among populations (r=0.193; P=0.050), and did not persist at plate number means greater than 10 (r=0.156; P=0.594).

The frequency of trout scars decreased significantly as both the armour PC increased (r=-0.426; P<0.001) and plate number mean increased (r=-0.310; P=0.001) among populations. This effect was strongest when plate number means increased from 0 to 10 among populations (r=-0.186; P=0.062), however, among populations with plate means greater than 10 the effect was still negative but was not as strong (r=-0.314; P=0.274).

Lateral plate number selection differentials

There were no significant selection differentials for mean lateral plate number for males or females in any population after sequential Bonferroni corrections. The number

Figure 10. Distribution of PC2, an armour component, among populations of threespine stickleback on Haida Gwaii.● = top (most heavily armoured) 25% quartile; ◉ = 25–50% quartile; ⊛ = 50-75% quartile; ○ = bottom (least heavily armoured) 25% quartile.

of populations with positive plate number differentials did not differ from the number of populations with negative plate number differentials for males (52 vs. 42; X^2=1.064; df=1; P=0.302), however there were significantly more populations with positive plate number differentials than negative for females (55 vs. 34; X^2=4.955; df=1; P=0.026).

There were no significant correlations between the frequency of either avian or trout scars and signed lateral plate selection differentials for either males or females (all P>0.250). This was consistent when the analysis was restricted to samples with lateral plate modes of less than 10 as well as those with plate modes greater than 10 (all P>0.150).

Multi-way analysis of variance indicated that there were no significant differences in the mean signed lateral plate number selection differentials among geographical areas, levels of water clarity, levels of water depth, population lateral plate mode or degree of overlap for either males or females (Table 3). The degree of overlap may be an indication of the ability of the plates to support the spines, since lack of any overlap would prevent lateral buttressing from occurring. Degree of overlap approached significance for both sexes, with plate selection differentials gradually becoming more positive as degree of overlap increased.

In addition, logistic regression indicated that there were no significant differences in the likelihood of positive or negative lateral plate number shifts being associated with different geographical areas, levels of water clarity, levels of water depth, population lateral plate mode or degree of overlap for males or females (Table 4).

Table 3. Results of ANOVA of mean lateral plate number signed selection differentials for females and males. All homogeneity of variance tests were insignificant.

Factor	Mean plate number *i* (males)		Mean plate number *i* (females)	
	F	P	F	P
Geographical area	1.760	0.180	0.932	0.399
Water clarity	0.014	0.987	0.231	0.794
Water depth	0.985	0.405	1.930	0.133
Plate number	1.220	0.311	0.245	0.864
Overlap	2.270	0.057	2.254	0.059

Table 4. Results of logistic regression of the direction of lateral plate number selection differentials (increase or decrease with age) for males and females as a function of 5 factors.

Factor	Direction of plate number *i* (males)			Direction of plate number *i* (females)		
	B	Wald	P	B	Wald	P
Geog. area*	0.347	0.739	0.390	0.582	1.823	0.177
Water clarity	-0.021	0.003	0.953	-0.026	0.005	0.946
Water depth	0.221	1.484	0.223	0.311	2.725	0.099
Plate number	-0.065	0.033	0.856	0.479	1.196	0.274
Overlap	-0.064	0.027	0.869	-0.347	0.572	0.449

*(0=mountain, 1=plateau, 2=lowland)

Lateral plate number differences between injured and uninjured fish

There were avian injuries present in 49 samples, and trout injuries present in 87 samples. Of those samples with injuries present, on average avian scars were present in 6.2% of the fish (+/-6.8 SD), and trout scars were present in 18.3% of the fish (+/-14.8 SD).

For those samples that had trout or avian scars present, there were no significant differences in mean lateral plate number between injured (either avian or trout) and uninjured fish for either males or females after sequential Bonferroni corrections. For avian scars, samples were given a + score if there were elevated plate numbers in injured fish and a – score if there were reduced plate numbers in injured fish. The same was done for trout injuries in each sample.

There were no significant differences between the number of samples that had greater lateral plate numbers in trout-injured vs. uninjured stickleback and those that did not for either females (X^2=2.600; df=1; P=0.107) or males (X^2=0.778; df=1; P=0.378).

Logistic regression indicated that whether a sample had greater or fewer lateral plates in trout-injured fish was not predicted by geographical area, level of water clarity, level of water depth, or lateral plate mode for either males or females (Table 5). Females showed a decrease in the likelihood of a sample having elevated plate numbers in trout-injured fish as degree of population overlap increased, but there was no significant effect in males.

Table 5. Results of logistic regression of the direction of plate number shifts between trout-injured and uninjured fish for females and males as a function of 5 factors.

Factor	Direction of plate number shift (males)			Direction of plate number shift (females)		
	B	Wald	P	B	Wald	P
Geog. area*	-0.511	0.870	0.351	-0.394	0.447	0.504
Water clarity	-0.037	0.006	0.937	0.628	1.422	0.233
Water depth	-0.110	0.310	0.578	-0.066	0.074	0.785
Plate number	-0.327	0.415	0.519	0.737	1.923	0.165
Overlap	0 .466	0.798	0.372	-1.444	6.240	0.012

*(0=mountain, 1=plateau, 2=lowland)

There were no significant differences between the number of samples that had greater lateral plate numbers in avian-injured vs. uninjured stickleback and those that did not for either females (X^2=1.125; df=1; P=0.289) or males (X^2=1.960; df=1; P=0.162).

Logistic regression indicated that whether a sample had greater or fewer lateral plates in avian-injured fish was not predicted by geographical area, level of water clarity, level of water depth, lateral plate mode or degree of overlap for either males or females (Table 6).

There were no significant correlations between the direction of lateral plate selection differentials and direction of plate number shifts between injured and uninjured fish for trout or avian injuries in either sex (Chi squared: all P>0.170).

Table 6. Results of logistic regression of the direction of plate number shifts between avian-injured and uninjured fish for females and males as a function of 5 factors.

Factor	Direction of plate number shift (males)			Direction of plate number shift (females)		
	B	Wald	P	B	Wald	P
Geog. area*	-0.953	0.985	0.321	0.784	0.986	0.321
Water clarity	0.231	0.077	0.782	0.184	0.082	0.775
Water depth	-0.001	0.001	0.996	0.275	0.858	0.354
Plate number	-0.194	0.065	0.799	1.143	1.832	0.176
Overlap	1.058	0.965	0.326	-0.514	0.398	0.528

*(0=mountain, 1=plateau, 2=lowland)

Discussion

This study demonstrated a strong cline in the expression of lateral plates in freshwater stickleback from the lowlands to the plateaus to the mountains on this archipelago. I expected to find trout scar frequencies to increase with this cline, and to find no effect with avian scars. However, trout scars decreased in frequency as plate numbers increased, and avian scars showed a suggestive but weak increase. There could be several explanations for this.

While the presence of compression-type avian scars are usually unambiguous and very easy to see even on top of lateral plates (indeed they often break the plates), trout scars are subtler and less likely to be visible on top of a hard bony scute. For this reason, there may be fewer trout scars scored in samples with more plates simply because they are less visible. If this were the case however, I would expect to see fewer plates in fish with trout scars *within* the majority of samples. Sixty percent of samples had fewer plates in females with trout scars, and 44% had fewer plates in males with trout scars, neither of which were the significant majority. However, lateral plate variation among populations is far greater than that within most samples, providing greater opportunity to make this type of scoring error. In addition, injuries in a species of tropical reef fish can heal and become indistinguishable after a couple of months (Foster 1985). While integument-healing rates have not been determined in stickleback, light trout-type injuries left on top of a plate will probably be shallower than those left directly on the soft integument, and therefore might heal more quickly.

Both the slight increase in avian scars and the decrease in trout scars as plate numbers increased were only significant (or approached significance) among those samples with lateral plate modes ranging from 0 to 10. With avian scars, this is to be expected. Diving birds swallow their prey head first (Sanford and Harris 1967; Douthwaite 1971; Reimchen and Douglas 1984b), and the large increase in cross-sectional diameter of a stickleback with erect spines can impede handling. In ponds on Haida Gwaii lacking predatory trout but large enough to contain diving birds, most stickleback have between 2 and 4 of the structurally important lateral plates, as well as the large spines (Reimchen 1994a). This suggests that integrity of the spines may be beneficial to stickleback subject to avian predation. Increasing plate numbers from 0 to 10 includes the addition of the structurally important plates that give lateral support for the spines, which may provide a selective advantage in these habitats. The additional acquisition of lateral plates beyond 10 may be detrimental to fast-start performance and selected against by diving birds (Bergstrom 2002), and this is consistent with the levelling off of the rate of avian injuries with increasing plate numbers. However, based on experimental evidence (Reimchen 1992a, 2000), I would expect the addition of lateral plates beyond 10 to be beneficial in trout predation regimes, as they protect the integument against puncture.

An alternative explanation is that the number of samples with 10 or fewer plates far exceeded the number of samples with more than 10 (101 vs. 14, respectively), and therefore may be resulting in stronger significance for both avian and trout scar effects simply because of greater sample size.

The more ambiguous trout-type scars may in fact be induced by other non-trout sources. Aggressive conspecific encounters are common in stickleback, especially in the breeding season and where there is strong competition for territories (Bakker 1994;

Rowland 1994). While small half-moon shaped scars are easily categorized as conspecific-induced based on their size, the larger ones may have been occasionally mistaken as trout-induced. There is a slight but significant positive correlation between the frequency of trout scars and the frequency of clear conspecific scars (r=0.212; P=0.023). While the frequency of conspecific scars does increase in populations with fewer numbers of plates, the increase is weak and insignificant (r=-0.103; P=0.272). If I mistook conspecific scars for trout scars, this may partly explain the increase in injury frequency as plate numbers decrease, at least for plate modes of 10 or less, as this may be an indication of increased competition for territories. Male stickleback nest and compete for territories in shallow littoral areas (Rowland 1994). Among populations with plate modes of 10 or less, there is a significant positive correlation between plate number and the proportion of littoral habitat (area/depth) in a locality (r=0.255; P=0.016), although there is no effect with plate modes of greater than 10. However, residual littoral habitat, with the effect of plate number removed, had no effect on the frequency of either trout scars (r=0.098; P=0.359) or conspecific scars (r=0.028; P=0.792) among populations with fewer or more than 10 plates (both P>0.150).

In addition, the proportion of injuries in a population at any one time may be a poor indicator of the average selection for subjugation escape acting in the population over many generations. If there is any ambiguity in the classification of injury type, or if there is unequal healing rates of injuries among individuals, this can obscure detectable effects even further.

This analysis only assessed morphological correlations with successful post-capture escape. There may be morphological associations with successful search or pursuit escape correlated with plate number that are not accessible with analysis of injuries. In some instances for example, there may be selection for plate reduction if it enhances fast-start swimming performance (Reimchen 1995; Bergstrom 2002). If multiple predators, such as trout and diving birds, are in a single locality there may be opposing directional selection on plate numbers if the predators have different foraging styles and levels of efficiency. This would likely confound overall comparisons between morphology and injury among populations.

It was surprising that there were no predictable geographical distributions to plate number selection differentials. If greater plate number in a population indicates a history of selection for post-capture escape structures, then I would expect to find positive selection for plate numbers in those samples. However, there were no correlations between the mean or direction of selection differential and plate number as well as other habitat characteristics that would affect the chances of capture (water clarity and depth). For signed mean selection differentials however, there was a weak increase in populations with greater overlap between the plates and the spine supports for both males and females (Table 3). Level of overlap is an indicator of how effective the plates are in providing support to the spines. If there were little to no overlap, selection for plate increase would probably be less likely to occur than if there were more overlap since the biomechanical advantages of the plates would be less pertinent.

However, in general lateral plate selection differentials were small and demonstrated no predictable geographical distribution. The lateral plate number variation among populations we find on the archipelago now are the result of ~12,000 years of evolution. Perhaps stronger directional selection differentials would have been detected immediately after colonization of a novel, freshwater habitat. The weak differentials at present are perhaps an indication that a state of equilibrium between the morphology of the stickleback and their habitats has been reached. I would expect to find stronger directional selection in some mainland freshwater stickleback, as these populations are more likely to experience winter freeze kill-off and subsequent re-colonization more frequently than waters on Haida Gwaii that rarely freeze over (Reimchen pers. comm.).

However, there is evidence that seasonal fluctuations in plate numbers may be responding to opposing selection forces generated by trout and diving birds within the same population (Reimchen 1995), and vertebral numbers respond to opposing selection forces within the same population at different ages (Swain 1992). As the abundance of diving birds increases relative to the abundance of trout in the summer, plate numbers decrease, then increase again in the autumn when trout once again become the dominant predator (Reimchen 1995). If phenotypic shifts in plate numbers are occurring more than once per generation, classical comparisons between two cohorts may not detect them. Selection differentials at one point in time will reflect both these short and long term effects, rendering meaningful phenotypic comparisons among unique habitats difficult, especially in populations that are close to equilibrium (Endler 1986).

Populations were more likely to have greater plate numbers in fish with trout injuries as the degree of overlap decreased, but only for females (Table 5). This is in contrast to my expectation and conflicts with the trend for plate differentials to become more positive as overlap *increased* (Table 3). In order for this result to be taken at face value and interpreted in an adaptive context, we would have to conclude that more plates provide a greater chance of escape from trout when overlap levels are *lowest*. This is contrary to what we know about the functional morphology of structural defenses in this species. However, while the effect for males was insignificant, it was in the predicted direction, and males may be under stronger selective pressure from trout than females (Moodie 1972). The enigmatic effect with females would be better addressed with experimental work that specifically measured the effect of the interaction between overlap and plate number on survivorship of both sexes under trout predation.

Clearly, the phenotypic comparison of injured and uninjured individuals with the aim of resolving selective pressure is problematic. Crucial is the ability to clearly distinguish the source of injury, as is evident from the patterns found with avian vs. 'trout' induced scars. While avian scars are strong and usually quite severe, 'trout' scars are subtle and may be healing more quickly, particularly when directly on top of a bony scute. The study would be improved by an estimate of the healing rates of different types of injuries in stickleback, as well as a clearer categorization of true trout scars.

However, some interesting trends have emerged from this study. Avian scars increase with plate numbers, but only up to plate modes of 10, beyond which no significant increase is found. This is consistent with the prediction that the integrity of spines is important to the chances of escape from avian predators after capture. However, the addition of more than 10 plates might pose a hydrodynamic cost that increases a stickleback's chances of being *captured* by diving birds (see next chapter), which may be responsible for the lack of correlation between avian injury frequency and plate number among populations with plate means greater than 10. Indeed, stickleback with more plates have decreased fast-start velocity (Bergstrom 2002). In addition, it appears that selection for greater plate numbers is strongest when overlap, and therefore the opportunity for spine support, is greatest. Yet, the low selection differentials for plate number and their lack of any predictive geographical distribution implies that these populations are at equilibrium with the selective regime of their habitats, and that differences among samples are reflecting the history of gradual selection over the last 12,000 years.

Chapter 3: Fast-start swimming performance, avian predation, and lateral plate reduction in threespine stickleback

Introduction

The reduction of defensive armour in derived groups can evolve due to historical factors, limiting nutrients, or to a decrease in predation intensity. In addition, armour reduction may result in biomechanical benefits that are specific to a recently colonized habitat. Marine threespine stickleback (*Gasterosteus aculeatus*) have colonized freshwater habitats across the northern hemisphere, resulting in large numbers of isolated populations in coastal regions (Bell 1976; see review in Wootton 1984). Stickleback have a predator defense apparatus consisting of dorsal and pelvic spines as well as a series of heritable, bony lateral plates (Hagen 1973; Hagen and Gilbertson 1973a; Avise 1976). The plates are used for protection against punctures from predatory fish (Reimchen, 1992a), and support the adjoining dorsal and pelvic spines (Reimchen 1983). This allows the spines to remain erect and to deflect lateral pressure during manipulation by predators (Hoogland *et al.* 1957).

Freshwater stickleback exhibit immense variance in lateral plate numbers, ranging from plate-less to possession of the complete set of 30-35 (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1977; Reimchen et al. 1985; Reimchen 1994a). This variance is in contrast to the homogeneous presence of the completely plated phenotype among marine ancestral stickleback (Bell 1976; see review in Wootton 1984).

At a climatic scale in freshwater populations, low plate morphs are associated with higher temperatures and reduced annual temperature fluctuations (Hagen and

Moodie 1982; Baumgartner and Bell 1984). Within drainages, low plated morphs tend to reside in slow-moving, low gradient habitats, while completely plated morphs are associated with fast-moving, high gradient habitats (Hagen 1967; Bell 1982; Baumgartner and Bell 1984; Baumgartner 1992). At smaller scales, localized environmental and selective forces have been shown to be predictors of lateral plate expression. Low dissolved calcium is associated with reduced armour (Giles 1983; Francis *et al.* 1986; Bell *et al.* 1993), and the absence of fast predatory fish is associated with fewer numbers of lateral plates (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1977; Bell and Richkind 1981). Reimchen (1992a) proposed that plate reduction might be an adaptation to certain predation regimes if it improves fast-start performance. Experimental demonstration of a negative relationship between swimming performance and lateral plate number in stickleback would support the hypothesis that plate reduction improves hydrodynamic efficiency in the wild.

Several studies have addressed trade-offs between body armour and hydrodynamic performance in stickleback. Nelson (1969) suggested that short spines in brook stickleback (*Culea inconstans)* could be the result of natural selection for escape during pursuit. Experiments have confirmed that *C. inconstans* individuals with reduced pelvic spine development have superior burst velocity and acceleration both among populations (Andraso and Barron 1995) and within (Andraso 1997). A comparison of swimming performance between anadromous and freshwater threespine stickleback found that freshwater fish had better fast-start performance than anadromous completely plated fish (Taylor and McPhail 1986). The low plated limnetic and benthic species of stickleback in Paxton Lake, British Columbia both had significantly higher velocities than completely plated marine stickleback (Law and Blake 1996).

However, none of these studies have specifically addressed the effect of lateral plate expression on swimming performance, and the studies with *G. aculeatus* have not compared swimming performance among individuals within populations. Variation in swimming performance among lateral plate morphs that come from different populations is likely to be confounded by other morphological and behavioural effects. Comparing swimming performance among individuals within populations would minimize variation due to these factors.

Lateral plate reduction is correlated with the absence of large fish predators in freshwater populations of Haida Gwaii, and plate numbers are greatly reduced in the northeastern lowland bog region of the archipelago (Moodie and Reimchen 1976). In addition to a reduction of predatory fish, the lowland region is characterized by an increase in the prevalence of avian piscivores (Reimchen 1994a), which place considerable foraging pressure on stickleback (Reimchen 1980, 1994a). Within Drizzle Lake on Haida Gwaii, decrease in plate numbers is concordant with annual seasonal increase in the relative activity of avian to trout predators (Reimchen 1995). These correlations suggest that, contrary to fish predators, avian predators may be placing either no selection pressure on plate numbers or selection for plate reduction. However, there have been no experimental demonstrations of selective predation by diving birds on plate numbers in stickleback.

The purpose of this study is twofold. First, I will assess variation in swimming performance in relation to lateral plate number within two populations of *G. aculeatus* that are polymorphic for this trait. Second, I will determine if captive Hooded Mergansers (*Lophodytes cuculatus*), a common predator of stickleback, are placing detectable selection on the numbers of plates under experimental conditions. If there is an opportunity for escape from pursuit by the mergansers, and if lateral plate number affects swimming performance, I would expect to see a shift in the mean plate number of samples before and after predation.

Materials and Methods

Assessment of swimming performance

Sample collections and experimental protocol

Gasterosteus aculeatus were collected from Fairy Lake, Vancouver Island, British Columbia and from Tlell Pond, Haida Gwaii, British Columbia, on May 15, 1999, and June 12, 2000, respectively. Fish from each locality were trapped with baited minnow traps within 3 m of shore. This restricted the analysis primarily to stickleback with benthic feeding modes and reduced the risk of confounding the study with variance in swimming performance between benthic and pelagic stickleback. Locality samples were kept in separate 50 gallon holding tanks in the aquatic facility at the University of Victoria.

The experimental tank was a 45 cm by 35 cm fibreglass container filled with 5 cm of de-chlorinated water and with a laminated 1.0 cm^2 grid placed on the bottom. A Hi-8 Sony Sports video camera was erected 1.5 m. directly above the centre of the tank. Stickleback were randomly selected from the collected samples and placed individually into the experimental tank. Fish that had obvious injuries and females that were visibly ripe were excluded from the experiment. After placement into the experimental tank, the fish were allowed to acclimate for 5 minutes. After this time had elapsed, I waited until the stickleback were stationary and facing away from the side of the tank, started the camera and then thrust the handle of a fish net directly towards the head in order to initiate a flight response. This technique has been used in other studies of burst flight performance in fishes and has produced conclusive results (Harper and Blake 1990; Brainerd and Patek 1998). The startle response in teleosts is an all-or-nothing response activated by one Mauthner cell and its spinal motor neurone pool (Eaton *et al.* 1977). Therefore, because of the threshold nature of the response, there should not be a graded relationship between degree of the response and intensity of the stimulus.

After the flight response had ceased, the video recorder was turned off and the fish allowed 3 minutes to recover before given the visual stimulus again. If the stickleback became unresponsive to the stimulus, no more replicates were performed for that individual. Between 1 and 4 replicates were performed per fish. Twenty-one fish were tested from Tlell Pond and 42 from Fairy Lake. All individuals were tested at water temperatures of 18-19° C.

After the burst swimming tests were completed, the fish were removed from the tank and lightly anaesthetised. Standard length (SL) in mm, body depth in mm, and the average number of lateral plates per side [(R+L)/2] were recorded using a dissecting microscope, after which the fish were returned to their holding tank. Body depth was measured at the point of maximum dorso-ventral depth of each fish and did not include spine or fin size. In stickleback, this is at the point just anterior to the base of the second dorsal spine. Mean lateral plate number was square root transformed. Means and variances of SL, body depth and lateral plate number did not significantly differ between Fairy Lake and Tlell Pond samples (Table 7). Minimum SL was 38.5 mm in the Fairy Lake sample and 45.1 mm in the Tlell Pond sample, both of which were above the minimum SL at which lateral plate development is complete (Bell 1981).

Video and statistical analysis

Video data were digitized and split frame images were de-interlaced, allowing for an image frequency of 60 frames/sec. Video image analyses were completed in Scion Image. Maximum velocity and maximum acceleration reached during the Mauthner-initiated flight response were calculated for each individual fish replicate, as well as the distance travelled (displacement) in mm in the first 6 frames (0.100 seconds). Other studies of burst swimming performance in *G. aculeatus* have found that maximum velocity and acceleration were reached in the first 0.040 to 0.050 seconds (Taylor and McPhail 1986; Law and Blake 1996).

Mauthner-initiated startle responses are preceded by a c-curvature of the body (Eaton *et al.* 1977). The initial angle of this curvature is positively correlated with burst velocity in *C. inconstans* (Andraso and Barron 1995; Andraso 1997). It is conceivable that additional plates could reduce the angle of curvature and thus affect swimming performance in threespine stickleback. Therefore, curvature was measured in each individual replicate in this study. The curvature coefficient (CC) was measured as the chord distance from the snout to the posterior tip of the caudal peduncle when the stickleback assume their c-shape (the bent length or BL), divided by the standard length (SL) of the fish when straight (Webb 1978; Figure 11). Smaller values of CC indicate greater curvature.

Table 7. Mean and variance of SL (standard length), body depth and lateral plate number for Fairy Lake and Tlell Pond stickleback samples. Results of Levene's tests and independent t-tests show lack of significant differences in variances or means (respectively) between the 2 samples.

	Fairy Lake	Tlell Pond	Levene's		T-test	
	Mean +/- SD	Mean +/- SD	F	P	t	P
SL	53.03 +/- 6.72	56.16 +/- 6.95	0.001	0.977	1.902	0.061
Depth	12.78 +/- 1.70	12.96 +/- 1.60	0.480	0.491	0.448	0.655
Plate number	9.83 +/- 6.88	12.80 +/- 8.11	1.971	0.165	1.667	0.100

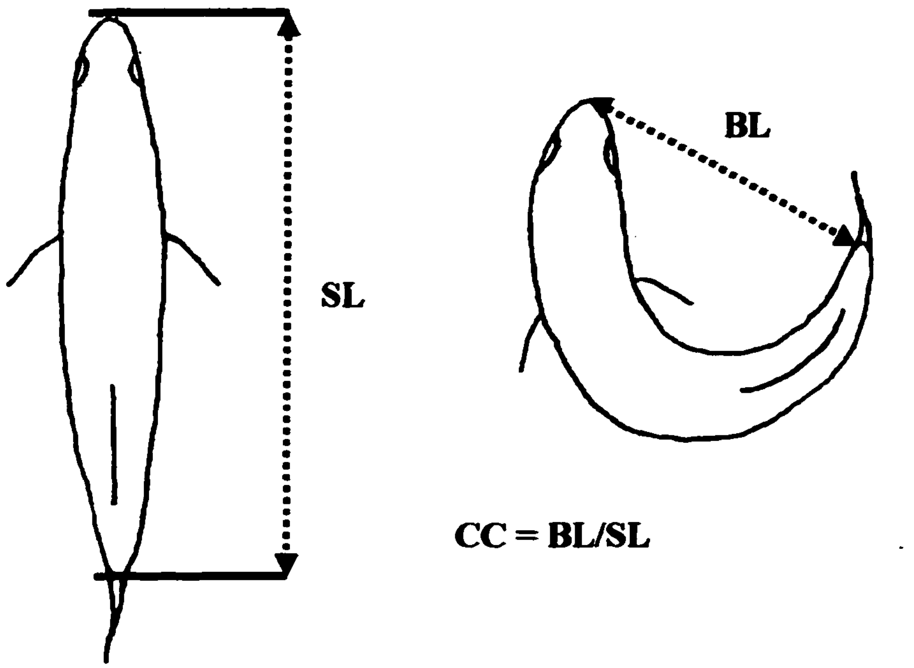

Figure 11. Calculation of the curvature coefficient (CC), modified from Webb (1978). BL = bent length, or chord distance, from snout to posterior tip of caudal peduncle of flexed fish. SL = standard length of straight fish at rest.

The collection of replicate performance data allowed me to determine if within-individual variation (among replicates) was significantly larger than differences among individuals. The numbers of replicates were low and variable among individuals (from 1 to 4), resulting in significant heteroscedasticity in performance variance among individuals for all performance variables. Therefore, non-parametric Kruskal-Wallis tests were run on individuals X maximum velocity, maximum acceleration, displacement and CC. Among individuals with more than one replicate fast-start performance measured, there was significantly greater variation among individuals than within individuals for maximum velocity (Kruskal-Wallis: X^2=59.944; df=42; P=0.028), maximum acceleration (Kruskal-Wallis: X^2=59.079; df=41; P=0.033) and displacement (Kruskal-Wallis: X^2=61.088; df=41; P=0.017). Curvature coefficient did not have significantly greater variance among individuals than within (Kruskal-Wallis: X^2=48.548; df=36; P=0.079), although significance was approached.

Analysis of covariance was performed to determine if the relationships between the 4 performance variables and the factors (SL, body depth and plate number) were similar enough between the two lakes for their samples to be pooled in multiple regression analyses. Multiple regression analyses were used to assess the individual residual effects of each of the factors on the performance variables. Distributions of the 4 performance variables did not differ significantly from normality (Kolmogorov-Smirnov: P>0.200 for each variable).

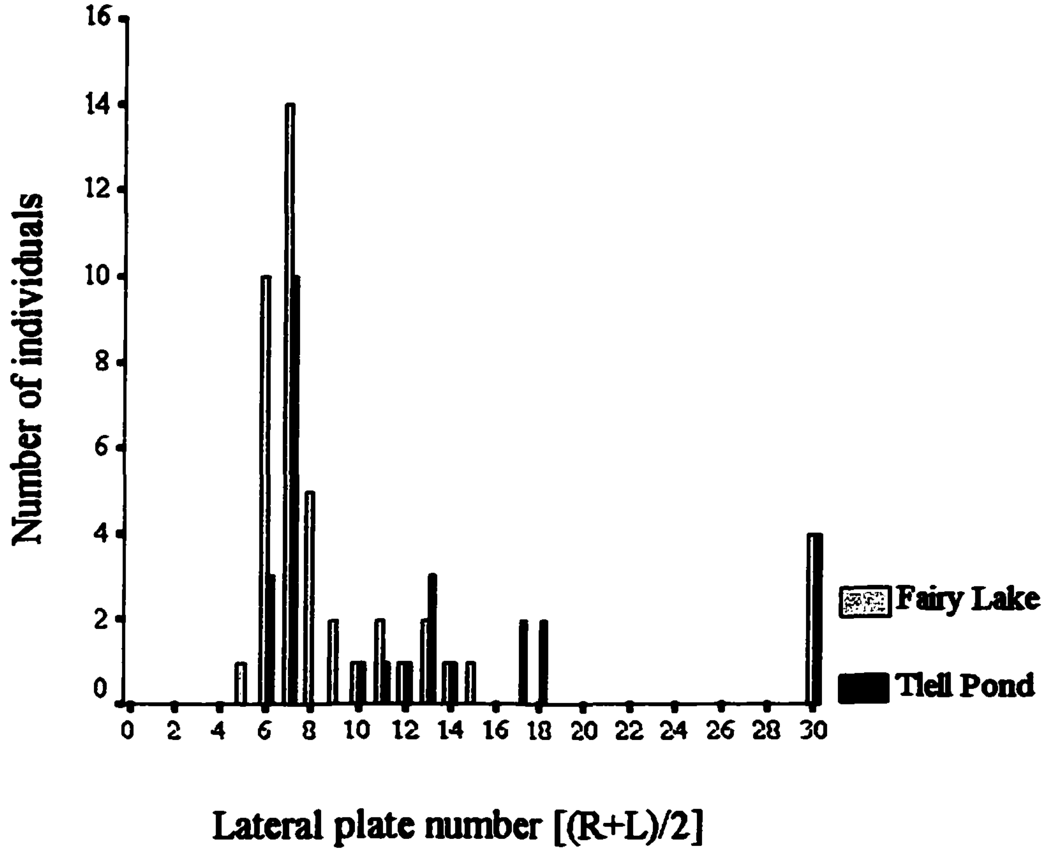

Figure 12. Frequency distribution of lateral plate number in Fairy Lake and Tlell Pond.

Lateral plate numbers ranged from 5 to 30 per side in Fairy Lake and from 6 to 30 in Tlell Pond. The combined plate number phenotypes from both samples formed a roughly continuous distribution from 5 to 18 only (Figure 12), followed by a group of outliers composed of 8 completely plated individuals (containing 30 plates per side). Performing regression analyses on data containing outliers is a serious violation of the underlying assumptions of most parametric tests (Zar 1999). I therefore ran the multiple regression analyses both excluding and including this group of outliers in order to determine if there were incongruities in the results. All statistical analyses were performed in SPSS-10.0.

Avian selective predation experiment

Experiments were conducted at the National Oceanic and Atmospheric Agency fisheries research facility in Port Orchard, on the Kitsap Peninsula, Washington. Individual stickleback were collected from two localities on the Kitsap Peninsula (Carpenter Lake N=120; Eglon Pond N=160) within three metres of shore with baited minnow traps. All stickleback were returned to the facility and the two samples were maintained separately in outdoor holding pens. Stickleback were allowed to acclimate for at least 48 hours before experimentation.

There were seven replicated experiments, three using fish from Carpenter Lake and four using fish from Eglon Pond. For each replicate, 40 stickleback were lightly anaesthetized with MS-222, and measured for standard length (SL), body depth, and number of lateral plates on the left and right side. SL and body depth were measured with callipers to the nearest 0.1 mm. Mean lateral plate number was calculated as the mean number of plates per side [(R+L)/2] per fish. There were no correlations between plate number and standard length or body depth in either population (all $P>0.200$).

Four resident captive raised Hooded Mergansers were kept in an outdoor aquatic aviary. A 4' wide by 12' long stream channel was kept filled with water to 2' deep. Stickleback were placed in the stream channel after measurements were completed, and allowed to acclimate overnight while the mergansers were kept separated in the nesting area of the aviary with no access to the fish. In the morning the birds were allowed to enter the stream channel and forage until approximately half (N=20) of the stickleback were consumed, after which the mergansers were herded out of the channel back into the nesting area. The stream channel was drained, the remaining stickleback collected and re-measured for the same traits that were measured before the experiment. Directional selection differentials (i) were calculated for lateral plate number for each replicate experiment (Endler 1986).

All experimental replicate runs were conducted between 7:00 AM and 10:00 AM on clear days. Five of the replicates (2 with Carpenter Lake stickleback, 3 with Eglon Pond stickleback) were video recorded in order to calculate pursuit and handling efficiency of the mergansers. Water flowing through the stream channel was diverted from a local stream, and drained into marine waters immediately adjacent to the mouth of the stream. The bottom of the white stream channel was clear of rocks and vegetation and swept between each replicate to removed silt and debris introduced with the intake of stream water.

Results

Assessment of swimming performance

There was a high degree of correlation among the four performance variables (Table 8). Velocity, acceleration and displacement all had strong positive correlations with each other and were significant at $P<0.001$ in each case. Curvature coefficient was negatively correlated with each of the other 3 variables, but was significant only with velocity at $P<0.050$.

All ANCOVAs passed Levene's test for homogeneity of variance. None of the slopes between the factors and the performance variables were significantly different between Fairy Lake and Tlell Pond (Table 9). This allowed me to test whether the adjusted means of the variables were similar enough between the two populations to pool them into one sample. Adjusted means of the performance variables were significantly different between Fairy Lake and Tlell Pond for maximum acceleration and displacement, approached significance for maximum velocity (Fairy Lake fish showed greater performance for all 3 variables) and was insignificant for CC (Table 9). I therefore included location as a factor in the regressions in order to account for the variance in adjusted means between samples.

Lateral plate number was significantly correlated with maximum velocity after the effects of SL, body depth and sample locality were removed (Table 10). Stickleback with fewer plates achieved a greater maximum velocity. However, this association only applied to the low and partially plated stickleback. When the completely plated outliers were included in the regression analysis, the correlation remained negative but became

Table 8. Correlation coefficient matrix showing levels of correlation among the 4 performance variables. CC=curvature coefficient.

	Velocity	Acceleration	Displacement
Acceleration	r = 0.673‡	-	
Displacement	r = 0.869‡	r = 0.642‡	-
CC	r = -0.318†	r = -0.177	r = -0.151

† P < 0.050 (2-tailed); ‡ P < 0.001 (2-tailed).

Table 9. Test for homogeneity of slopes and differences of adjusted means (ANCOVA) between stickleback samples from two lakes for each of the performance variables (maximum velocity, maximum acceleration, displacement and curvature coefficient) and 3 factors (SL, depth and lateral plate number). Plate number is square root transformed. SL=standard length; CC=curvature coefficient.

	SL		Depth		Plate number		Adjusted means	
Dependant	F	P	F	P	F	P	F	P
Velocity	0.813	0.371	0.032	0.858	0.374	0.543	3.451	0.068
Acceleration	0.914	0.343	0.002	0.962	0.001	0.972	8.925	0.004†
Displacement	0.927	0.340	0.079	0.779	0.144	0.706	7.191	0.009†
CC	0.007	0.934	2.872	0.096	0.121	0.730	0.036	0.851

† $P < 0.050$.

Table 10. Partial correlations, t-scores and significance levels from multiple regression analyses. SL=standard length; CC=curvature coefficient.

Dependant	Factor	Partial r	T	P
Velocity	SL	0.032 (0.018)	0.234 (0.134)	0.816 (0.893)
	Depth	0.037 (0.017)	0.271 (0.129)	0.788 (0.898)
	Plate number	-0.267 (-0.232)	-2.015 (-1.817)	0.049† (0.074)
	Site	-0.140 (-0.180)	-1.026 (-1.392)	0.310 (0.169)
Acceleration	SL	0.152 (0.151)	1.118 (1.164)	0.269 (0.249)
	Depth	-0.118 (-0.113)	-0.867 (-0.869)	0.390 (0.388)
	Plate number	-0.023 (-0.131)	-0.171 (-1.007)	0.865 (0.318)
	Site	-0.351 (-0.356)	-2.725 (-2.905)	0.009† (0.005) †
Displacement	SL	0.016 (0.003)	0.116 (0.026)	0.908 (0.979)
	Depth	0.057 (0.054)	0.415 (0.412)	0.680 (0.782)
	Plate number	-0.290 (-0.315)	-2.188 (-2.503)	0.033† (0.015) †
	Site	-0.221 (-0.262)	-1.637 (-2.046)	0.108 (0.045) †
CC	SL	-0.186 (-0.173)	-1.361 (-1.311)	0.180 (0.195)
	Depth	0.127 (0.153)	0.928 (1.155)	0.358 (0.253)
	Plate number	0.057 (0.055)	0.421 (0.409)	0.676 (0.684)
	Site	0.103 (0.071)	0.757 (0.535)	0.453 (0.595)

Note: resulting values in parentheses include outliers in the analysis.

† $P < 0.050$ (2-tailed).

insignificant. SL, body depth and location had no significant effect on maximum velocity, with or without the outliers.

Maximum acceleration was not significantly correlated with SL, body depth or lateral plate number, with or without the outliers (all $P<0.249$; Table 10). However, sample location was a significant predictor of acceleration both with the completely-plated outliers ($P=0.005$) and without ($P=0.009$). Fairy Lake stickleback had greater acceleration than Tlell Pond stickleback in both cases.

Displacement increased significantly as lateral plate number decreased, both with ($P=0.015$) and without ($P=0.033$) the outliers (Table 10). None of the other three factors had a significant effect when the completely-plated outliers were excluded (all $P>0.109$), but sample location was a significant predictor of displacement when the outliers were included (Fairy Lake>Tlell Pond; $P=0.045$).

There were no significant correlations between SL, body depth, lateral plate number or location and CC (all $P>0.180$), regardless of the inclusion or exclusion of the outliers (Table 10).

Avian selective predation experiment

Predation by *L. cuculatus* did not generate significant directional selection on lateral plate number in any of the seven replicate experiments (all $P>0.500$; Table 11). The differentials on the combined data for each population sample were also insignificant (all $P>0.330$; Table 11), as was the overall differential that included all replicates from both samples ($i=0.018$; $P=0.746$). Furthermore, there were no consistent trends in the direction of selection among replicate experiments.

Table 11. Lateral plate means before and after predation by *L. cuculatus* (+/- SD), resulting selection differentials *i*, and significant levels (P). C1, C2, and C3 represent the first, second, and third replicates using Carpenter Lake stickleback. E4, E5, E6, and E7 represent the fourth, fifth, sixth, and seventh replicates using Eglon Pond stickleback. CT and ET represent pooled replicates for stickleback from Carpenter Lake and Eglon Pond, respectively.

Replicate	Plate # Before	Plate # After	*i* (P)
C1	6.8+/-.5	6.8+/-.5	0 (n/a)
C2	6.5+/-.5	6.4+/-.5	-.200 (.692)
C3	6.6+/-.6	6.7+/-.6	.167 (.502)
CT	**6.6+/-.5**	**6.7+/-.5**	**.200 (.334)**
E4	11.5+/-6.9	12.5+/-8.4	.145 (.596)
E5	11.1+/-7.3	11.3+/-7.6	.027 (.942)
E6	10.4+/-6.1	9.4+/-5.5	-.164 (.530)
E7	10.9+/-6.8	11.4+/-7.3	.074 (.784)
ET	**11.0+/-6.7**	**11.0+/-7.3**	**0 (n/a)**

The range of lateral plate number morphs was 6 through 8 in Carpenter Lake, 5 through 30 in Eglon Pond, and the lateral plate mode for both was 7. However, in Eglon Pond there was an additional discrete mode at 30, representing the completely plated morph. While approximately half of the fish with 7 plates were consumed, only 3 of the 12 completely plated stickleback were eaten. Stickleback from Eglon Pond with fewer than 30 plates represented a relatively continuous and normal distribution, although slightly positively skewed. I therefore calculated lateral plate number selection differentials for Eglon Pond stickleback with the completely plated stickleback excluded. The selection differentials for the remaining low and partially-plated stickleback from Eglon Pond were not significant (Table 12), but 3 of the 4 replicates showed negative differentials (the 4th showed a differential of zero) and the overall selection differential for the combined replicates from Eglon Pond was -0.122 (P=0.381), rather than zero with the completes included. The overall differential that included all replicates from both Carpenter Lake and Eglon Pond samples, with the completes excluded, was negative (i=-0.088) but insignificant (P=0.467)

For five of the seven replicate experiments, I was able to videotape and record pursuit and handling efficiencies rates of the mergansers. The average pursuit efficiency (frequency of dives that resulted in a fish brought to the surface) was 0.40 +/- 0.11 S.D., while the average handling efficiency (frequency of fish brought to the surface that were successfully swallowed) was 0.86 +/- 0.10 S.D. Neither pursuit efficiency nor handling efficiency changed with experience (Spearman rank correlation: P=0.285 and P=0.747, respectively) nor did they differ between the two populations of stickleback (Kruskall-

Table 12. Lateral plate means before and after predation by *L. cuculatus* (+/- SD) for Eglon Pond stickleback of plate numbers less than 30, resulting selection differentials *i*, and significant levels (P). E4, E5, E6, and E7 represent the fourth, fifth, sixth, and seventh replicates. ET represents pooled replicates for stickleback from Eglon Pond.

	Plate # before	Plate # after	*i* (P)
E4	9.9+/-4.6	9.3+/-3.8	-.130 (.580)
E5	9.0+/-3.7	8.9+/-3.6	-.027 (.935)
E6	9.4+/-4.2	8.3+/-2.8	-.286 (.312)
E7	9.4+/-4.1	9.4+/-3.9	0 (n/a)
ET	**9.4+/-4.1**	**8.9+/-3.5**	**-.122 (.391)**

Wallis test: P=0.083 and P=0.564, respectively), although pursuit efficiency was slightly greater with Carpenter Lake stickleback.

Discussion

Assessment of swimming performance

In freshwater threespine stickleback, lateral plate number was negatively associated with maximum velocity and displacement but not with maximum acceleration or minimum curvature coefficient. These results suggest that, while plate number was negatively associated with some components of fast-start performance as predicted, it did not appear to affect performance by limiting flexibility (CC) or acceleration. In addition, flexibility was not strongly correlated with the other performance variables, and had only a weak significant association with velocity (Table 8). Hence, flexibility itself was not a good predictor of fast-start performance in these 2 populations.

There could be two explanations for this. The first is that the error variance of CC may have been too great to detect significant correlations between it and other factors. While the variance of CC was greater among individuals than within, it was not significantly so (P=0.079). Measurement error in the extraction of data from the video could have swamped correlations, if present, between plate number and curvature as well as between curvature and the other performance variables. Problems with accurate extraction of video data have been demonstrated, and instantaneous information such as minimum body curvature or maximum acceleration is frequently 'smoothed over' when using frame-by-frame analysis with low film speeds (Harper and Blake 1989).

Secondly, associations between swimming performance and lateral plate number may have been confounded by other morphological or behavioural factors that directly affect swimming performance. Taylor and McPhail (1986) found that stream-dwelling stickleback, presumably with fewer plates, had superior fast-start performance over marine stickleback, and attributed this to differences in body shape. The benthic stickleback of Paxton Lake, British Columbia have fewer plates than the limnetic stickleback, but lateral plate number is correlated with other morphological and trophic characteristics as well (McPhail 1992). If body form was correlated with plate number in Fairy Lake and Tlell Pond, this could have produced complex and unresolved interaction effects on swimming performance. However, all of the stickleback used were taken from benthic littoral regions of the lakes, reducing the likelihood of confounding the results with benthic/limnetic associated body shape differences. Nonetheless, subtle body shape variance among benthic stickleback from these lakes could have contributed to unexplained variance in swimming performance in this study, and confounded the weak associations between plate number and performance variables (Table 10).

Lateral plate number may have directly affected velocity and displacement by generating drag. The effect of drag on hydrodynamic performance in fishes has been well documented (Webb and Skadsen 1979; Vogel 1981; Webb 1982), and projections or 'roughness' of the surface can reduce fast-start performance. The lateral plates in stickleback form slight lateral projections at their posterior edges. The projection length of the plates were very close to but slightly greater than the largest admissible size before disruption of the boundary layer occurs (Schlichting 1960; Aleyev 1977), raising the possibility that the plates may have generated enough drag to affect performance.

The group of outliers in the fast-start assessment was composed entirely of completely plated individuals: one of the 3 genetically determined lateral plate morphs described in this species. It is conceivable that other behavioural or morphological traits could have been associated with this phenotype that also affected swimming performance independently of the lateral plates. Indeed, the inclusion of the completely plated outliers in the multiple regressions had some subtle effects on the results (Table 9). The complete morphs acquired slightly higher velocities than predicted by the regression of fish with 18 plates or less, reducing the negative partial r-value to just below significance. This could have been due to behavioural or morphological attributes of the complete morph that affected velocity independently of any biomechanical effects of the plates. The inclusion of the completely plated outliers also resulted in a significant effect of sample locality on displacement (Table 9). Interestingly, the inclusion of completes resulted in greater partial r values for sample effects for every performance variable except CC, suggesting that the complete morphs in Fairy Lake may have other behavioural or morphological attributes that enhanced their burst swimming performance, but which are lacking in the Tlell Pond complete morphs.

Avian selective predation experiment

The demonstration of a negative relationship between swimming performance and lateral plate number in this study supports the hypothesis that plate reduction is associated, either directly or indirectly, with improved hydrodynamic efficiency. This association is predicted by Reimchen's (1992a) hypothesis that plate reduction may be a defensive adaptation to predators with low pursuit efficiency. Reduced pursuit efficiency could occur where swimming speeds of predators are similar to their prey and in habitats of poor visibility caused by water staining or dense aquatic vegetation. The average fast-start velocity of stickleback from this study (Fairy Lake and Tlell Pond) was 1.36 m s $^{-1}$ and 1.13 m s $^{-1}$, respectively, while maximum velocities ranging from 0.90 m s $^{-1}$ to 1.65 m s $^{-1}$ have been reported for other freshwater stickleback (Taylor and McPhail 1986; Law and Blake 1996). Submerged swimming speeds of freshwater avian piscivores range from 1.0 m s $^{-1}$ in grebes (Johansson and Norberg 2001) to 1.5 m s $^{-1}$ in cormorants (Schmid *et al.* 1995). Reports of maximum fast-start velocities for rainbow trout in one study are 2.77 m s $^{-1}$ (Harper and Blake 1990) and in another range from 1.91 to 2.65 m s $^{-1}$ (Webb 1976). Based on these reports, the diving birds are within, but towards the upper edge of the stickleback velocity range, while rainbow trout achieve maximum velocities between 20 to 300% greater than their stickleback prey. In addition, foraging efficiency in diving birds will be affected by restrictions in submergence time due to the need to return to the surface for air. Average dives for loons on Drizzle Lake, Haida Gwaii, were only 30 – 40 seconds long (Reimchen and Douglas 1980). This may produce a further premium on rapid swimming in the prey since birds are not capable of prolonged pursuits. This suggests that there is considerably greater likelihood of stickleback escaping pursuit from diving birds than from trout, as was originally suggested by Reimchen (1992a). The slight improvement in burst velocity due to a reduction in plate numbers could make the difference between capture and escape to a stickleback fleeing a pursuing diving bird, yet be irrelevant to the chances of escape by a large fast predatory trout. If this is the case, lateral plate reduction may be advantageous in avian predation regimes because of its association with enhanced fast-start swimming performance.

However, while I did detect a hydrodynamic advantage to fish with reduced numbers of lateral plates, there were no significant selection differentials resulting from predation by captive *L. cuculatus*. This can be interpreted in several ways. First, the diving velocity of the mergansers may have swamped any differences in velocity generated by variation in plate number. The avian diving velocities reported above are averages, and may be considerably greater during prey strikes. The nature of the experimental set up was such that there was very little chance for escape after detection; the aquarium was relatively small and shallow, and there was no detritus on the bottom in which to hide. This may have artificially inflated the pursuit efficiency of the mergansers to a level where virtually no stickleback escaped that otherwise would have in a natural setting. In addition, the swimming performance of the stickleback may have been reduced because of induced stress from the experiment. It is common for biomechanical performance to be dissimilar between field and experimental conditions (Irschick and Garland 2001). Second, the stickleback had no visual refuge from the birds, given the relatively small size of the white stream channel and lack of vegetation or detritus on the bottom. Original strikes by the birds may have targeted slower stickleback, but with no refuge, the fish were continuously subject to subsequent strikes and potential capture regardless of phenotype. Third, at least in Carpenter Lake, the variance in lateral plate number may not have been large enough to detect differences in velocity and predation success.

When completely plated fish were excluded from Eglon Pond samples, the differentials were still insignificant, but 3 of the 4 replicates had reductions in plate number after avian predation and the overall differential was negative (Table 12). This suggests that, from a plate range of 5 through 25, there was a slight but insignificant increase in the chance of succumbing to predation as plate numbers increased, but no effect if the 12 completely plate fish were included. The swimming performance experiments found a greater than expected velocity in completely plated stickleback, which could explain this effect in the predation experiment. There may be other behavioural or morphological correlates with the completely plated morph that are enhancing swimming and pursuit escape performance.

Regardless, even with the exclusion of the complete morph, the differentials were small and statistically insignificant. This is a common problem when measuring natural selection, and a recent review of the strength of natural selection found that the median differential magnitude was 0.150 (Kingsolver *et al.* 2001). Weak selection differentials (<.1), especially in large sample sizes (N=1000), were common among the reviewed studies. However, even differentials of small magnitude could, given enough generations, generate large phenotypic shifts (Conner 2001). In addition, testing for significant differences between groups before and after selection rather than between selected and unselected groups with t-scores tends to underestimate significance (Endler 1986). Comparing plate numbers between eaten and surviving stickleback would have given me more statistical power than comparing plate numbers before and after predation. While replication of this experiment would give more confidence to the estimated selection differential, it is conceivable that even the weak overall differential of

–0.088 found in this study could result in significant reduction of plate numbers over large numbers of generations.

In conclusion, this study has demonstrated that there is a significant increase in fast-start velocity and displacement, but not acceleration or curvature, in threespine stickleback that have reduced numbers of lateral plates. However, selection differentials for lateral plate numbers before and after predation by captive Hooded Mergansers were insignificant but overall slightly negative. While weak selection differentials can result in significant phenotypic changes over time, these results must be interpreted with caution given the contrived nature of the predation experiment. Regardless, lateral plate reduction enhances fast-start swimming performance in threespine stickleback, and while evidence of direct selection for plate reduction by one species of diving bird remains ambiguous, it has the potential to be advantageous in some predation regimes.

Chapter 4: Geographical variation in asymmetry in threespine stickleback

Introduction

Over the last 3 decades, fluctuating asymmetry (FA) has been subject to an expanding repertoire of investigations encompassing genetics, development, behaviour, evolution, conservation and parasitology (for review see Møller and Swaddle 1997). FA is generally thought to be associated with developmental instability (Mather 1953; Van Valen 1962; Soulé 1967) and to reflect the failure of an individual to correct subtle and random departures from perfect symmetry during ontogeny (Waddington 1942; Zakharov 1992). Ability to correct developmental errors can be compromised by internal factors such as elevated homozygosity (Soulé 1979; Palmer 1986; Mitton 1995), as well as by the disruption of co-adapted gene complexes through hybridization (Vrijenhoek and Lerman 1982; Graham 1992; Zakharov 1992; Clarke 1993). Developmental errors can also stem from physiological stress caused by extreme environmental conditions (Beardmore 1960; Gest *et al.* 1986; Hosken *et al.* 2000). Elevated levels of FA are potentially relevant for conservation as these can indicate demographic bottlenecks and environmental deterioration (Wayne *et al.* 1986; Pankakoski *et al.* 1992; Manning and Chamberlain 1993; Clarke 1995; Gomendio *et al.* 2000; Lens *et al.* 2000). However, such inferences can be misleading without knowledge of the range in FA among undisturbed populations (Palmer 1996).

FA can also reflect relative fitness of individuals as it is associated with reduced immunocompetence (Rantala *et al.* 2000), increased susceptibility to parasitism (Escos *et al.* 1995; Bonn *et al.* 1996; Reimchen 1997; Hunt and Allen 1998), reduced survivorship

(Packer and Pusey 1993; Ueno 1994; Pelabon and van Breukelen 1998; Nosil and Reimchen 2001), and avoidance by potential mates (Møller and Pomiankowski 1993; Watson and Thornhill 1994; Simmons and Ritchie 1996). If FA is reflecting the general intrinsic fitness and developmental stability of an individual, there should be correlations in the levels of FA among different traits in that individual. However, diverse studies have found little support for such a correlation (VanValen 1962; Mason *et al.* 1967; Ames *et al.* 1979; Dufour and Weatherhead 1996; Evans and Marshall 1996; Clarke 1998). This suggests that FA/fitness associations are heterogeneous among traits, and this heterogeneity may reflect the degree to which asymmetry compromises the functional integrity of some traits but not others (Moodie 1977; Balmford *et al.* 1993; Allen and Simmons 1996; Gummer and Brigham 1995; Crespi and Vanderkist 1997; Swaddle 1997a; Bergstrom and Reimchen 2000). Combined indices of FA from multiple traits can provide a more reliable estimate of developmental stability (Soulé and Baker 1968; Dufour and Weatherhead 1996; Gangestad and Thornhill 1999; Bryden and Heath 2000).

Asymmetry in threespine stickleback

Here I assess natural levels of FA in multiple traits among endemic populations of freshwater threespine stickleback (*Gasterosteus aculeatus*) that have colonized the archipelago of Haida Gwaii, British Columbia, Canada approximately 12,000 years ago (Moodie and Reimchen 1976). Populations on this archipelago inhabit geographically isolated freshwater lakes, ponds and streams, the majority of which are relatively pristine and undisturbed by human activity. The localities encompass a range of natural environmental variation making this system an ideal one with which to investigate baseline levels of FA in the wild as well as sensitivity of FA to natural levels of environmental variation. I will investigate geographical variation in a multi-trait composite FA index in response to several environmental factors that vary among habitats from this archipelago. I will also test for the predicted negative correlation between FA and survivorship among these natural populations by comparing mean FA between 2 age classes and compare FA among parasitized and unparasitized fish, as recent studies from one of these populations show broad temporal trends between FA and parasitism (Reimchen 1997; Reimchen and Nosil 2001). If fitness and FA are negatively associated, and if FA is an accurate indicator of organism-wide developmental stability, there should be a reduction in FA in adults relative to juveniles as well as a correlation in FA among traits within individuals. I will investigate differences in asymmetry of the individual traits used in the multi-trait composite FA index to determine if there is evidence of differential levels of developmental stability among them. Consistent variation in the level of asymmetry among traits across a range of habitats implies that FA may have value as an indicator of the relative functional importance of the traits. Such an examination of geographical patterns of both composite and trait-specific asymmetry will provide context to the importance of environmental stress and natural selection in the expanding analyses of intra-individual variation among natural populations.

Materials and Methods

T.E. Reimchen obtained samples of *G. aculeatus* from lakes and streams throughout the archipelago during multiple expeditions between 1969 and 1997. Some samples contained stickleback that lacked the traits pertinent to this analysis and were therefore excluded. Of these original collections, 87 samples from the 3 major geographical areas of the archipelago (lowlands, plateaus and mountains) were used for this study (Figure 13). The majority of *G. aculeatus* samples were collected from April to July using standard-mesh minnow traps placed in the littoral zones. Fish were fixed in 10% formalin and stored in 95% ethanol. Water chemistry and lake data were collected from a subset of the localities at the time of collection. This included pH, percent light transmission at a wavelength of 400 nm (T400), specific conductance (_mhos cm^{-1}), water depth (m), and lake area (hectares). Specific conductance, water depth and lake area were log transformed to normalize the skew in their distributions.

From each sample of *G. aculeatus*, comprising approximately 50 juveniles (ca. 35-45 mm SL) and 50 adults (>45 mm), I measured up to 100 individuals for standard length (SL), sex, and parasite infection (*Schistocephalus solidus, Eustrongylides* sp., *Diplostomum* sp.). I also measured asymmetry in 10 bilateral traits on the predator defense apparatus (Figure 14). These traits included: number of forks on the dorsal edge of the ascending process (FORKS), amount of overlap between lateral plates 4 through 7 (if present) and the basal plates (BP4, BP5, BP6 and BP7), amount of overlap between lateral plates 5 through 7 (if present) and the ascending process (AP5, AP6 and AP7), ascending process height (APH) and ascending process width (APW). Overlap values ranged from 0 (not touching) to 3 (strong overlap). The overlap in this defensive apparatus provides structural support for the spines and dispersal of forces during

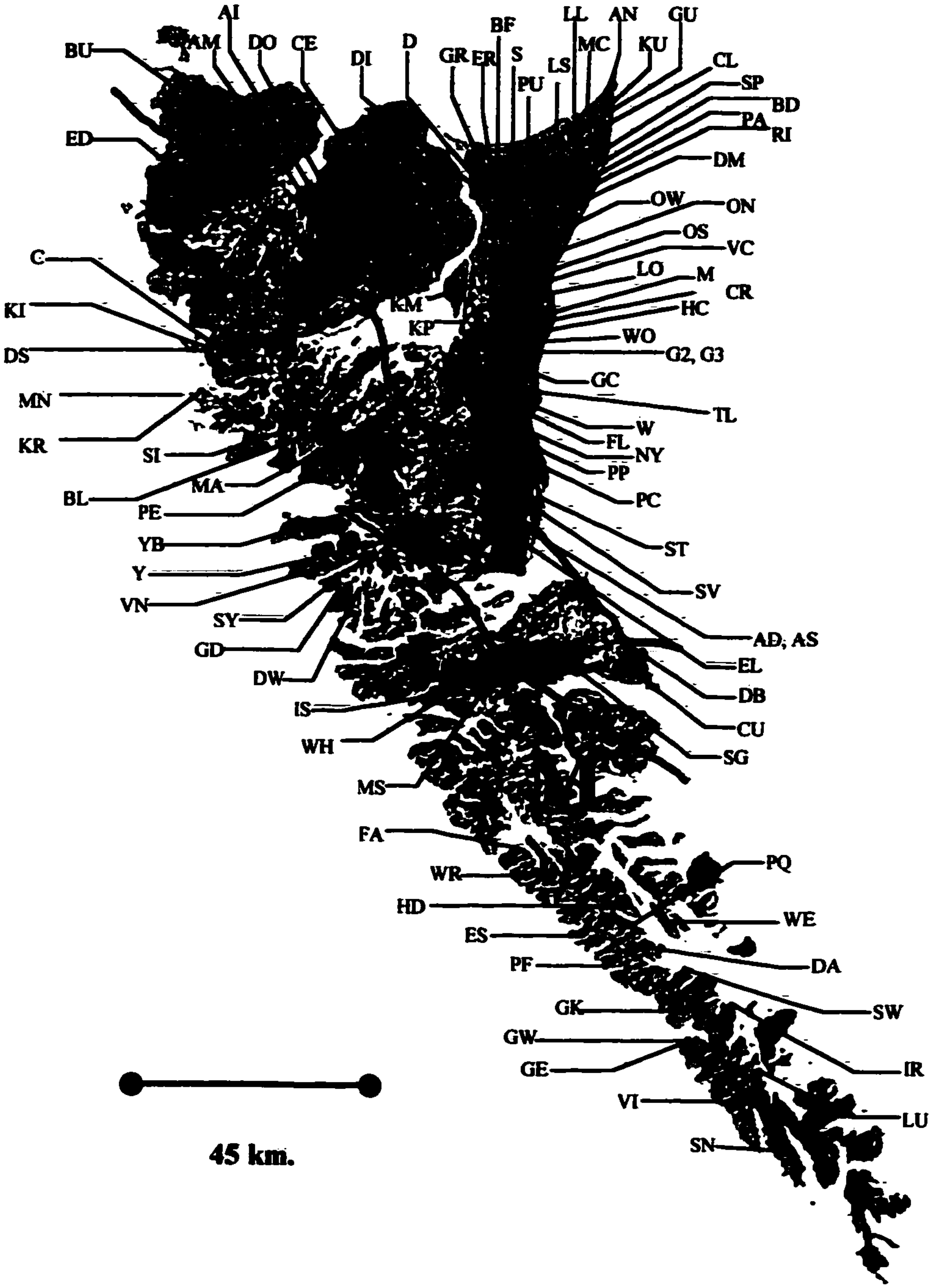
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Figure 13. Map of Haida Gwaii showing sampled localities used for this analysis. Three major geographical zones of the islands defined as L (lowlands), P (plateaus) and M (mountains) as defined in Brown (1968). N=87. AI=Ain L., AM=Amber L., AD=Anderson N. L., AS=Anderson S. L., AN=Anser L., BF=Bigfish L., BL=Blackwater Cr., BD=Blue Danube L., BU=Bruin, CR=Capeball R., CE=Cedar L., CL=Clearwater L., C=Coates L., CU=Cumshewa L., DM=Dam L., DA=Darwin L., DW=Dawson L., DB=Debris L., DS=Desolate L., DO=Downtree L., D=Drizzle L., DI=Drizzle Inlet, ED=Eden L., EL=Elk Survey Cr., ER=Eriophorum L., ES=Escarpment L., FA=Fairfax L., FL=Florence Cr., G2=Geikie 2 Cr., G3=Geikie 3 Cr., GC=Gold Cr., GK=Goski L., GE=Gowgaia L. (east), GW=Gowgaia L. (west), GR=Gross L., GU=Grus L., GD=Gudal L., HC=Hickey L., HD=Hidden L., IS=Inskip, IR=Irridens L., KI=Kiokathli L., KR=Krajina L., KU=Kumara L., KM=Kumdis L., KP=Kumdis P., LO=Loon L., LL=Lumme L., LS=Lumme Swamp, LU=Lutea L., MA=Marie L., M=Mayer L., MN=Menyanthes L., MC=Mica L., MS=Mosquito L., NY=New Years L., ON=Otter North L., OS=Otter South L., OW=Oeanda R., PA=Parkes L., PE=Peter L., PC=Pontoon Centre L., PP=Pontoon Tlell P., PQ=Poque L., PF=Puffin L., PU=Pure L., RI=Richter L., SI=Seal Inlet L., SG=Skidegate L., S=Skonun L., SN=Snub L., SP=Spence L., ST=Stellata L., SY=Stiu L., SW=Sundew L., SV=Survey Cr., TL=Tlell Estuary, VC=Vaccinium L., VN=Van Inlet L., VI=Victoria L., WE=Wegner L., WH=White Swan P., WI=Wiggins L., WO=Woodpile L., WR=Wright L., Y=Yakoun L., YB=Yakoun Backwater R.

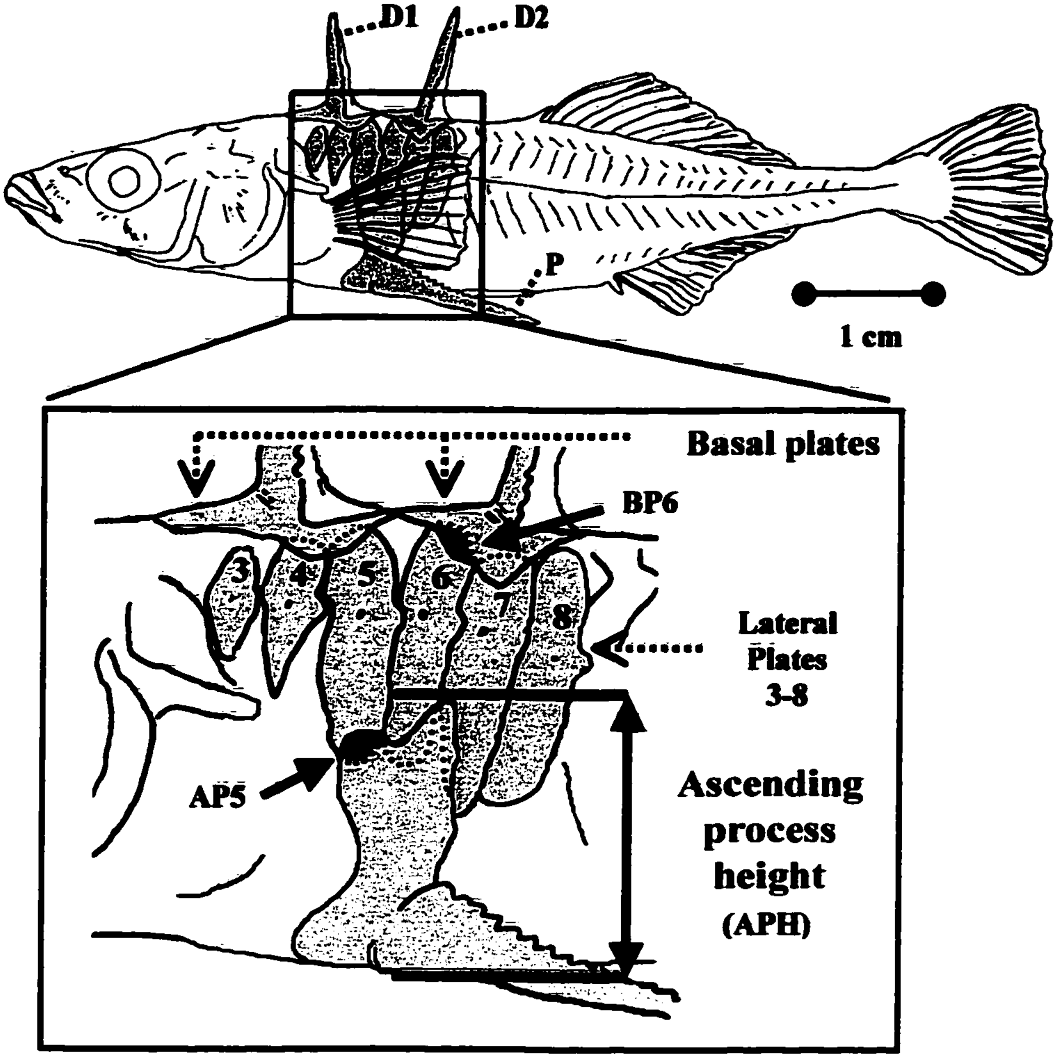

D1
D2
P
1 cm
Basal plates
BP6
3
4
5
6
7
8
Lateral Plates 3-8
AP5
Ascending process height (APH)

Figure 14. Camera lucida sketch of the left side of a *G. aculeatus* showing the first dorsal spine (D1), second dorsal spine (D2), left pelvic spine (P), bony lateral plates number 3 through 8 highlighted in grey, basal plates and ascending process. Inset shows traits used in the preliminary FA analysis: number of forks (FORKS) on dorsal edge of the ascending process ranged from 1 – 4 (2 in this individual); BP4, BP5, BP6, BP7 shown in dotted lines designating overlap between lateral plates 4 – 7 and the basal plates; AP5, AP6 and AP7 shown in dotted lines designating overlap between lateral plates 5 – 7 and the ascending process; ascending process height (APH) measured at the highest point, dorsal to ventral; and ascending process width (APW) measured at the widest point of the ascending process, anterior to posterior. Final traits used in the FA analysis (designated by heavy solid arrows) were: ascending process height (APH), overlap between lateral plate 6 and the basal plate (BP6), and overlap between lateral plate 5 and the ascending process (AP5).

predator manipulation (Reimchen 1983). All measurements were made to 0.010 mm (where applicable) using a dissecting scope and micrometer. The lateral plates and other defensive structures were completely absent in at least some individuals in 28 samples. I therefore excluded these samples from further analysis, as it is impossible to assess the degree of symmetry in absent characters.

After preliminary measurements were completed, 12 samples were randomly chosen for analysis of measurement error. From each sample, 20 individuals were randomly selected and re-measured. A 2-way mixed model ANOVA was run for each trait with side (fixed) and individual (random) as factors and trait as the dependent (Palmer 1994) in order to determine whether measurement error variance contributed significantly to between sides variance. One of the traits (AP6) had significant measurement error after Bonferroni correction (Table 13) and was therefore excluded from further analysis.

Directional asymmetry and anti-symmetry among juveniles were examined with T-tests of the signed asymmetry (R-L) of each trait against a mean of zero, and by calculating the kurtosis of the R-L distributions (Table 14). Two traits showed significant directional asymmetry (FORKS and APW were both right side dominant) and were thus excluded from further analysis. Kurtosis values (g^2) for each trait were positive, evidence that the R-L distributions were unimodal and leptokurtic (Palmer and Strobeck 1986).

The remaining traits varied in the frequency of their occurrence in individuals. Some of the populations have a reduction in the numbers of structural lateral plates and it was not possible to measure overlap with the spine supports when these plates were

Table 13. 2-way mixed model ANOVA of sides (fixed) by individual (random). Results are from the subset of re-measured populations only. Significant *Side* effects are evidence of directional asymmetry, significant *Individual* effects are evidence of size variance in the trait among individuals, and significant *Side X Individual* effects are evidence that FA contributes significantly more to between sides variance than measurement error (Palmer 1994). Significance values are before Bonferroni corrections. See Figure 14 caption (p. 82) for description of trait abbreviations.

Trait	*Side*		Individual		Side X Individual	
	F	*P*	*F*	*P*	*F*	*P*
FORKS	2.687	0.103	5.173	<0.001	2.481	<0.001
BP4	0.250	0.617	5.877	<0.001	8.847	<0.001
BP5	0.056	0.813	4.435	<0.001	3.828	<0.001
BP6	0.494	0.483	10.872	<0.001	4.271	<0.001
BP7	0.127	0.722	4.160	<0.001	13.756	<0.001
AP5	3.193	0.075	12.143	<0.001	2.059	<0.001
AP6	0.221	0.639	33.572	<0.001	1.234	0.038
AP7	0.038	0.845	15.447	<0.001	2.047	<0.001
APH	0.029	0.865	74.097	<0.001	3.397	<0.001
APW	5.652	0.018	29.766	<0.001	3.147	<0.001

Table 14. T-tests (2-tailed) against a mean of zero and kurtosis (g^2) of (R-L) distributions (all juveniles from the data set included). A significant result is evidence of directional asymmetry. Kurtosis values greater than 0 are evidence of the absence of anti-symmetry (platykurtosis). Significance values are before Bonferroni corrections. See Figure 14 caption (p. 82) for description of trait abbreviations.

Trait	*t*	P	g^2
FORKS	3.059	0.002	n/a
BP4	-0.742	0.458	6.772
BP5	-0.749	0.454	14.251
BP6	-0.114	0.909	1.792
BP7	1.039	0.299	113.350
AP5	-1.210	0.227	5.991
AP6	-1.861	0.063	101.505
AP7	0.846	0.398	34.580
APH	-0.533	0.594	8.807
APW	5.583	<0.001	n/a

missing. The plates at positions 5 and 6 are the most conserved, and consequently, for this study, I used the amount of overlap between lateral plate 5 and the ascending process (AP5), the amount of overlap between lateral plate 6 and the basal plates (BP6) as well as the ascending process height (APH) (Figure 14). None of the samples demonstrated significant trait size/asymmetry associations for these traits. Asymmetries from these 3 traits were coalesced into a composite FA index (CFA). Because the variables represent both continuous and discrete distributions, I used the sum of the ranks of all 3 traits (see Leung *et al.* 2000). Each individual in the pooled dataset was given a rank according to the absolute (R-L) score for each of the 3 traits, and the ranks were then added to give a summed CFA score for each individual. There were no significant differences in mean CFA between sexes in any of the samples after sequential Bonferroni corrections ($P>0.100$ in all cases), and the number of samples that had greater mean CFA for males did not differ significantly from those that had greater mean CFA for females (N=37 and 43, respectively; $X^2=0.450$; df=1; $P>0.500$; 7 samples had only one sex present). Sexes were therefore pooled for the remaining analyses.

Selection differentials (*i*) between sub-adults and adults for CFA and for FA of each trait separately were calculated (see Endler 1986) for 73 localities that had both age classes represented in the samples. I tested for presence of an individual asymmetry parameter in juveniles only, in order to reduce the potential impact of selection on asymmetry. Individuals were ranked (within each sample) based on the relative asymmetry of each trait, and the ranks of individuals were tested for concordance among all 3 traits. Comparisons of the relative asymmetry among traits were performed using $|\log(L/R)|$ in order to express the asymmetry scores on a common scale (Clarke 1998).

Results

Mean total CFA (adults and juveniles combined) varied significantly among *G. aculeatus* samples collected from the 87 localities on Haida Gwaii (Kruskal Wallis: X^2=365.68; df=86; P<0.001). This differed among the 3 geographical regions (Kruskal Wallis: X^2=9.210; df=2; P=0.010), with lowland *G. aculeatus* samples having the highest FA and mountain samples having the lowest. In the plateaus and mountains, the most symmetric populations tended to be on the west coast (Figure 15). There were no significant differences between mean total CFA of creeks and their adjoining lakes (Paired t-test: t=0.630; df=9; P=0.540).

Mean total CFA differed with respect to limnological factors among the lakes. For bivariate comparisons, it was inversely correlated with lake pH (r=-0.460; P=0.003) and water colour (r=-0.390; P=0.001) but not with water depth (r=-0.090; P=0.390), lake area (r=-0.060; P=0.590) or specific conductance (r=-0.180; P=0.270). Water colour (T400) and pH are strongly positively correlated among localities (r=0.790; P<0.001) and both increase in value from the lowlands to the mountains, tending to be highest on the west coast of the archipelago, which also contains the least asymmetric samples. Of the 10 localities with the highest CFA, 9 were from lowland habitats, which are characterized by very shallow, acidic and dystrophic lakes (low values of T400). The 3 west coast lakes with the highest asymmetry (Bruin L., Seal Inlet L. and Dawson L.; Figure 15; Appendix 1) also have the highest levels of water staining in this area (pH was not collected from these 3 localities). However, the independent residual effects of pH, T400, specific conductance, lake depth and lake area on total CFA were insignificant (Table 15).

Figure 15. Distribution of mean total CFA among populations. Black circles designate populations with mean CFA scores greater than 6000 (see Appendix 1) and grey circles designate populations with mean CFA scores less than 6000. The 3 populations with black circles on the northwest coast are Bruin L., Seal Inlet L. and Dawson L.

Table 15. Multiple regression of total and juvenile sample mean CFA by pH, T400, specific conductance, water depth and lake area. Model results for total samples: $R^2=0.007$, $P=0.115$; model results for juveniles: $R^2=0.009$, $P=0.304$.

Factor	Total		Juvenile	
	Partial r	P	Partial r	P
pH	0.031	0.276	-0.020	0.659
T400	0.016	0.569	0.067	0.132
Sp.cond. (log)	0.038	0.176	0.057	0.205
Water depth (log)	0.006	0.822	0.043	0.337
Lake area (log)	0.034	0.233	0.002	0.972

I also examined mean CFA for juvenile fish in relation to limnological factors among lakes. While there were significant differences among populations (Kruskal Wallis: X^2=173.96; df=70; P<0.001), there were no differences among geographical regions (Kruskal Wallis: X^2=0.380; df=2; P=0.830), or between creeks and their adjoining lakes (Paired t-test: t=0.518; df=9; P=0.620). There were no significant bivariate correlations between population mean juvenile CFA and any limnological variable (pH: r=-0.070; P=0.690; T400: r=-0.050; P=0.680; specific conductance: r=-0.260; P=0.120; water depth: r=-0.020; P=0.850; lake area: r=-0.010; P=0.930) and no significant residual effects from these variables resulting from multiple regression analysis (Table 15).

In order to determine if asymmetry of the 3 individual traits used to compute the CFA index varied in their sensitivity to environmental fluctuations, I ran multiple regressions of the 5 environmental variables and mean juvenile absolute asymmetry for each trait. While APH and AP5 demonstrated no significant associations with the environmental variables, the regression model for BP6 was significant and this was primarily due to a negative correlation with specific conductance (Table 16).

I examined whether some individual traits were consistently more asymmetric than others. Six populations showed significant concordance (Kendall's *W*) of trait asymmetry rank after sequential Bonferroni corrections (Escarpment L., Gowgaia West L., Gudal L., Inskip Lagoon, Lutea L., and Yakoun River). In each of these populations, ascending process height ranked highest for asymmetry relative to the other 2 traits.

Table 16. Multiple regression of mean absolute asymmetry for 3 traits, APH, AP5 and BP6 by pH, T400, specific conductance, water depth and lake area. Model results for APH: R^2=0.011, P=0.297; model results for AP5: R^2=0.005, P=0.733; model results for BP6: R^2=0.036, P=0.001. See Figure 14 caption (p. 82) for description of trait abbreviations.

Factor	*APH*		*AP5*		*BP6*	
	Partial r	P	Partial r	P	Partial r	P
pH	-0.015	0.730	0.005	0.730	0.032	0.452
T400	0.061	0.149	0.061	0.149	-0.047	0.266
Sp. Cond. (log)	-0.058	0.170	-0.058	0.170	-0.146	0.001
Water depth (log)	-0.067	0.112	-0.067	0.112	0.073	0.086
Lake area (log)	0.054	0.206	0.054	0.206	-0.063	0.137

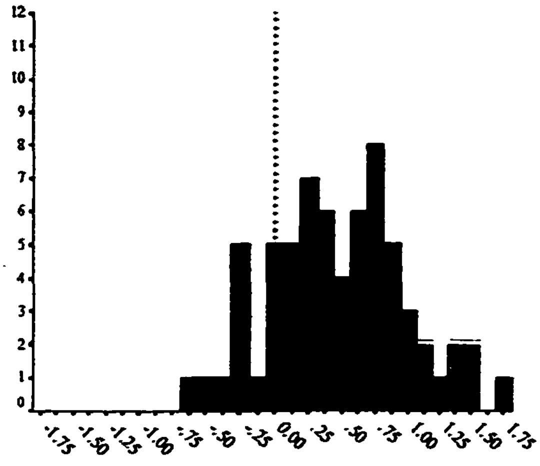
Consistent with this pattern among individuals, the rank of mean trait asymmetries showed significant concordance among all populations (Kendall's *W* = 0.29; X^2=41.000; df=2; P<0.001), with APH having the highest mean asymmetry rank (2.480) and AP5 having the lowest (1.440).

In order to determine if developmental stability had a genome-wide effect, I assessed the degree of concordance of FA among these 3 traits within individuals in each sample using Kendall's coefficient of concordance *(W)*. I found no significant intra-individual correlation of asymmetry among traits in any sample, implying that the relative asymmetry of one individual was not predictable among traits in any sample.

There were no significant differences between parasitized and nonparasitized fish in CFA or in individual trait FA within any population (T-tests: P>0.050). However, parasitized fish had a higher CFA than nonparasitized fish in a significant majority of populations containing parasites (41 of the 63 populations, Binomial test: P<0.025) although this did not occur for comparisons with individual trait FA (Binomial test: P >0.100).

I assessed whether there was evidence of a correlation between survivorship and CFA in the samples by calculating selection differentials between the age classes, and determined whether there was any geographical predictability to the distribution of these selection differentials. Among the 73 localities, no significant (Bonferroni corrected) selection differentials were detected but contrary to prediction, there were significantly more positive than negative differentials (N=52 and 21 respectively; Binomial test P<0.001). This trend occurred in each of the 3 geographical areas but was strongest in the lowland samples (lowlands 20 vs. 6, respectively; Binomial P<0.050; plateau: 13 vs.

7; Binomial $P=0.260$; mountains: 19 vs. 8; Binomial $P=0.050$). I found no relationships among the selective differentials between creeks and their adjoining lakes (Paired t-test: $t=1.410$; $df=9$; $P=0.190$) or with any environmental variables using bivariate comparisons and multiple regressions. I also examined mortality selection on asymmetry in the three individual traits. While there were no significant selection differentials for FA in any population, differentials varied significantly among traits (Kruskal-Wallis $X^2=1096.14$; $df=2$; $P<0.001$; Figure 16). The differentials of the 3 traits did not differ among the 3 geographical areas and were not correlated with the limnological variables ($P>0.050$).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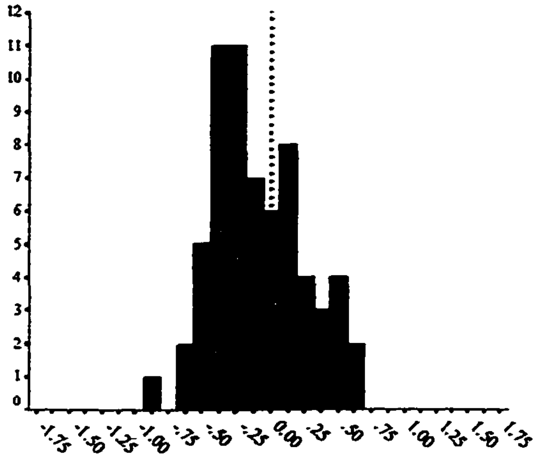
(A)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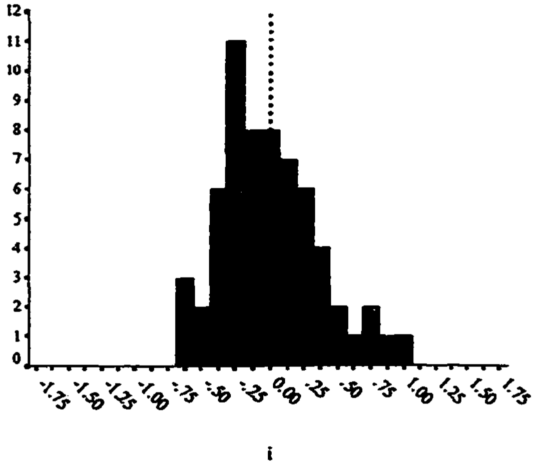
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(C)

Figure 16. Population selection differential (*i*) frequency histograms for: (A) ascending process height asymmetry (APH), (B) lateral plate 6 overlap asymmetry with basal plate (BP6), and (C) lateral plate 5 overlap asymmetry with ascending process (AP5). Selection differentials for the 3 traits vary significantly (Kruskal-Wallis X^2=1096.14; df=2; P<0.001; APH mean=0.42, AP5 mean=0.03, BP6 mean=-0.05).

Discussion

These results have demonstrated high levels of variance in multi-trait bilateral asymmetry among natural populations of freshwater *G. aculeatus* residing in pristine habitats. Inter-population variance in CFA differed geographically with highest values occurring in lakes with increased acidity and increased water staining, these tending to occur in the lowlands regions of the archipelago. In contrast, low levels of CFA were most prevalent in the oligotrophic lakes that were more similar to lakes in the mountain regions that have higher pH and higher water clarity. The abiotic associations were detected only in the complete samples (adults and juveniles) and did not occur in the subset of juvenile fish from each locality.

Previous studies have found elevated levels of FA in organisms subjected to stress during embryonic development, including acidic conditions (Jagoe and Haines 1985; Ostbye *et al.* 1997; Mazzi and Bakker 2001), exposure to UV radiation (Midgley *et al.* 1998), food deprivation (Swaddle and Witter 1994; Roy and Stanton 1999; Stoks 2001) and temperature fluctuations (Beardmore 1960; Gest *et al.* 1986; Clarke and McKenzie 1992; Leary *et al.* 1992; Hosken *et al.* 2000). As these factors can generate FA in newly emerging young, I expected to see elevated FA in juvenile stickleback in the small bog lakes of the lowlands, as these are acidic, dystrophic and shallow. That I did not observe this suggests that the specific environmental variables measured were not generating detectable FA in the young. However, several other factors may account for these results.

Other studies that have investigated environmental impacts on population FA in the wild have focused on short-term disturbances caused by human activity (Valentine and Soulé 1973; Valentine *et al.* 1973; Sanchez-Galan *et al.* 1998; Kirchhoff *et al.* 1999).

Stickleback populations on Haida Gwaii may have adapted their developmental buffering capabilities to these limnologically extreme but historically stable habitats. However, this still does not explain the large variance in juvenile FA among populations. It is likely that developmental stability in these populations was not sensitive to the particular variables measured, but rather to other ecological and/or abiotic sources of stress. In addition, the environmental variance among the pristine habitats used in this study may not have been great enough to generate detectable differences in FA. The ranges of environmental conditions in the lab that generate differences in FA are in many cases greater than environmental fluctuations in the wild (Zakharov 1992). Comparing FA among populations encompassing a greater range of conditions may reveal some more general associations.

This study involving 87 natural populations found no clear evidence that asymmetric individuals were suffering reduced survivorship. While several studies have demonstrated a negative correlation between FA and survivorship (Møller 1994; Ueno 1994; Nosil and Reimchen 2001), others have not (Bjorksten *et al.* 2001) and many have failed to find a correlation between FA and other fitness measures (Eggert and Sakaluk 1994; Swaddle and Cuthill 1995; Tomkins and Simmons 1998). In some cases, a positive correlation between FA and reproductive success or functional performance has been reported (Moodie and Moodie 1996; Seligmann 1998). What was unique to this study was the positive correlation between survivorship and asymmetry in some of the populations. High metabolic costs of developmental stability could sustain epigenetic errors in populations (Schlichting and Pigliucci 1998). Survivorship may be the result of interactions between the metabolic costs of maintaining symmetry and the fitness benefits associated with that symmetry. In habitats where the fitness benefits of symmetry are minimal, this might result in a reduction of survivorship in highly symmetric individuals.

While I found no negative correlation between survivorship and FA, I did find that parasitized fish were slightly more asymmetric than unparasitized fish among 2/3 of the populations. Other studies have found elevated FA in parasitized individuals from diverse taxa (Escos *et al.* 1995; Bonn *et al.* 1996; Hunt and Allen 1998) as well as in stickleback (Reimchen 1997; Reimchen and Nosil 2001), suggesting that FA is a reasonably consistent indicator of susceptibility of the immune system to parasite infestation. The variability in FA/fitness associations in this and other studies implies that the association is specific to particular fitness components.

I observed that asymmetry in one trait was a poor predictor of asymmetry in another trait in the same individual, indicating no suggestion of genome-wide developmental instability. Rather, FA appeared to be trait specific. For example, asymmetry of overlap between the basal plate with the 6th lateral plate (BP6) in juveniles was inversely associated with conductance, while neither ascending process height asymmetry (APH) nor asymmetry of overlap between the ascending process and the 5th plate (AP5) showed correlations with any habitat parameter in my analysis. BP6 could be more sensitive to environmental stress than the other traits, possibly due to differences in the developmental stability or timing of development among these 3 traits.

Asymmetry among the 3 traits varied significantly and demonstrated strong concordance in their relative levels of asymmetry among the populations. Ascending process height (APH) had the highest level of FA among populations and ascending process overlap asymmetry with lateral plate 5 (AP5) had the lowest. That the ranking of asymmetry among these 3 traits is similar among populations implies a common developmental process among localities and potentially a common ranking of the functional importance of these 3 traits in different habitats. Consistent differences in asymmetry among specific lateral plates exist as well, as lateral plate asymmetries in the structural plates that support the spines are relatively rare compared to asymmetry in the other plates (Bergstrom and Reimchen 2000), presumably due to increased functional costs. In the present study, there may be more serious costs of asymmetry in BP6 or AP5 than of APH, resulting in relatively stronger selection for developmental stability in BP6 and AP5 over the last 12,000 years. If this is correct, then there should be less evidence for selection against asymmetry in APH than in BP6 or AP5 and this is indeed the case, as selection differentials for APH asymmetry are significantly more positive than for the other 2 traits (Figure 16).

In summary, this study has demonstrated a large variance in CFA among 87 natural populations of *G. aculeatus*. I found significantly elevated FA in adults from lowland dystrophic localities that tend to be highly acidic, stained and shallow, and significant but weak negative bivariate correlations between adult CFA and pH and T400. However, mean CFA in juveniles was randomly distributed across the archipelago and not strongly statistically associated with pH, T400, conductance or water body size. The differences in the distribution and habitat correlates of mean CFA between these 2 age classes imply that there are other potential selective factors associated with these major geographical areas of Haida Gwaii that are generating differences in asymmetry in adults, but not juveniles. I found little evidence of reduced survivorship in asymmetric individuals or for correlations in asymmetry among traits within individuals, although the significant majority of samples had slightly elevated CFA in parasitized fish. Relative asymmetry among the individual traits was consistent among populations, and only one of the traits showed an association between FA and one limnological variable. The variance in selection differentials for FA among the 3 traits may reflect variability in their functional importance. These cumulative data imply that FA/fitness associations are character-specific and may be the result of interactions between the costs and benefits of symmetric development.

Chapter 5: Functional implications of fluctuating asymmetry among endemic populations of threespine stickleback

Introduction

Quantification of the fitness of an individual is simultaneously one of the most important tasks in evolutionary ecology and one of the most difficult. Fitness itself is theoretically simple in definition, but the practical measurement of such a quality quickly becomes a highly multidimensional and complex task (Endler 1986). There is need for a simple index of fitness that can be measured at one point in time and is relieved of the burden of a complete assessment of the selective factors in an individual's habitat. Fluctuating asymmetry (FA) has been proposed as such an index of individual fitness (Soule' 1967; Møller 1994), as it is thought to reflect an organism's genome-wide ability to buffer against stress during development (Leary *et al.* 1992). FA is manifested as a population-wide pattern of asymmetry in a bilateral trait that is normally distributed around a mean of zero (Van Valen 1962; Palmer and Strobeck 1986). FA is indicative of random errors in the phenotypic development of an organism in response to environmental or genetic stress (Adams and Niswander 1967; Gest *et al.* 1986; Clarke and McKenzie 1992; Imasheva *et al.* 1997; Campbell *et al.* 1998).

There is considerable evidence that FA is selected against both in sexually selected traits (Arcese 1994; Møller 1994; Swaddle and Cuthill 1994; Watson and Thornhill 1994; Hansen *et al.* 1999), and in traits important for locomotion (Alexander *et al.* 1984; Møller 1991; Balmford *et al.* 1993). Symmetry in freely moving animals is conceivably the ideal state, as this allows equal between-sides efficiency in response of function and behaviour in a

3-dimensional, symmetric world where the direction of stimuli is unpredictable (Bradshaw and Rogers 1993). The symmetry of functional traits used for locomotion or defense would presumably be selected for in a population, and result in reduced numbers of individuals asymmetric at these traits in older age classes.

In this chapter, I assess FA among natural isolated populations of freshwater threespine stickleback from the Haida Gwaii archipelago in northern British Columbia, Canada. Stickleback have a defensive apparatus which is composed of a series of heritable bony lateral plates (Hagen 1973) located in parallel on both sides of the body, as well as 2 large dorsal spines and 2 large pelvic spines (Figure 17). Freshwater stickleback from Haida Gwaii exhibit tremendous variation in the number of lateral plates, ranging from none to the full set of approximately 30 plates (Moodie and Reimchen 1976; Reimchen *et al.* 1985). The lateral plates are functionally important for survival, protecting the underlying integument of stickleback during predator manipulation, as well as providing structural support for the dorsal and pelvic spines (Reimchen 1983, 1992a; review in Reimchen 1994a).

Several studies have found asymmetries in the number of lateral plates in stickleback (Hagen 1973; Hagen and Gilbertson 1973a; Moodie and Reimchen 1976; Moodie and Moodie 1996), although interpretations of functional implications have been limited. Moodie and Reimchen (1976) found a correlation among stickleback populations between the degree of asymmetry and the presence of certain predators, suggesting that asymmetric stickleback are at a disadvantage as prey. Here, I have greatly expanded the investigation of inter-population variation in asymmetry to include 115 populations,

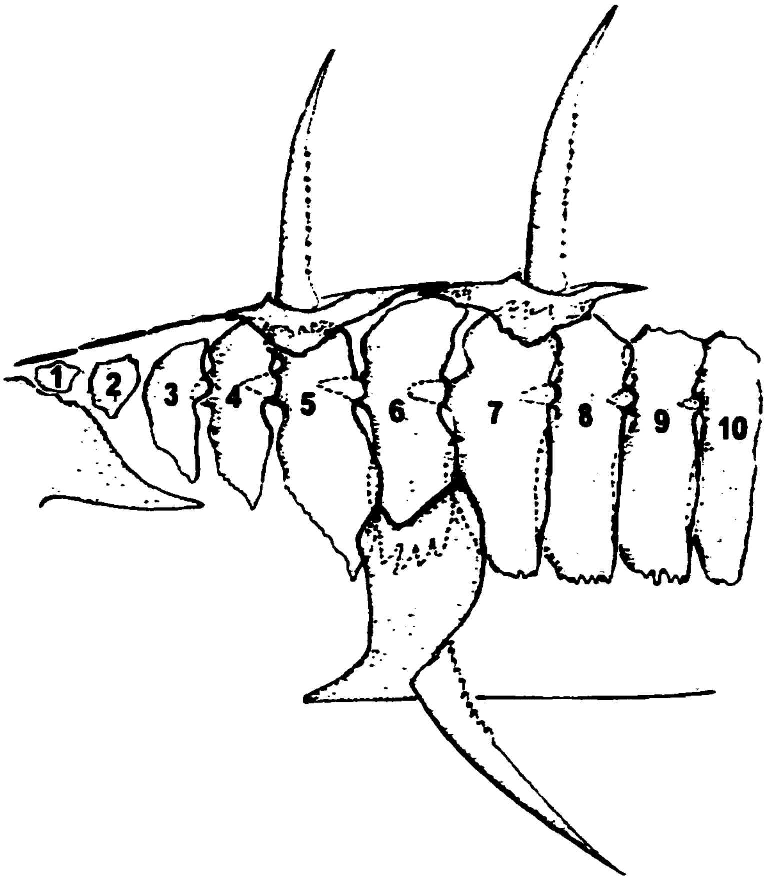

Figure 17. Schematic of lateral plates on the anterior half of a stickleback possessing plates 1 through 10, showing areas of overlap between lateral plates and spine supports. Lateral plate positions are labelled by number. Modified from Reimchen (1983).

and have used an additional and more rigorous estimate of lateral plate asymmetry than has been used before.

My first prediction is that asymmetries in the number and positions of lateral plates will compromise their effectiveness as defensive structures, therefore increasing the individual's chances of predator-mediated mortality. This will result in overall low levels of asymmetry in the lateral plates among natural populations of stickleback, as well as a decrease in relative frequency of asymmetric individuals with age.

Second, I assess variation in the FA of lateral plates among 115 natural populations in order to give an estimate of baseline levels of developmental stability. Other investigators have assessed FA among populations in the wild in order to compare their relative health under varying levels of stress generated by human disturbance (Wayne *et al.* 1986; Clarke 1995; Manning and Chamberlain 1993; Lens *et al.* 1999). However, it is difficult to interpret differences in FA among populations without first knowing what baseline levels of FA are found naturally (Palmer 1996). The benefit of using freshwater populations from Haida Gwaii for this purpose is that the habitats are relatively undisturbed and pristine, therefore giving an estimate of 'natural' levels of FA with which to compare to disturbed habitats. This is the first study to look at levels of FA among such a large number of wild populations.

Thirdly, I ascertain whether there is variation in the incidence of asymmetry among lateral plate positions. The assumption that FA is an indication of overall developmental stability predicts that I should find roughly equal frequencies of asymmetry among the plate positions. However, other studies have not found high correlations in asymmetry between traits within individuals (Van Valen 1962; Palmer and Strobeck 1986; Dufour and

Weatherhead 1996). One explanation for this lack of correlation is that different traits will vary in their susceptibility to stress as a result of different developmental pathways (Møller and Swaddle 1997). The lateral plates of stickleback are ideal to test this prediction with, as they provide a series of structures which presumably are under similar developmental constraints and which develop in close temporal proximity of each other. Therefore, variance in developmental processes should be minimised among plates.

Materials and Methods

Sampling and study area

T.E. Reimchen obtained samples of stickleback from lakes and streams throughout the archipelago during multiple expeditions between 1969 and 1997. The majority of stickleback samples were collected from April to June using standard-mesh minnow traps placed in the littoral zones. Fish were fixed in 10% formalin and stored in 95% ethanol.

Morphometrics

Morphometrics were completed on up to 50 sub-adults and 50 adults randomly selected from each sample. Morphometrics involved the following traits: standard body length (SL), sex, position of each lateral plate on the left, position of each lateral plate on the right, total plate number on the left and total plate number on the right. Each lateral plate overlies a single myomere, and can be identified by a number (P1, P2, P3, ..., P30) that is consistent between individuals (Reimchen 1983).

Asymmetry was calculated in two ways: plate number asymmetry (PNUM) and plate position asymmetry (PPOS). PNUM was calculated by subtracting the total number of plates on the left from the total number of plates on the right for each individual. This gave a signed asymmetry value for each fish (0 being symmetric), allowing me to test statistically for the presence of FA. I tested whether plate number asymmetry (PNUM) differed significantly from zero by performing 2-tailed single sample t-tests on juveniles in each sample. Two samples (Slim and Spraint) were comprised of entirely plateless stickleback and were thus excluded from further analysis of plate asymmetry. Seventeen samples did not have sufficient numbers of juveniles to run the tests for FA. Five of the remaining 96 samples showed significant but weak departures from a mean of zero (4 were left-side biased and 1 was right-side biased), but none remained significant after sequential Bonferroni corrections. Ten samples had means of exactly 0, and of the 86 samples with non-zero means, 43 were positive and 43 were negative. Kurtosis values for the signed asymmetry distributions for each sample indicated no evidence of bi-modality (anti-symmetry).

In order to calculate plate position asymmetry (PPOS), each plate along the trunk was given an asymmetry score by subtracting its presence (1) or absence (0) on the left side from the right side. Plate positions that had no plates on either side were given a null value for that position. PPOS was then calculated by summing the absolute asymmetry scores for each plate position.

Measurement error is often disguised as FA, and thus can artificially inflate asymmetry estimates (Palmer 1994). It is crucial to determine if measurement error is equal to, greater than or less than the FA of a trait. Therefore, after initial measurements were complete, 12 localities were randomly chosen and 20 individuals from each were re-measured. A 2-way mixed model ANOVA with plate number as the dependant and individual and side as the factors was used to determine if the variance among sides was significantly greater than the variance among replicate measurements. The side by individual interaction term was significant ($P<0.001$), demonstrating that asymmetry variance was significantly greater than measurement error variance (Palmer and Strobeck 1986).

Previous published studies have used PNUM as their only measure of lateral plate asymmetry in stickleback (Hagen 1973; Hagen and Gilbertson 1973a; Moodie and Reimchen 1976; Moodie and Moodie 1996). PPOS provides a more informative assessment of asymmetry than PNUM. In the context of this study, it allows for the inclusion of fish into the asymmetric group that have a plate present at a designated position on one side but not the other even if total plate number is symmetric (*e.g.* plates 2 to 7 present on the left, and 3 to 8 on the right). I considered a fish to be asymmetric if it was asymmetric at least at one plate position. Fish with no plates were given missing values for plate asymmetry.

I compared PPOS between sexes from each locality using 2-tailed t-tests. None of the differences were significant at the $P\leq0.050$ level after Bonferroni corrections, and there was no significant difference in the number of localities with higher PPOS in females than with higher PPOS in males (55 vs. 50, respectively; $X^2=0.238$; df=1; $P>0.500$). The sexes were therefore pooled for further analysis.

The relative frequency of asymmetric individuals was calculated among sampled populations. I also compared the frequency of asymmetric sub-adults and adults at each location to determine if the asymmetric individuals were being selected out of the populations. Some localities contained a mixture of stickleback with lateral plates and others that were completely naked. The validity of scoring a fish as symmetric for a trait that is absent on both sides is questionable. Thirty-four samples contained at least one naked stickleback, and were therefore excluded from population comparisons of frequencies of asymmetric individuals.

Results

Distribution of asymmetries among populations

The frequency of stickleback asymmetric for PNUM among the 81 samples with no naked individuals varied from 0% to 63% among localities (mean = 39%), while the frequency of those asymmetric for PPOS varied from 0% to 76% (mean = 41%). As expected, the frequency of stickleback asymmetric for PNUM was in all cases equal to or less than the frequency of stickleback asymmetric for PPOS. The correlation of these 2 asymmetry indices was significant and positive among populations (Pearson's $r=0.980$, $P<0.001$). Approximately 89% of the samples were composed of at least 25% asymmetric stickleback, and the majority of the samples from this archipelago contained at least 42% asymmetric individuals.

Mean PPOS was not significantly different between sub-adults and adults for any locality after Bonferroni corrections. The number of localities that had greater FA in juveniles than adults was not significantly different from the number that had greater FA in adults than juveniles (41 vs. 54, respectively; $X^2=1.779$, $df=1$; $P>0.100$).

Distribution of asymmetries among lateral plate positions

The frequency of asymmetric plates among the 30 possible plate positions for the pooled samples was highly variable (χ^2= 211.41; df=29; P< 0.001; Figure 18). Asymmetries occurred frequently in positions 1 and 2, with a sharp decrease in positions 4 to 7 followed by an increase again in positions 8 and 9. Posterior to position 9 there was a progressive reduction in asymmetry.

Threespine stickleback have three genetically distinct lateral plate morphologies: low plated, partially plated and completely plated (Wootton 1984), all three of which are represented in the samples used for this study. The low-plated morph has anterior plates only, usually in the range of positions 1 to 9, and no keel on the caudal peduncle. The completely plated morph has the full set of plates extending to the anterior tip of a well-developed keel. The partially plated morph has the anterior plates and a keel, as well as some intermediate plates in various positions along the trunk. Asymmetries occurred very rarely in the completely plated morph (4.4% of the individuals of this group) compared to the partially plated (72.7%) and low plated (49.5%) morphs. Therefore, in order to remove any confounding effects of genetic predisposition and varying susceptibility to FA among the 3 morphs, frequency of asymmetries among plate positions were evaluated for each.

The distribution of lateral plate asymmetries among positions for the low plated morphs was highly variable and significantly deviated from an equal distribution (χ^2=218.28; df=8; P<0.001; Figure 19). The distribution was U-shaped, with the most anterior and posterior positions having the highest asymmetry, and central positions 4 to 7 having the lowest.

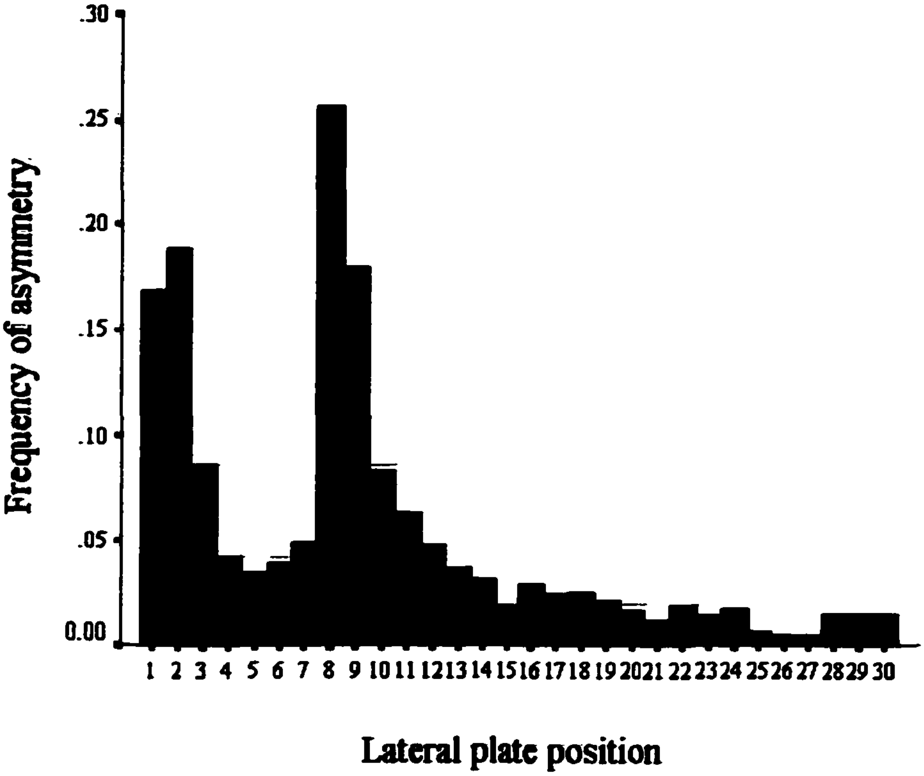

Figure 18. Total frequencies of asymmetries among lateral plate positions for pooled locality samples. Total N=3,702.

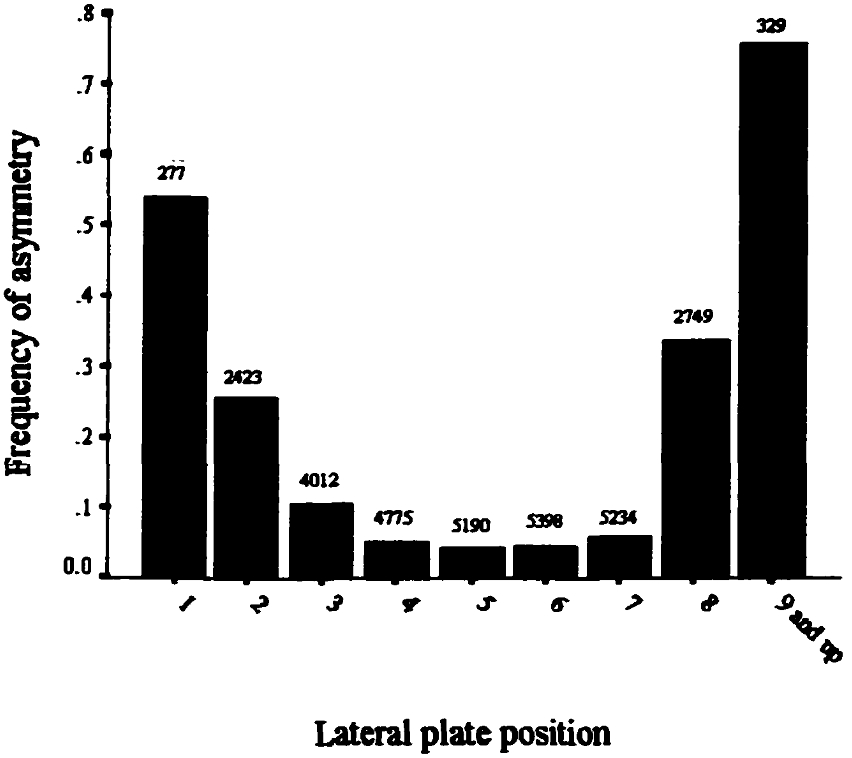

Figure 19. Frequencies of asymmetries among lateral plate positions for the low-plated morphs. Locality samples are pooled. Numbers above bars denote the number of symmetric and asymmetric stickleback at that plate position. Fish with no plates present at a position are not included in that position's sample size.

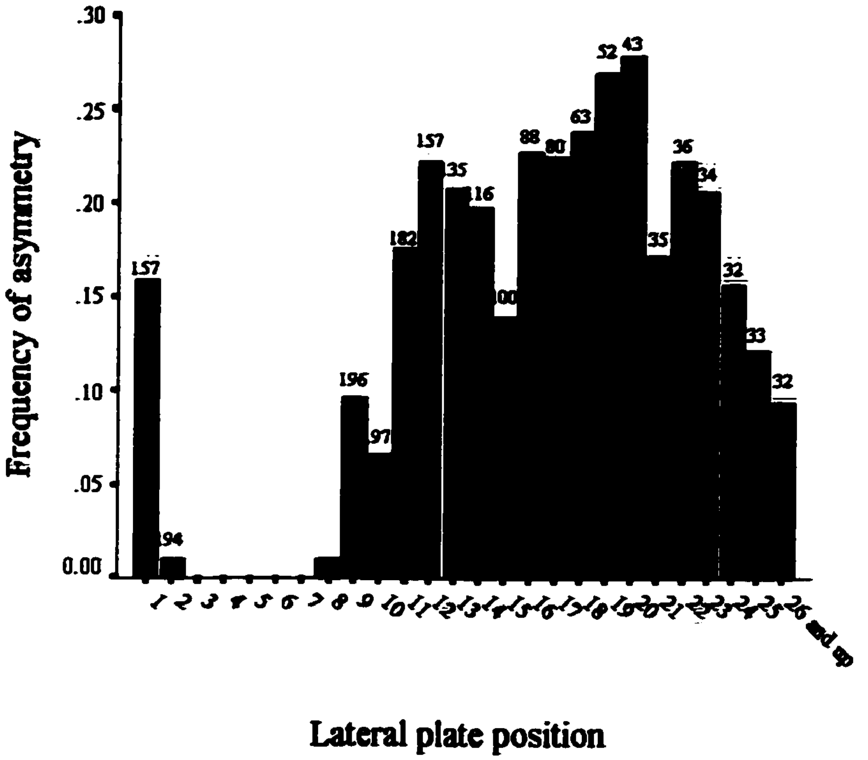

Figure 20. Frequencies of asymmetries among lateral plate positions for the partially-plated morphs. Locality samples are pooled. Numbers above bars denote the number of symmetric and asymmetric stickleback at that plate position if different from the total sample size. Fish with no plates present at a position are not included in that position's sample size. Total N=233.

The distribution of lateral plate asymmetries among positions for the partially plated morphs was also highly variable and significantly deviated from an equal distribution (χ^2=171.53; df=25; P<0.001; Figure 20). In this group the asymmetry was greatly reduced in positions 3 to 7 relative to more anterior and posterior plates, similar to the low plated morphs, and decreased gradually towards the caudal peduncle (22 to 26).

The completely plated morph had very low overall plate asymmetry relative to the other two morphs (4.4% of all completely plated individuals). The distribution of lateral plate asymmetries among positions for the complete morph was variable but did not deviate significantly from an equal distribution (χ^2=22.26; df=29; P>0.75; Figure 21). Asymmetries were relatively infrequent throughout plate positions (at most 14 individuals, or ~2% of the completely plated fish, were asymmetric for a given position) and were completely absent in the majority.

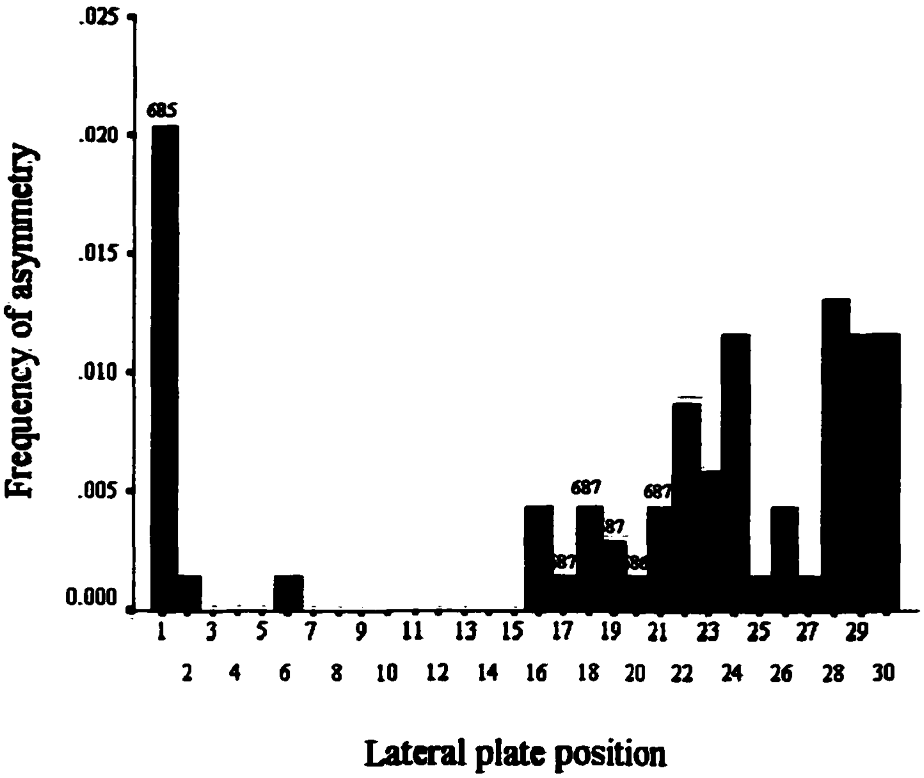

Figure 21. Frequencies of asymmetries among lateral plate positions for the completely-plated morphs. Locality samples are pooled. Numbers above bars denote the number of symmetric and asymmetric stickleback at that plate position if different from the total sample size. Fish with no plates present at a position are not included in that position's sample size. Total N=690.

Discussion

Total frequency of asymmetric individuals

My prediction of low levels of asymmetry in the lateral plates of *G. aculeatus* was not supported, as the incidence of asymmetry among these natural populations was exceptionally high (average frequency was 41%). Other studies have predicted that asymmetry in functional traits would be selected out of the gene pool (Van Valen 1962), and in many instances, this has been demonstrated (*e.g.* Balmford *et al.* 1993; Evans *et al.* 1994; Swaddle 1997b). It may be that asymmetric individuals in these lakes and ponds are in fact suffering increased relative mortality, but the level of asymmetry in the pre-selected sub-adults is so high each year, that the frequency of asymmetric adults remains high. However, if this were the case I would expect to see a decrease in the frequency of asymmetric individuals from sub-adults to adults, and this has not been found in any of these samples, or in other studies of plate number asymmetry in stickleback (Moodie and Reimchen 1976; Reimchen unpublished data).

It is possible that the asymmetry may actually provide an advantage to the stickleback, and this advantage is maintaining the high frequency of asymmetric individuals. Other studies have found subtle advantages to asymmetry in stickleback. Moodie and Moodie (1996) found that male stickleback asymmetric for plate number were more likely to contain fry in their nests than symmetric males, and Reimchen (1997) found that juvenile stickleback asymmetric for pelvic girdle development had a significantly lower incidence of parasite infection. Asymmetry studies in other taxa as well have shown evidence that subtle asymmetries can be advantageous (*e.g.* Seligman 1998). A functional advantage to asymmetry of the posterior lateral plates in stickleback may be an increase in flexibility. Stickleback perform a C-start burst acceleration when startled (Taylor and McPhail 1985) and a missing plate on one side may allow a greater angle to be acquired and thus a greater velocity. However, experiments on the effects of lateral plates on body flexibility have demonstrated little effect, and C-shape angle was not a good predictor of fast-start performance in this species (Bergstrom 2002).

Distribution of asymmetries among populations

The large variation in the frequency of asymmetric individuals among populations (Figure 18) was unexpected. Clearly there is not a homogenous baseline level of lateral plate asymmetry among freshwater stickleback populations. I suggest that this variation may be due to any one or more of the following factors.

First, the variation may be due to differences in the severity of natural environmental stress (temperature, pH, productivity, inter- and intra-specific competition) among localities (*e.g.* Auffray *et al.* 1999). For example, increased competition or low productivity may result in resource depletion for gravid females. Lower allocation of energy and nutrients into the eggs may equate to increased stress during development from this maternal affect, and inflated FA in the offspring. However, there were no correlations with environmental variation and asymmetry in three other traits among stickleback populations from this archipelago (Bergstrom and Reimchen in press). The possible effects of habitat characteristics on lateral plate asymmetry will be assessed in the next chapter.

Second, the isolated populations might vary in their genetic quality, resulting from the disruption of co-adapted gene complexes or elevated levels of homozygosity; both proposed as causes of FA (review in Møller and Swaddle 1997). Hybridization is unlikely between these populations, as the majority of them are geographically isolated lakes and ponds. However, some hybridization is possible with marine stickleback because of flooding and/or non-assortative mating between lake and stream forms, but the exact degree to which this takes place is not known. Lower Victoria Lake, which has the highest frequency of asymmetric individuals, is geographically isolated from marine waters with a high gradient stream separating them, leaving no opportunity for hybridization with marine stickleback. The inlet streams draining into Lower Victoria also have a high gradient, and thus are unlikely to have resident stream populations of stickleback, minimising the chances of hybridization between lake and stream forms. If hybridization were a primary cause of FA, one would expect to find low levels of asymmetry at this lake, contrary to our results. Levels of genetic homozygosity in the populations have not been thoroughly assessed. A quantification of the impact these factors have on inter-population variation in FA can only be obtained with rigorous genetic analysis.

Third, the variation may be due to differential selective pressures on asymmetric individuals among populations. Moodie and Reimchen (1976) found a negative correlation among stickleback populations from Haida Gwaii between lateral plate number asymmetry (PNUM) and the presence of predatory fish. It is possible that variation in predation regime or intensity among localities is generating a differential in selective pressure on asymmetric stickleback prey. This will also be addressed in the next chapter.

Distribution of asymmetries among lateral plate positions

The distributions of lateral plate asymmetries among the positions exhibit unexpectedly high levels of heterogeneity, contrary to my prediction. Lateral plates at positions 4 through 7, which exhibit a drastic decrease in incidence of asymmetry, provide the major structural support for the dorsal and pelvic spines (Reimchen 1983). There may be strong selection against asymmetry in these structural plates. The large spines on stickleback provide protection against predators (Hoogland *et al.* 1957) by increasing the cross-sectional diameter, often to sizes beyond the capabilities of gape-limited predators (Reimchen 1991). If the absence of a plate on one side of a structural position equates to the spines' easy deflection during manipulation by a predator, the cost of this asymmetry may be high. This reasoning is consistent with the anterior U-shaped distribution of asymmetries that are found in low and partially plated stickleback (Figures 20, 21). If the cost of asymmetry decreases with increasing distance from the structural plate positions, this may explain the increase in incidence of asymmetry posterior to position 8 and anterior to position 4 in these 2 morphs.

The second explanation of this variation in asymmetry among positions is a developmental one. The lateral plates do not develop simultaneously at each position, but rather in series. The structural plates 5 and 6 develop first, followed by the more anterior and posterior plates in sequence (7, then 4, 8, 3, etc.) until development is complete (Igarashi 1964; Bell 1981). This happens to correspond roughly with increasing asymmetry found among the anterior plate positions. The window of susceptibility to stress during ontogeny may vary temporally, such that lateral plates developing later may be more sensitive to stress than plates that develop earlier. Clearly, this issue is in need of experimental investigation.

The pattern of asymmetry among plate positions for the completely plated stickleback was statistically homogeneous, although the distribution of frequency of plate asymmetry was suggestively similar to the low and partially plated distributions (Figure 21). Strong selection for post-capture survival during predation events results in the maintenance of the lateral plates in complete morphs in freshwater habitats (Reimchen 1994a). If the benefits of plate symmetry in complete morphs out-weigh the potential developmental costs of symmetry, plate development in this morph may be highly canalized in all positions. In other words, there may be strong selection against developmental errors that compromise the functional integrity of a structure when optimal function of that trait is crucial to the survival of the organism in its natural ecological context. If this is the case for lateral plates in stickleback, a decrease in plate asymmetry in the complete morph, as well as in the structural plates of the low and partially plated morphs, is not surprising.

Conclusions

The results of this study suggest that the use of trait FA as an estimate of fitness is clearly problematic. In order to interpret associations between trait FA and fitness, it is crucial to have a thorough understanding of how the trait functions in an ecological context, and of the significance of selective pressures acting on it. I have demonstrated that there is high discordance of asymmetry among serial, homologous structures within and among populations, and that the structures may be under different levels of selection. The accuracy of an estimate of fitness based on the FA of one or a few traits is highly dependent on the magnitude of variation of the selective pressures on the traits in question. Proceeding with a study of FA on a set of traits with less than a thorough understanding of their function will lead to questionable interpretations. Ideally, the assessment of asymmetry in numerous traits not subject to selection would be ideal, but the satisfactory demonstration of true neutrality is extremely problematic.

The use of FA to assess the health of a population also needs to be exercised with caution, as this study demonstrates that natural levels of FA can fluctuate drastically. Clearly more work is needed to investigate the causes of FA in the wild before this measure can be trusted with confidence as an indicator of environmental stress.

Chapter 6: Asymmetry in structural defenses: insights into selective predation in the wild

Introduction

Fluctuating asymmetry (FA) reflects small random errors made during the development of a trait (Waddington 1942; Zakharov 1992). There has been growing interest in the evolutionary implications of (FA) in the last three decades, as a large range of taxa exhibit negative correlations between FA and components of fitness (Packer and Pusey 1993; Watson and Thornhill 1994; Hunt and Allen 1998; Rantala *et al.* 2000; Nosil and Reimchen 2001). In some cases, FA is an indication of fitness reduction that is associated with developmental instability (Mather 1953; Van Valen 1962; Soulé 1967). In other cases, FA can result in fitness loss by reducing the functional efficiency of traits (Allen and Simmons 1996), particularly in the context of locomotion (Moodie 1977; Møller and Hoglund 1991; Thomas 1993; Gummer and Brigham 1995; Swaddle *et al.* 1996; Swaddle 1997b; Martin and Lopez 2001). When the functional consequences of asymmetry directly reduce biomechanical efficiency, it is difficult to resolve the cause/effect relationship between FA and fitness.

The outcome of predator/prey interactions can be dependant on asymmetry of the participants. Asymmetric houseflies are more susceptible to predation by barn swallows (Møller 1996), while asymmetric barn swallows are more susceptible to predation by European Sparrow hawks (Møller and Nielsen 1997). Houseflies captured by dung flies have elevated tibial asymmetry relative to non-predated houseflies, while unsuccessful predatory dung flies have elevated tibial asymmetry relative to successful individuals (Swaddle 1997a). However, it is unknown whether susceptibility to predation is a direct functional consequence of asymmetry or an indirect effect of reduction in other fitness components that are associated with developmental instability.

Given the ubiquity of selective predation in the wild, it is likely that there will be functional costs of asymmetry in traits used for predator defense. Selection can reduce trait specific asymmetry over time (Reeve 1960; Møller and Thornhill 1997); but see (Perfectti and Camacho 1999; Leamy *et al.* 2000) and consequently, the degree of character asymmetry may reflect its relative biomechanical importance (Mather 1953; Palmer and Strobeck 1986; Balmford *et al.* 1993; Gummer and Brigham 1995; Clarke 1998). Given this, one would predict that structures that are important to the survival of prey would have reduced asymmetry in those habitats where predation intensity is relatively high.

Threespine stickleback (*Gasterosteus aculeatus*) possess structural predator defenses that include two large dorsal spines, a pair of ventral pelvic spines, and a series of heritable, bony lateral plates (see review in Wootton 1984), some of which buttress the dorsal and pelvic spine supports (Figure 22). Experimental work and fieldwork have demonstrated that the numbers of lateral plates are under selection by predators (Hagen and Gilbertson 1973b; Moodie and Reimchen 1976; Gross 1977; Bell and Haglund 1978; Bell and Richkind 1981; Banbura *et al.* 1989; Reimchen 1992a, 2000). The lateral plates provide protection against puncture during predator handling (Reimchen 1992a), interfere with the pharyngeal actions of gape-limited predators (Reimchen 2000) and provide structural support for adjoining dorsal and pelvic spines (Reimchen 1983). The dorsal and pelvic spines are erect during predator handling, and the subsequent

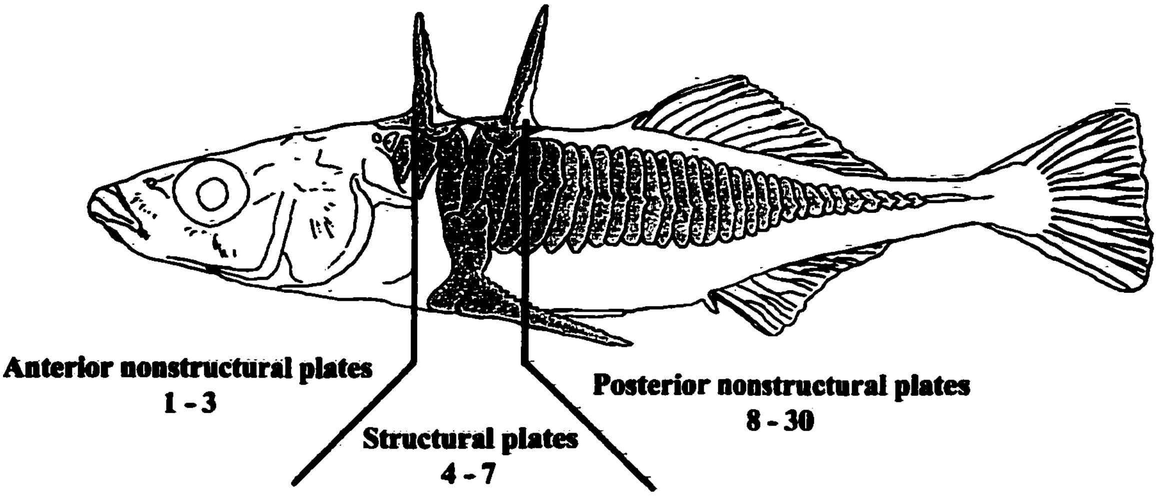

Figure 22. Camera lucida drawing of a completely plated threespine stickleback. The two large dorsal spines, left pelvic spine, structural, and non-structural lateral plates are highlighted in grey.

increase in cross-sectional diameter interferes with the handling efficiency of large fish predators (Hoogland *et al.* 1957; Reimchen 1991) and is greater in stickleback residing with predators (Hagen and Gilbertson 1972; Gross 1978). Artificial removal of the structural plates (those that lie directly below the spines) results in easier spine deflection, an effect that would have serious repercussions to a stickleback trying to escape manipulation by a predator (Reimchen 1983). Therefore, it is likely that asymmetry in the presence of structural plates would be to the detriment of stickleback during predator handling. Indeed, these plates exhibit reduced levels of asymmetry relative to non-structural plates among freshwater populations of stickleback from the islands of Haida Gwaii, British Columbia (Bergstrom and Reimchen 2000), suggesting that structural asymmetries may put stickleback at a selective disadvantage.

Frequencies of stickleback asymmetric for lateral plate numbers and positioning (structural and non-structural) vary from 1% to 76% among freshwater populations on Haida Gwaii (Bergstrom and Reimchen 2000). While plate number asymmetry is reduced in populations residing with predatory trout (Moodie and Reimchen 1976), we don't know whether the variance in asymmetry among populations is due more to structural or to non-structural plates, whether the asymmetry is associated with the degree of structural defense expression, or whether the asymmetry is dependant on the chances of capture by predators.

Here I investigate variation in lateral plate asymmetry among 115 endemic natural freshwater populations of *G. aculeatus* from Haida Gwaii. I determine if the frequency of structural and non-structural lateral plate asymmetry is negatively correlated with the degree of expression of structural defenses in stickleback, as measured by total plate number and cross-sectional diameter. I also determine if plate asymmetry is greater in populations where visibility, and therefore the chance of capture, is reduced. Within each population, I compare the frequency of lateral plate asymmetry between stickleback with predator injuries and those without, between parasitized and unparasitized stickleback, as well as between 2 age classes (juvenile and adult). This allows me to investigate the association between lateral plate FA and 3 fitness components (susceptibility to predator subjugation, parasitism and total survivorship). Knowledge of the function and sources of selection on lateral plates allows for the determination of whether geographical distributions of plate FA among populations reflects differences in developmental stability or differences in the relative biomechanical importance of structural defenses against predators. This is the first investigation to address the ecological and functional implications of geographical variation in structural defense asymmetry among wild populations.

Materials and Methods

T.E. Reimchen collected samples of threespine stickleback from lakes and streams throughout the archipelago during multiple expeditions between 1969 and 1997. Of these original collections, 115 samples were used for this study. The majority of stickleback samples were collected from April to July using standard-mesh minnow traps placed in the littoral zones. Fish were fixed in 10% formalin and stored in 95% ethanol. Water chemistry and lake data were collected from a subset of the localities at the time of collection. This included pH, percent light transmission at a wavelength of 400 nm (T400), specific conductance (μmhos cm^{-1}), water depth (m), and lake area (hectares).

Specific conductance, water depth and lake area were log transformed to normalize the skew in their distributions. All localities were categorized as belonging in one of the three recognized geographical areas on the archipelago: lowlands, plateaus or mountains (Brown 1968).

From each sample of stickleback, up to 100 individuals comprising approximately 50 juveniles (ca. 35 - 45 mm SL) and 50 adults (> 45 mm) were measured for standard length (SL), sex, parasite infection (*Schistocephalus solidus, Eustrongylides* sp., *Diplostomum* sp.), cross-sectional diameter with the spines erect, degree of overlap between the plates and the spine supports, number of lateral plates per side, position of each lateral plate on the left, and position of each lateral plate on the right. Each lateral plate overlies a single myomere, and can be identified by a number (P1, P2, P3, ..., P30) that is consistent between individuals (Reimchen 1983). Asymmetry at each position was also calculated ($R_{P1} - L_{P1}$, $R_{P2} - L_{P2}$, etc.), and a fish was considered asymmetric if it was asymmetric at one or more plate positions. Lateral plate number was square root transformed and cross-sectional diameter was log transformed.

Lateral plate number asymmetry did not show evidence of directional asymmetry, anti-symmetry, or significant measurement error in any population sample (Bergstrom and Reimchen 2000). Stickleback with no plates and two samples that were comprised entirely of plateless stickleback (Slim and Spraint) were excluded from further analysis of plate asymmetry. There were no significant differences in the frequency of lateral plate position asymmetry between sexes in any population sample (Bergstrom and Reimchen 2000). The sexes were therefore pooled for further analysis.

Plate positions were subdivided into 'structural' and 'non-structural'. Structural plates are comprised of plates 4 – 7, which lie directly underneath the two large dorsal spines and above the pelvic spines (Figure 22). These plates provide the greatest lateral buttressing support for the dorsal spines (Reimchen 1983). Non-structural plates are those plates anterior and posterior to the structural plates (1–3, and 8–30; Figure 22). A fish was considered to have structural plate asymmetry if it was asymmetric at one or more structural positions (4 – 7) and was considered to have non-structural asymmetry if it was asymmetric at one or more non-structural positions (1 – 3 or 8 – 30).

Each stickleback was scored for the presence or absence of predator-induced injuries, as described in Reimchen (1988). Briefly, injuries were categorized as avian-caused if there were parallel, compression-like, dorsal/ventral directed scars on both sides of the body, and as trout-caused if there were punctures or long comb-like anterior/posterior directed scars (Figure 9).

Results

Mean frequency of total plate asymmetry differed significantly among the three geographical areas (Kruskal-Wallis X^2=19.784; df=2; P<0.001), and increased from the mountain to the plateau to the lowland samples. Total plate asymmetry was comprised of both non-structural and structural plates and there were geographical clines in the distributions of population frequencies of both types of asymmetry. Non-structural asymmetries were present in 43% of all stickleback while structural asymmetries were present in 12%. Ninety-eight percent of the sampled populations had a greater incidence of non-structural than structural asymmetry. The mean population frequency of asymmetric fish differed significantly among the three geographical areas for both non-structural (Figure 23; Kruskal-Wallis $X^2=18.693$; df=2; $P<0.001$) and structural (Figure 24; Kruskal-Wallis $X^2=44.590$; df=2; $P<0.001$) plate asymmetries, and both demonstrated an increase in mean frequency from the mountain to the lowland samples. The variance of frequencies also differed significantly among the three areas for non-structural (Levene's test: $F=4.935$; $P<0.050$) and structural plate asymmetries (Levene's test: $F=81.249$; $P<0.001$), being greatest in the lowlands for both.

I ran a multiple regression to determine if there were correlations between population frequency of juvenile stickleback asymmetric for lateral plate position and several environmental variables that may affect developmental stress (pH, specific conductivity, T400, water depth, and lake area). There was a significant negative correlation with pH, but not with any of the other environmental factors (Table 17). However, lateral plate number may have confounded this effect, as pH and mean number of lateral plates are strongly positively correlated among population samples ($r=0.657$; $P<0.001$). When mean number of plates was included in the regression model, it was the only significant correlate with population frequency of asymmetric juveniles (Table 17). Not all samples were included in the multiple regression analysis since pH, specific conductivity, and T400 were only collected at a subset of localities. I had water depth, lake area and a categorical measure of water staining for all localities, and a 3-way ANOVA of these variables on population frequency of asymmetric juveniles demonstrated no significant main effects or interaction terms (all $P>0.250$), consistent with the multiple regression.

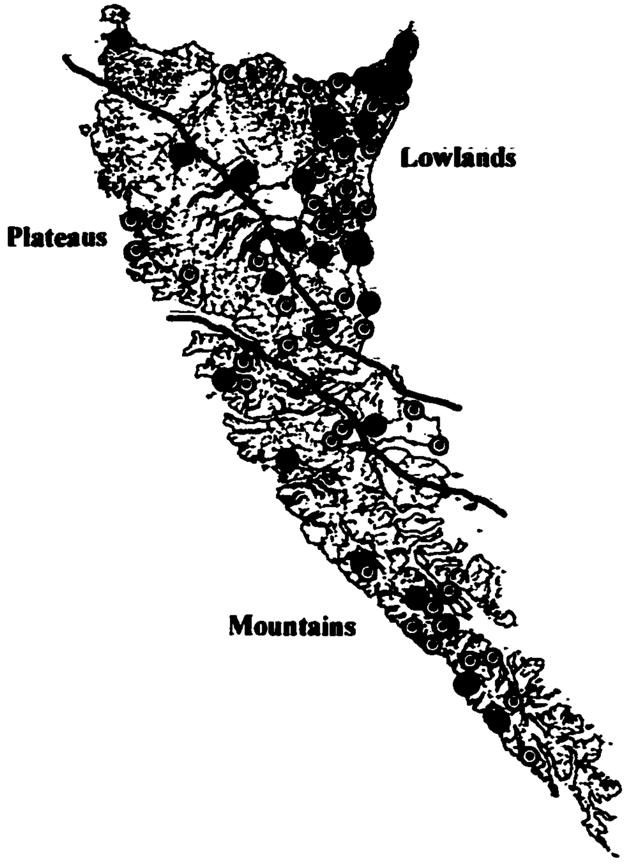

Figure 23. Distribution of population frequencies for non-structural plate asymmetry. ● = > 66% fish asymmetric for non-structural plate positions; ◉ = 33 – 66% fish asymmetric for non-structural plate positions; ⊛ = < 33% fish asymmetric for non-structural plate positions. Lowland samples contained the largest proportion of populations with more than 66% asymmetric stickleback, while the mountain samples contained the largest proportion with fewer than 33% asymmetric stickleback (X^2=17.766; df=2; P=0.001).

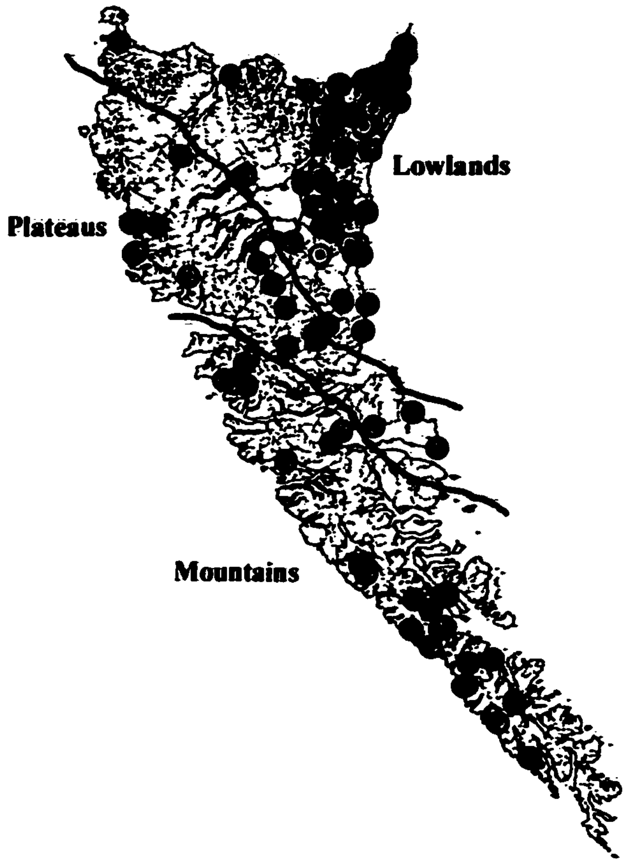

Figure 24. Distribution of population frequencies for structural plate asymmetry. ● = > 66% fish asymmetric for structural plate positions; ◉ = 33 – 66% fish asymmetric for structural plate positions; ◎ = < 33% fish asymmetric for structural plate positions. All of the population samples with greater than 33% asymmetric fish were found in the lowlands, with the plateaus and mountains only containing population samples with fewer than 33% asymmetric fish (X^2=26.242; df=2; P<0.001).

Table 17. Multiple regression analysis showing correlations between frequency of juveniles asymmetric for lateral plate position and pH, T400, specific conductivity (log-transformed), water depth (log-transformed), and lake area (log-transformed) with and without lateral plate number (square-root transformed) included as a factor. Multiple regression model results without plate number included: R^2=0.249; F=1.458; df=27; P=0.243. Multiple regression model results with plate number included: R^2=0.611; F=5.494; df=27; P=0.001.

	Without plate number		With plate number	
	Partial r	P	*Partial r*	*P*
Lateral plate number	.	.	-0.694	<0.001
pH	-0.454	0.026	-0.143	0.516
T400	0.262	0.217	0.155	0.480
Specific conductivity	0.075	0.729	0.133	0.613
Water depth	0.038	0.858	0.279	0.198
Lake area	-0.223	0.296	-0.363	0.089

I wanted to determine if frequency of lateral plate asymmetry was associated with the expression of post-capture defenses in stickleback, as measured by the number of lateral plates and cross-sectional diameter of the stickleback. There was a significant negative correlation between population lateral plate mode and frequency of asymmetric individuals, including juveniles and adults ($r=-0.798$; $P<0.001$). The correlation was significant for both non-structural ($r=-0.696$; $P<0.001$) and structural ($r=-0.680$; $P<0.001$) plate asymmetries (Figure 25). Frequency of non-structural asymmetry gradually decreased from close to 100% to less than 10% with increasing plate number. Frequency of structural plate asymmetry decreased sharply from close to 100% to about 2% as population plate modes increased from 0 to 7 plates per side ($r=-0.948$; $P<0.001$). In populations with plate modes greater than 7, the frequency of structural plate asymmetries remained at or below 2% and no longer decreased significantly with plate number ($r=0.174$; $P=0.471$). In addition, after the effect of plate number was removed, residual cross-sectional diameter was significantly negatively correlated with the frequency of structural plate asymmetry among populations (residual $r=-0.261$; $P=0.005$) but not with non-structural plate asymmetry (residual $r=-0.118$; $P=0.242$).

Lateral structural support of the spines by the lateral plates is dependant on the degree of overlap between the plates and the adjoining spine buttresses. The degree of overlap varies among individuals from very strong (>1 mm) to no contact at all (Reimchen 1983). In populations with little overlap, I would expect to find that structural asymmetry frequencies are comparable to non-structural asymmetries, as the biomechanical difference between these two types of plates would be minimized. As the degree of overlap increases, I would expect to find significantly reduced structural plate

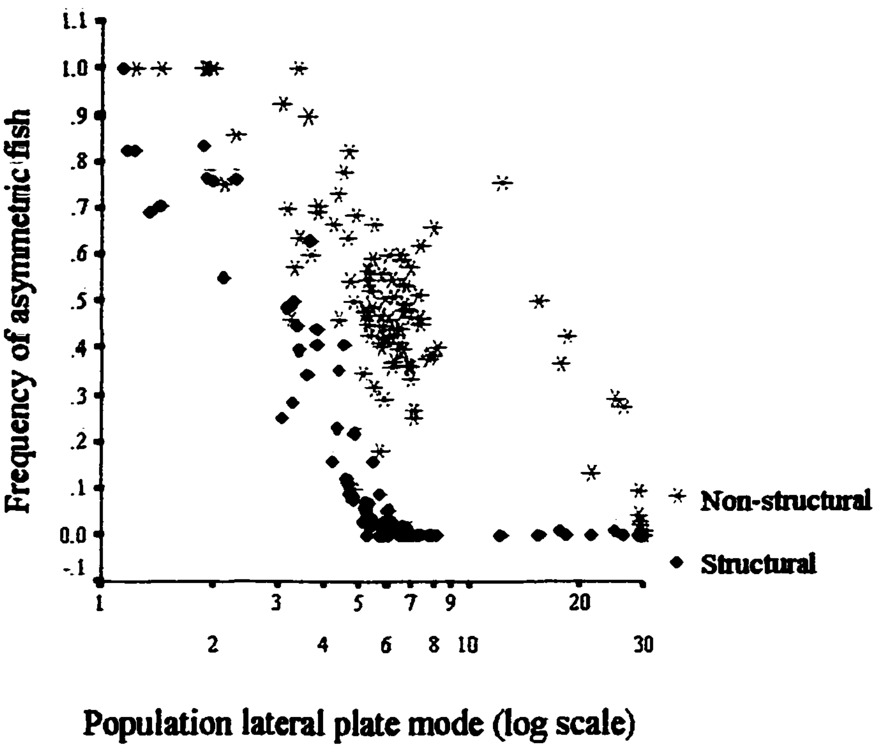

Figure 25. Negative correlations between lateral plate mode and the frequency of non-structurally and structurally asymmetric stickleback among population samples.

asymmetries relative to non-structural. I compared the frequency of asymmetric individuals among populations of a given lateral plate number mode but with varying degrees of mean overlap. Only populations with plate modes of 3 and 4 provided suitable samples sizes of both types of asymmetry to make interpretable comparisons. As predicted, at the lowest levels of overlap, the frequency of structurally asymmetric fish was not significantly different from non-structural asymmetry for either plate mode (Figure 26, 27). However, as population overlap mode increased, structural asymmetries became significantly less frequent than non-structural asymmetries among populations of both plate modes.

Another indicator of the biomechanical importance of structural defenses to prey in aquatic systems is visibility. While T400 did not appear to affect developmental stability and FA in juveniles, it may be an indicator of the strength of selection on structural defenses in older fish, as it is associated with predator reaction distance and likelihood of prey capture (Vinyard and O'Brien 1976; Utne 1997). Therefore, I compared population frequencies of plate asymmetry (including both juveniles and adults) among 3 different categorical levels of water staining that were assigned in the field at the time of collection: stained ($T400<70$), moderately stained ($70<T400<85$) and clear ($T400>85$) (Reimchen, pers. comm.). Mean plate asymmetry frequency increased significantly as water staining increased (ANOVA: $F=12.846$; $df=2$; $P<0.001$; Table 18), and multiple comparisons showed that heavily stained localities ($T400< 70$) contained significantly more asymmetric fish than both moderately stained ($70<T400<85$; $P<0.025$) and clear ($T400>85$; $P<0.001$). The increase in plate asymmetry with water staining was

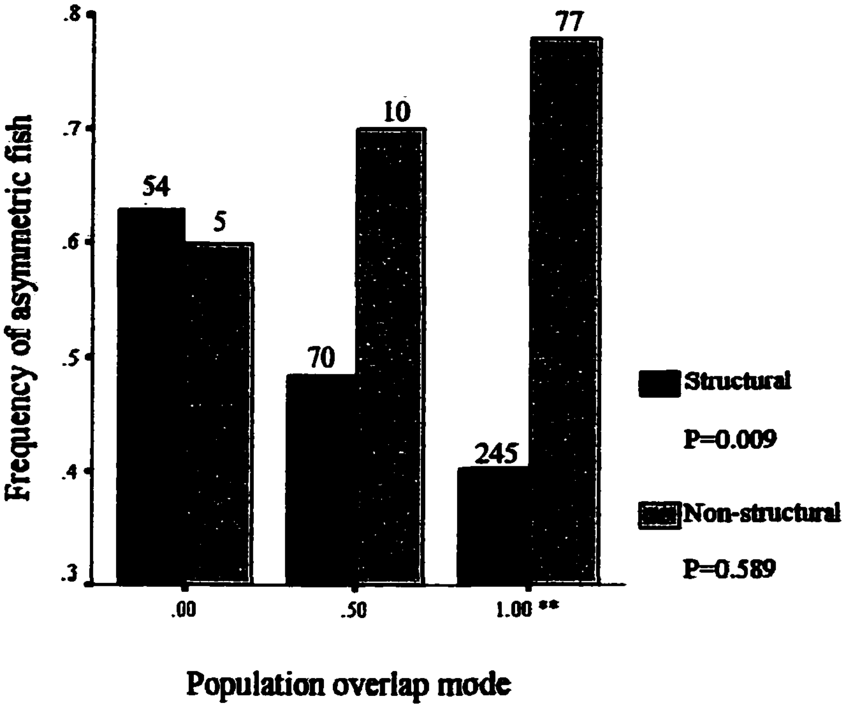

Figure 26. Frequency of structurally and non-structurally asymmetric stickleback among populations with lateral plate modes of 3 but with different levels of overlap between the plates and the spine supports. Numbers above bars designate sample size. $^{**}P<0.001$.

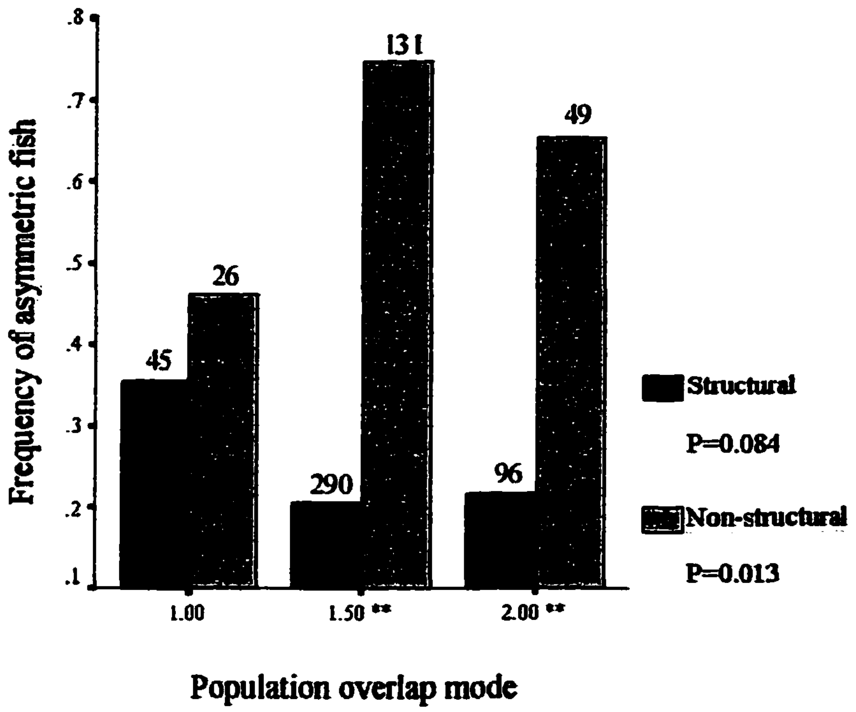

Figure 27. Frequency of structurally and non-structurally asymmetric stickleback among populations with lateral plate modes of 4 but with different levels of overlap between the plates and the spine supports. Numbers above bars designate sample size. **P<0.001.

Table 18. Means and standard errors of frequencies of asymmetric fish and lateral plate numbers of populations from localities of 3 different levels of water staining.

Level of staining	Frequency of asymmetric fish	Lateral plate number
	Mean +/- SE	Mean +/- SE
Heavy	0.591 +/- 0.028	3.695 +/- 2.034
Moderate	0.486 +/- 0.037	8.305 +/- 7.645
Clear	0.368 +/- 0.034	11.128 +/- 8.994

evident in both non-structural (Kruskal-Wallis: X^2=21.420; df=2; P<0.001) and structural (Kruskal-Wallis X^2=43.191; df=2; P<0.001) plate asymmetries.

The reduction in frequency of asymmetric stickleback in clear water habitats may have been confounded by population lateral plate mode, as plate number increases significantly from stained to clear water systems (Kruskall-Wallis X^2=47.755; df=2; P<0.001; Table 18). Populations with plate modes of 5 through 7 were present at all three staining levels, allowing me to compare asymmetry among staining levels while minimizing plate mode variance. Non-structural plate asymmetries did not increase significantly in mean frequency from clear to stained localities for this lateral plate range (Kruskal-Wallis X^2=3.979; df=2; P=0.137; Figure 28), but structural plate asymmetries did show a significant increase (Kruskal-Wallis X^2=15.210; df=2; P<0.001; Figure 29).

To determine if fish with lateral plate asymmetries differed in injury rate from symmetric fish, I compared the incidence of avian and trout scars between asymmetric (both non-structural and structural) and symmetric adult fish. There were no significant differences in the relative proportion of asymmetric (non-structural or structural) fish with injuries (avian or trout induced) within any population after sequential Bonferroni corrections (all P>0.150). However, significantly more populations had a greater incidence of trout induced injuries in symmetric rather than structurally asymmetric stickleback than the number of populations that did not (Table 19).

To determine if asymmetry was associated with relative parasitism, I compared the relative proportion of infected asymmetric to symmetric stickleback in each population. There were no significant differences in the number of infected asymmetric or symmetric fish in any population after Bonferroni corrections, and the number of

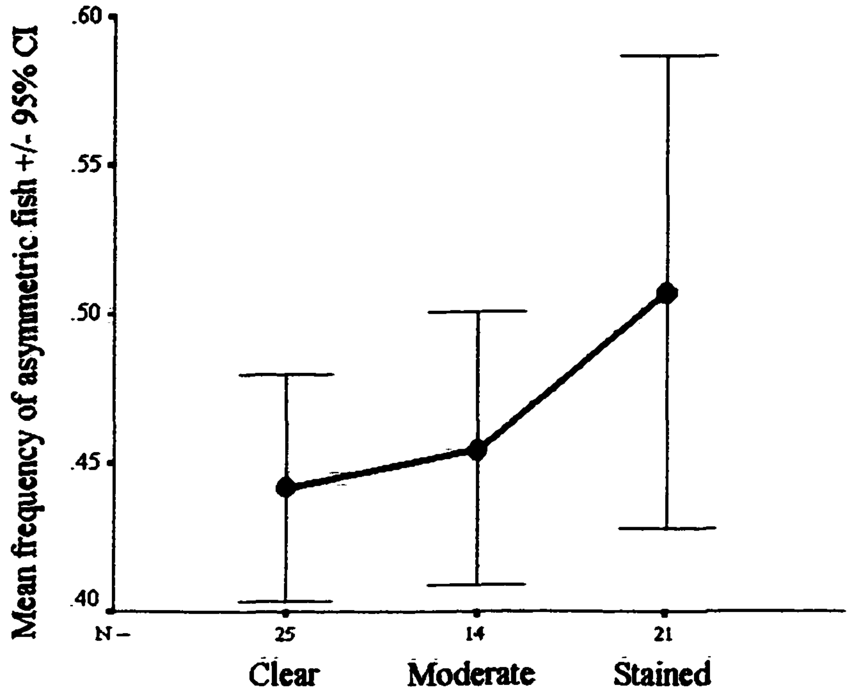

Figure 28. Mean population frequency of non-structurally asymmetric fish among different levels of water staining. Includes populations with plate modes of 5 through 7 only.

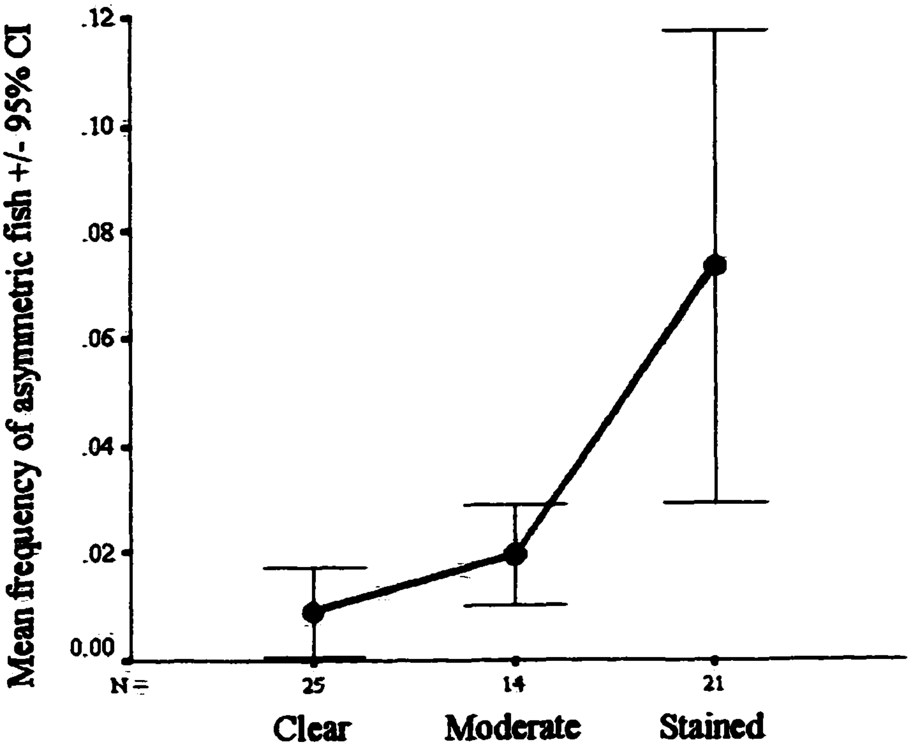

Figure 29. Mean population frequency of structurally asymmetric fish among different levels of water staining. Includes populations with plate modes of 5 through 7 only.

Table 19. Number of populations that have more scars on symmetric fish vs. the number that have more scars on asymmetric fish. Includes both avian and trout scars and is segregated into non-structural and structural asymmetries.

	# of populations with more avian scars on fish that are:			# of populations with more trout scars on fish that are:		
	Symmetric	Asymmetric	X^2	Symmetric	Asymmetric	X^2
Non-structural	16	19	0.257	33	34	0.015
Structural	16	11	0.926	37	19	5.786 *

* $P<0.025$

populations with greater infection rates in symmetric fish was not significantly different than the number of population with greater infection rates in asymmetric fish (50 vs. 39, respectively; Binomial: P=0.289).

However, the proportion of populations with inflated parasite infection in asymmetric fish increased significantly in populations with lower plate modes (X^2=9.939; df=3; P=0.019; Figure 30). Each of the three species of parasites was represented in similar proportions among populations from the different lateral plate mode groups (all P>0.450).

In order to determine if asymmetric fish had reduced survivorship, I calculated frequency shifts of symmetric fish between juvenile and adult age classes within each locality. There were no significant differences in the proportion of either structurally or non-structurally asymmetric stickleback between age classes in any population after Bonferroni corrections (all P>0.200). The number of populations that had increases in asymmetry with increasing age was not significantly different from the number that had decreases for either structural asymmetries (32 vs. 28, respectively; Binomial P=0.699) or non-structural asymmetries (47 vs. 36, respectively; Binomial P=0.272). For those populations that had a greater incidence of trout induced injuries in symmetric stickleback than structurally asymmetric stickleback, there was no significant difference between the numbers of populations with positive or negative shifts in structural

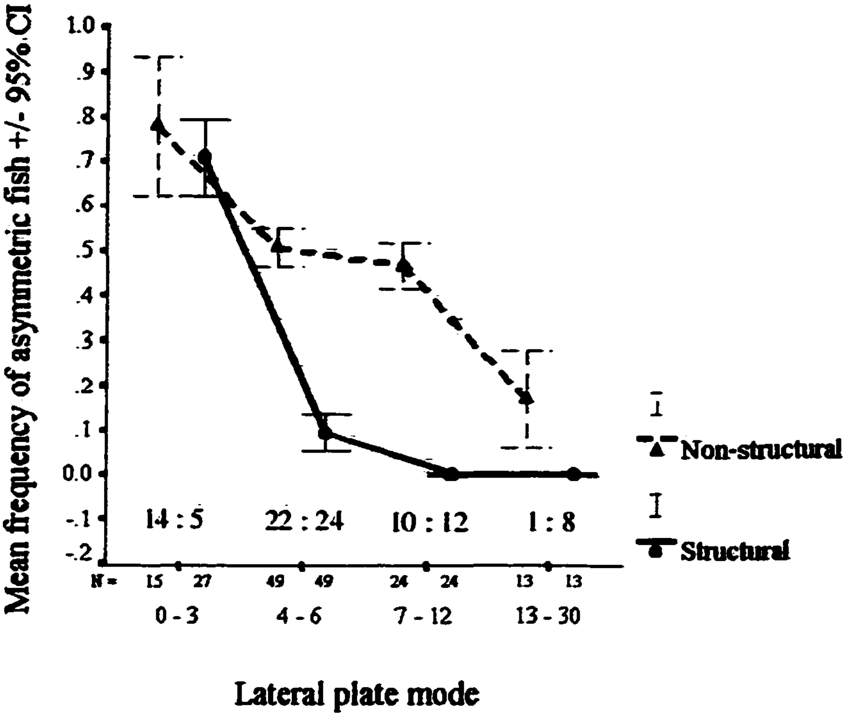

Figure 30. Mean population frequencies of structural and non-structural plate asymmetry among four different lateral plate mode categories. Ratios designate the number of populations per plate mode category that have elevated parasite infection rates in asymmetric fish vs. the number of populations that do not. The relative proportion of the two types of populations varies significantly among the four lateral plate mode categories (X^2=9.939; df=3; P=0.019).

asymmetry with age. There were no significant differences among the three levels of water staining in signed frequency shifts of structural asymmetries (Kruskal-Wallis: X^2=0.081; df=2; P=0.961) or in signed frequency shifts of non-structural asymmetries (Kruskal-Wallis: X^2=0.727; df=2; P=0.695). Among populations within clear and moderate levels of staining, neither structural or non-structural asymmetry frequencies were significantly correlated with plate mode, cross-sectional diameter or mean plate overlap (all P>0.250). Among heavily stained sites, there were suggestive but insignificant negative correlations between signed structural asymmetry frequency shifts and both cross-sectional diameter and mean level of plate overlap (P=0.090 and P=0.067, respectively). However, there was a significant negative correlation between signed frequency shift of structural asymmetry between juveniles and adult fish and population plate mode (r=-0.316; P=0.050) among the stained sites. The correlation was negative but insignificant (r=-0.013; P=0.944) for non-structural frequency shifts.

Discussion

These results demonstrate that there is a strong cline in the distribution of asymmetry in structural defenses among freshwater populations of stickleback from Haida Gwaii, and this cline is correlated with two measures of predation regime: reaction distance and the expression of defensive armour. The northeastern lowlands of the archipelago contain the most asymmetric populations, and are characterized by muskeg bog swamps of low pH, heavy water staining, low conductivity and relatively shallow waters with high activity by avian piscivores (Reimchen 1994a). Populations of stickleback in this region tend to have reduced expression of body armour (shorter spines, reduced numbers of lateral plates), a result either of relaxed selection for post-capture defenses (Moodie and Reimchen 1976) or direct selection for improved hydrodynamic performance (Reimchen 1992a; Bergstrom 2002). I found no strong evidence that the distribution of asymmetry, at least in juveniles, was a function of geographical variation in developmental stress generated by several abiotic factors (T400, conductivity, lake area or water depth), although there was greater asymmetry in lakes with low pH. However, this correlation appeared to be a function of total lateral plate number, as plate numbers are lower in the acidic lowlands and the inclusion of plate number into our multiple regression rendered the association between FA and pH insignificant (Table 17).

There were strong correlations between the mean number of lateral plates and the frequency of both non-structural and structural plate asymmetries among populations. Non-structural asymmetry decreased gradually as plate number increased, while structural asymmetry decreased sharply from plate modes of 1 to 7, then remained constant at frequencies of less than 2% at higher plate numbers. In addition, non-structural asymmetries were ubiquitously more common than structural asymmetries within populations. Populations with greater expression of lateral plate numbers are associated with predation regimes where the functional importance of structural defenses is high (Reimchen 1994a). Asymmetric fish may be at a functional disadvantage in these predation regimes and therefore have been gradually selected out of the gene pool over the last 12,000 years. The disparity in frequency between non-structural and structural asymmetry supports this interpretation as it indicates that the functional consequences of structural plate asymmetry may be more serious than non-structural asymmetry. Presence of structural plates on both sides of the fish is crucial to lateral rigidity of the adjoining spines (Reimchen 1983), while symmetry and expression of non-structural plates only maximizes the surface area of integument that is protected from puncture (Reimchen 1992a).

Alternatively, the negative association between plate asymmetry and plate number could be the result of directional selection for armour reduction in freshwater stickleback, since directional selection has disrupted developmental stability in other taxa (Leamy and Atchley 1985). However, a multi-trait index of FA in three other characters associated with structural defenses of these populations did not show a negative correlation with mean plate number (r=0.105, P=0.334; Bergstrom and Reimchen unpublished data) which would be expected if directional selection for armour reduction was generating developmental instability. Directional selection for plate number reduction might affect developmental stability of the lateral plates only, and therefore inflate levels of FA in the plates but not in other traits. If plate FA is solely determined by directional selection for plate number reduction, there should be no association between plate FA and other measures of armour robustness that are independent of plate number. I therefore compared plate asymmetry frequency with two other measures of structural defense robustness: cross-sectional diameter of adult stickleback with erect spines, and the degree of overlap between the structural plates and spine supports. Cross-sectional diameter was significantly negatively correlated with the frequency of structural plate asymmetry among populations but not with non-structural plate asymmetry after the effect of plate number was removed. In addition, structural asymmetry decreased as the degree of overlap increased, even when population lateral plate mode was held constant. It is possible that directional selection for reduction in other traits associated with armour, including cross-sectional diameter and level of overlap, is elevating developmental instability and FA in lateral plates. However, if this were the case I would expect to see similar levels of asymmetry in both structural and non-structural asymmetries rather than the strong dissimilarity found in the data. While unlikely, it is possible that directional selection is responsible for a portion of the asymmetry we find in these populations, and a detailed experimental analysis of the effects of directional selection on FA in this species would help to clarify this issue.

The likelihood of prey capture increases with reaction distance in aquatic systems (Vinyard and O'Brien 1976) and can therefore be used as an indirect measure of the importance of post-capture defenses independent of prey morphology. These results show an increase in plate asymmetry as water staining increases, although this effect was clearly confounded by a congruent decrease in total plate numbers. However, I found that for a restricted plate number range (from 5 to 7 plates per side) represented in all three staining levels, there was significantly greater structural asymmetry in stained localities than clear, but an insignificant difference in non-structural asymmetry (Figures 28, 29). While this association was weak, it does suggest that structural plate asymmetries may be functionally disadvantageous in habitats where the chances of capture are high. In heavily stained water with poor visibility reaction distances are reduced, therefore increasing the chances of escape during the pursuit phase of predation. In these situations, morphological attributes that enhance fast-start performance may be more crucial to survival than post-capture structural defenses, and thus selection against asymmetry that compromises the integrity of structural defenses would be relaxed. In clear water with good visibility, the reaction distance is increased as well as the chance of capture, which may result in stronger selection for the strength of post-capture defenses and against structural asymmetries.

These results demonstrated that structurally asymmetric fish had fewer trout injuries than symmetric fish in a significantly greater number of populations than those that did not, but there was no effect with non-structural asymmetries or with avian scars (Table 19). One interpretation of this is that symmetric stickleback are more likely to escape after capture from predatory fish than are stickleback with asymmetry in one or more of their structural plates. This is consistent with Reimchen's (1983) experimental work that found a significant decrease in the integrity of the spines when these structural plates were absent. If the spines are collapsed more easily in stickleback with structural plate gaps, gape-limited trout will be more likely to successfully swallow the individual. These results are also consistent with a previous study of lateral plate number asymmetry among a sub-set of these populations that found that asymmetry was significantly reduced in localities in which trout predators were present (Moodie and Reimchen 1976). Avian piscivores are less efficient at prey capture than are predatory fish (Bergstrom and Reimchen unpublished data), but enjoy relatively high post-capture success rates (Reimchen 1994a) which may result in little to no opportunity for generating selection on post-capture defenses (Vermeij 1982; Reimchen 1992a). While the relationship between predator efficiency and strength of selection remains largely unexplored experimentally, it may explain the lack of an association between avian injuries and structural plate asymmetry, especially if spine deflection is largely unrelated to the sticklebacks' chances of escaping handling by a large bird predator.

An additional explanation of the association between trout injuries and structurally asymmetric fish is that the symmetric fish may be captured more frequently, resulting in elevated injury rates. One way to resolve this is to look at asymmetry frequency shifts between juvenile and adult stickleback. If symmetric fish are being captured more frequently, there should be a general decrease in their frequency with age. I found no evidence of reduction in frequency of symmetric fish with age. However, in heavily stained localities, signed frequency shifts of structural, but not non-structural, asymmetry from young to old became significantly more negative as lateral plate mode increased. This suggests that fish with structural asymmetries were being selected out of those populations with greater plate numbers in stained sites. Lateral plate modes range from 0 to 7 in stained sites, and greater plate modes in general are associated with the presence of large fish predators (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1977; Bell and Richkind 1981). In Mayer Lake, a heavily stained lake on Haida Gwaii with a lateral plate mode of 7, the frequency of asymmetric stickleback in the guts of cutthroat trout was greater than a simultaneous seine-netted sample (31%, n=49 vs. 25%, n=295, respectively) although the difference was not significant (Moodie 1972, pers. comm.). These results, combined with the association between trout-induced injuries and structural asymmetries, implicate large predatory fish as potential agents that are selecting against structural asymmetries across the archipelago. This appears to be due to biomechanical weakness in structurally asymmetric fish, as there are no associations between non-structural asymmetries and trout injuries or survivorship. The lack of correlation between structural asymmetry frequency shifts and plate mode in moderately stained and clear localities is probably due to the lack of structural asymmetry variance among these sites. There could be two explanations for this. First, the reduced visibility in stained sites may reduce reaction distances enough to minimize the magnitude of selection acting on structural defenses. This relaxed selection may have allowed FA in plates to persist, even though it is still selected against in the trout-dominated sites of greater plate numbers. Secondly, it is possible that directional selection for plate number reduction is responsible for elevated levels of FA in the low-plated populations, the majority of which reside in heavily stained habitats. This might provide large enough asymmetry variance for the statistical detection of selection against structural asymmetry in the low-plated stained but not the high-plated clear sites.

However, I found no general trend for reduced survivorship in asymmetric stickleback across the archipelago, as the overall number of populations with positive shifts with age did not differ significantly from the number of populations with negative shifts with age. This conflicts with the broad generalization that FA and fitness are negatively correlated (Møller 1997), but is consistent with other studies of FA in stickleback that have failed to find evidence of reduced survivorship in asymmetric individuals (Moodie and Reimchen 1976; Bergstrom and Reimchen 2000; Bergstrom and Reimchen in press).

There were significantly more populations with an elevated incidence of parasite infection in asymmetric stickleback than populations without, but only among populations with lateral plate modes of three or less (Figure 30). The relationship between FA and susceptibility to parasites is inconsistent among studies and taxa (Folstad *et al.* 1996; Polak 1997; Thomas *et al.* 1998; Ward *et al.* 1998; Quek *et al.* 1999) and therefore it has been difficult to detect any general patterns. These results suggest that increased susceptibility to parasites in asymmetric fish may be restricted to habitats and traits where functional costs of, and direct selection against, trait asymmetry are minimized. Other studies have found increased parasite infection rates in asymmetric adult stickleback in Boulton Lake, Haida Gwaii (Reimchen 1997; Reimchen and Nosil 2001). This lake has a population with a lateral plate mode of 3, and virtually no overlap between the plates and the spine supports. Relaxation of selection for structural integrity of the spines in this population may have allowed the asymmetry to reach high enough levels to reflect underlying developmental stability and immunocompetence. The inconsistency of the association between FA and parasitism in other taxa could be due to undetected biomechanical selection against asymmetry that is obscuring the sensitivity of the trait to underlying developmental stability.

In summary, this study provides evidence that the geographical distribution of asymmetry in structural defenses among natural populations is a reflection of their relative biomechanical importance in different habitats. Structural plate asymmetry is generally greatly reduced relative to non-structural plate asymmetry, especially in samples from the mountainous, clear-water regions of the archipelago that are dominated by trout predators, high plate numbers and large reaction distances. The exception is in those samples where there is little overlap between the spines and the structural plates. In these cases, structural asymmetry is elevated to non-structural asymmetry levels, presumably because their functional importance is reduced. Among populations, mean asymmetry of the structural plates is also negatively correlated with mean cross-sectional diameter of the stickleback, a characteristic that has demonstrated importance in value as a post-capture predator defense (Hoogland *et al.* 1957; Reimchen 1991). Stickleback with structural plate asymmetries appear to suffer greater reductions in survivorship than non-structurally asymmetric stickleback, but only in populations with greater plate numbers in stained water sites. Structurally asymmetric fish also appear to escape after capture by trout predators less frequently than symmetric fish, an effect not seen in non-structurally asymmetric fish. In addition, increased susceptibility in asymmetric fish to parasite infection only appears in samples with low plate numbers. These results suggest that fitness reductions in asymmetric fish due to associations with developmental stability are only found in localities where the functional selection against the asymmetry is lifted. In localities where predator reaction distances are relatively high and post-capture defenses of stickleback are well developed, fitness reductions in asymmetric stickleback are due primarily to biomechanical disadvantages in predator-prey interactions.

Chapter 7: General Discussion

The adaptive significance of armour reduction

Determining whether divergence among insular populations has adaptive significance has been one of the cornerstones of evolutionary biology. Variation in colour, size, shape, life-history strategies, and behaviour among isolated closely related groups has been the classical means with which divergence has been described. In island populations in particular, characteristics that are used as defenses against predators such as vigilance, crypsis, and chemical and structural deterrents tend to differ from, and are generally reduced compared to, mainland populations. In some island systems, predation pressure may be more variable than it is in the corresponding mainland populations, resulting in concordant evolution of variation in defense mechanisms in the resident island prey. It is the degree and repeatability of covariance between phenotype and habitat among insular populations, in ecological and functional contexts, which indicates the relative importance of adaptive versus stochastic causes of diversification.

In this thesis, I have investigated variation in structural predator defenses among insular populations of freshwater threespine stickleback from the Haida Gwaii archipelago, with a focus on the ecomorphology of armour reduction and asymmetry. A ubiquitous characteristic of freshwater stickleback is their immense variation in structural defenses relative to the ancestral marine population. The system of populations that have resulted from colonization of freshwater habitats of Haida Gwaii approximately 12,000 years ago exhibit variation in these traits that parallels variation found in the entire species range of the northern hemisphere. Habitats on these islands also encompass a large range of parameters, from large clear pelagic lakes to small shallow stained ponds and slow moving creeks. Previous work has demonstrated extremely strong correlations between habitat type on these islands and defensive morphology of resident stickleback. The large clear lakes contain stickleback that most resemble the ancestral marine population, while the smallest, heavily stained ponds contain stickleback that have reduced armour and in many cases have completely lost their structural defenses.

In chapters 2 and 3 I have addressed whether there is evidence that armour reduction is a cost minimization strategy that occurs in response to relaxed selection from large predatory fish, or whether it endows stickleback with direct benefits that may be of adaptive value in the alternate predation regime, primarily avian, with which it coincides. I demonstrated that greater numbers of lateral plates are detrimental to fast-start swimming performance, and therefore reduction in plate number has the potential to prove beneficial during pursuit by predators. However, despite strong geographical correlations between plate number reduction and avian predation regimes, I found no consistent differences in plate number between uninjured stickleback and those with either avian or trout injuries within populations. In addition, I found that captive Hooded Mergansers were not generating significant selection differentials on lateral plate number, although the differentials tended to be negative with the exclusion of the completely-plated forms.

If lateral plate reduction is not resulting in successful escape from pursuing diving birds, why is plate reduction so common in habitats dominated by these predators? One explanation is that plate reduction really is providing a chance for pursuit escape from avian piscivores, but the magnitude of selection is too small to detect with the methods used in this thesis. This is a common problem in studies that attempt to detect natural selection in the wild (Endler 1986 Conner 2001, Hoekstra *et al.* 2001). Another explanation is that the expression of bony lateral plates is restricted in habitats with low levels of dissolved calcium. However, among freshwater sites on the Haida Gwaii archipelago, there is not a significant correlation between calcium concentration and lateral plate number (r=0.065; P=0.670). A third explanation is that plate reduction is the result of genetic bottlenecks that occurred during colonization. However, an overwhelming degree of repeatability and replication of pattern between morphology and habitat is evident on these islands, which one would not predict if all variability was simply the result of random shuffling of genetic information. If lateral plate reduction were the result of founder effects rather than adaptive responses to habitat ecology, the distribution of lateral plate morphs throughout the archipelago would be random. Yet, there is a strong cline in plate reduction from the mountainous regions of Moresby and western Graham Island through to the muskeg bog lowlands of northeastern Graham. In addition, the two marine genetic lines that colonized the archipelago have both independently diversified into low-plated and, in some sites, naked stickleback in similar habitats (Deagle *et al.* 1996). In those localities where both lineages are present, they are morphologically indistinguishable. This implies that adaptation to local habitats has been the cause of diversification of stickleback morphology among populations, rather than random founder effects.

I suspect that lateral plate reduction occurs in response to a combination of factors: the first being a relaxation of selection for strong structural defenses in the absence of predatory fish, and the second being selection for enhanced hydrodynamic performance in the presence of predators whose swimming speed more closely matches that of their stickleback prey. The quantification of the relative importance of these two factors is presently unknown. Experiments that more accurately assess whether selective predation by diving birds is occurring on armour in stickleback and other fishes would help to elucidate its importance. Providing a visual refuge for the prey in experimental conditions, either with reduced visibility or with suitable benthic substrate in which to hide, would help to generate a greater opportunity for pursuit escape.

Studies of the implications of armour reduction across a range of taxa have typically focussed on relaxation of selective predation as the cause, or have found evidence of behavioural attributes associated with armour reduction that make up for the weakness in structural defenses (Carroll 1988; McLean and Godin 1989; Abrahams 1995; Losos *et al.* in press). However, evidence of armour reduction may be an indication of selection for bio-mechanically enhanced mobility. Therefore, I suggest that it is important to include an assessment of functional ecomorphology of armour reduction in order to address the possibility of direct biomechanical benefits to reduction. In addition, while the evidence these studies present is strong, they all compare species, and therefore are not excluding variance that is attributable to phylogenetic divergence. The strength of the research presented in this thesis is its restriction of comparisons to populations within one species, hence minimizing phylogenetic effects. Efforts to resolve what factors are generating selection for reduction in armour that enhances locomotion in other taxa will contribute to our understanding of the mechanisms that drive evolution among allopatric groups.

Trait function and asymmetry

Some traits contribute more to the fitness of an individual than others, and determination of relative fitness consequences among traits is one of the focuses of evolutionary biology. This is a difficult task, as traits rarely function independently of each other, and the statistical detection of fitness covariance with a trait does not necessarily imply cause. The study of island taxa illuminates traits that are tightly linked to fitness, as these are often rapidly changed after colonization, are quite different from the source population, and may be tied to novel ecological and selective components of the new habitat with relative ease.

How traits change on islands or among closely related allopatric populations is usually determined by comparing trait means and variances among samples. The research presented in this thesis emphasizes that another trait characteristic, bilateral asymmetry, may shed additional light on the relative biomechanical importance of traits among populations. Previous studies have shown that trait asymmetry may be an indication of the importance of its mechanical integrity (Mather 1953; Palmer and Strobeck 1986; Balmford *et al.* 1993; Gummer and Brigham 1995; Clarke 1998). However, no other studies have investigated variation in asymmetry in a functional context among such a large number of closely related populations. My research has demonstrated that asymmetry among similar traits (lateral plates) varies immensely and appears to be correlated with their biomechanical importance. Asymmetry of the structural plates that support the spines was drastically reduced relative to the non-structural plates (Figure 18). The relative difference in asymmetry between these two trait types varied among populations as a function of the overall numbers of plates expressed and therefore, the dependence on structural defenses in predator/prey interactions (Figure 25). In populations with very low plate numbers, the degree of asymmetry between structural and non-structural plates was comparable. However, as plate numbers increased, the degree of asymmetry in structural plates decreased at a much faster rate than non-structural plates. In addition, structural plate asymmetry was reduced relative to non-structural asymmetry when the degree of overlap between the plates and the spines increased (Figure 26, 27) as well as when water clarity (and thus the chances of capture by a predator) was greater (Figure 29).

Interestingly, there appeared to be little to no effect of natural environmental variation in abiotic factors on the degree of asymmetry expressed in the lateral plates or three other traits (Tables 15, 16, 17). While many studies have found elevated asymmetry in populations subjected to unnatural or extreme environmental conditions (Wayne *et al.* 1986; Pankakoski *et al.* 1992; Manning and Chamberlain 1993; Clarke 1995; Gomendio *et al.* 2000; Lens *et al.* 2000) few have investigated the effects of natural levels of environmental fluctuation on asymmetry. The results I have presented here lend credence to the use of asymmetry to detect environmentally threatened populations, as they suggest that it is unlikely that variance in asymmetry is due solely to natural fluctuations in the environment. However, there was a considerable amount of unexplained variance in asymmetry that was not due to functional or environmental differences in the variables I assessed. It is certainly possible that FA is responding to variation in some environmental or genetic stress that I have not detected. Therefore, the use of FA to assess the health of a population needs to be exercised with caution.

One possible explanation for the large amount of unexplained variation in FA among populations is that the presence of predators might be inducing stress in the developing embryos. The presence of predators has been shown to increase wing asymmetry in damselfly larvae (Stoks *et al.* 2001). In stickleback, a high density of predators in a lake could disturb parental care and the benefits this provides to the developing embryos. Males provide the nest for the egg clutches, and spend a large portion of their time protecting the eggs from cannibalism and predation, as well as increasing water flow and oxygenation by fanning with the pectoral fins (see review in Wootton 1984). Frequent disturbance of this process may stress the embryos by reducing oxygen flow, and thus increase developmental instability and FA. As discussed previously, the determination of the activity and abundance of predators among habitats with indirect means is difficult. Experimental assessment of the effects predator presence has on the parental care of stickleback and their resulting developmental stability would be an interesting and worthy pursuit

My data has found little evidence to support the widely accepted hypothesis that asymmetry is an indicator of individual intrinsic fitness. Rather, FA/fitness associations appear to be trait and habitat specific. There was no overall reduction in survivorship in asymmetric individuals among populations on this archipelago, and only weak associations between FA and susceptibility to infection by parasites. Correlations between plate asymmetry and parasitism only occurred where total plate numbers were low and overall plate asymmetry was high. This suggests that the ability of a trait to reflect intrinsic fitness may be dependant on the strength of selection for functional symmetry in that trait. If selection for structural integrity has minimized the sensitivity of a trait to developmental stability, any developmental errors that occur and result in asymmetry may not be tightly correlated with inherent fitness components, including immunocompetence. However, this interpretation is speculative and would be strengthened with experimental work comparing fitness correlations with FA among groups with different histories of selection for structural symmetry.

It was surprising to find correlations between trout-injury rates and structural asymmetries, but not between trout-injuries and total plate number. My results suggest that fish with structural asymmetries were escaping subjugation by trout less frequently than symmetric fish (Table 19). One explanation for increased susceptibility to trout predation in asymmetric fish relative to fish with fewer plate numbers is that fish with plate asymmetries may suffer from both intrinsic fitness reduction and functional costs because of the asymmetry. Fish with reduced numbers of plates however, would only suffer from functional costs. Selective predation on functional aspects of plate numbers by trout may be so weak at any single point in time as to be undetectable with classical means. However, when the same functional disadvantages of low plated fish are achieved with asymmetry, and that asymmetry is associated with other intrinsic reductions in fitness, the selection may be strong enough to detect.

Many previous studies suggest that fitness reduction in asymmetric individuals is because of elevated homozygosity, reduced immunocompetence, or other intrinsic fitness components. The possibility of fitness reduction due to functional costs has been demonstrated to be very strong in this thesis, yet most studies, with some exceptions, do not address the effects functional costs may have on fitness reductions in asymmetric individuals. The large degree of heterogeneity in correlations between fitness and FA among studies in the last 30 years may be the result of the focus of investigators on either intrinsic or functional fitness costs, rather than a comprehensive consideration of both.

The high degree of variation in asymmetry among populations in this study has demonstrated strong correlations with habitat type and general predator distributions, but only for some traits. Structural asymmetries in particular show strong geographical correlations with reduction in dependence on structural defenses for survival, but less so for non-structural plate asymmetry and for the three other traits analyzed in chapter 4. This heterogeneity in asymmetry/habitat associations among traits has provided insight into the relative biomechanical importance of the traits studied that would not have been gleaned from simply analyzing differences in trait means. I suggest that relative asymmetry of traits be used as an additional character with which to describe variation in adaptive radiation and among insular populations. The following gives two examples of classic studies of adaptive radiations that may be enhanced by the inclusion of comparisons of FA in functional contexts.

First, studies of variation among populations and species of lizards could possibly be enhanced by the inclusion of comparisons of femur FA, since elevated femur asymmetry in the lizard *Psammodromus algirus* significantly reduces overall escape sprinting speeds (Martín and López 2001). The adaptive radiation of *Anolis* lizards in the Greater Antilles is primarily a function of divergence in limb length and locomotor performance (Losos 1990; Losos and Irschick 1996). Relative limb length is tightly correlated with the width of the vegetation the lizards rest on as well as the method of escaping pursuit from predators. Even between groups with similar limb lengths, a comparison of FA in locomotor-associated traits may give additional insight to the relative functional importance these traits have to lizards living in different habitats.

Secondly, analysis of wing asymmetry among species and populations of Darwin's finches might give insight into locomotor variation, a characteristic that has not received as much attention as beak size or courtship behaviour (Grant 1986). The 13 species of finches reside in various habitats that differ in the degree and heterogeneity of vegetative cover. Studies of feather asymmetry in barn swallows have shown that elevated FA interferes with manoeuvrability around tight corners (Thomas 1993). I would predict that a simple comparison of wing asymmetry between groups that reside in open vs. complex habitats would reveal reduced asymmetry in birds from the complex habitat, providing an additional example of ecologically-driven variation within this group of species.

Stochasticity and adaptation

This research has focussed on the evolutionary implications of reduction and asymmetry of defensive armour, yet also exemplifies the larger role adaptation plays in the geographical distribution of phenotypes. The relative importance of stochastic vs. adaptive factors in the generation of biological diversity has been a controversial topic and the source of heated dispute among evolutionary biologists for many decades. Similar phenotypes that arise through drastically different developmental mechanisms (convergent evolution; for example, the eyes of vertebrates and cephalopods), suggest that they have been moulded independently by selection to suit a similar purpose. Similarity between morphology, behaviour, and habitat use between marsupial and placental mammal species is a striking example of convergent phenotypes evolving in similar selection regimes. Convergent evolution has proven difficult to explain in stochastic terms. Examples of parallel evolution (convergence between closely related groups with similar developmental pathways) also provide evidence that adaptation to local habitats is responsible for divergence. The multiple and independent occurrence of similar 'ecomorphs' in similar island habitats in *Anolis* lizards of the West Indies is an excellent example of parallel evolution (Losos *et al.* 1998), as is the repeated and predictable divergence between stream/lake pairs of stickleback (Bell 1982; Reimchen *et al.* 1985; Thompson *et al.* 1997). The multiple and independent colonization of freshwater habitats on Haida Gwaii provides an excellent case study of parallel evolution on a large scale. While examples of convergence and parallel evolution among species or larger taxonomic groups are valuable, repeated independent evolution of similar forms in similar habitats within species is a powerful tool with which to study adaptation, as it minimizes the effects of historical and phylogenetic effects and constraints.

Each freshwater population of stickleback represents a natural experiment with which to address the relative importance of stochasticity versus local adaptation. Do similar body forms occur in similar habitats? Or is the resulting phenotype relatively unrelated to habitat, and the result of random genetic founder effects and subsequent drift? The geographical pattern of phenotypes among populations of stickleback on this archipelago strongly supports the former. Repeatedly, we see reduced plate numbers and black breeding colours in populations from shallow stained water bodies (Reimchen 1989), and a greater number of long slender gill rakers in populations from deep, steep-sided pelagic habitats (Reimchen *et al.* 1985). Even in characters such as fluctuating asymmetry, which are supposedly the result of stochastic errors during development, we see strong patterns in their geographical distribution that are consistent with eco-morphological predictions. The research presented here, in addition to enriching our understanding of the evolutionary and functional implications of armour reduction and asymmetry, has on a larger scale reinforced confidence in the importance of local adaptation to the generation of diversity.

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Appendix 1

Lakes from which samples were collected listed in ascending order from lowest population mean CFA to highest, showing mean CFA (including both age classes), pH, T400, specific conductance, water depth and lake area. All variables are untransformed. *=Stream sample.

Locality	Geographical area	CFA	*pH*	T400 (%)	*Sp. Cond. (μmhos cm^{-1})*	*Water depth (meters)*	*Lake area (hectares)*
Inskip	Mountain	4810.77		99		< 1	
Ain	Lowland	4826.30	5.1	87	37	5	78
Hidden	Mountain	5039.25		93	80	50	18
Gowgaia W.	Mountain	5115.61		78		8	25
Lumme Swp.	Lowland	5128.95				1	<1
Capeball Rv.*	Lowland	5257.65		47	118	< 1	
Yakoun Rv.*	Plateau	5257.82	6.5	90		< 1	
Kiokathli	Plateau	5262.31	6.0	75	45	15	9
Lutea	Mountain	5296.91	6.8	94	2200	2	3
Peter	Plateau	5354.28	6.9	92		20	41
Poque	Mountain	5396.37	6.0	91		25	17
Mosquito	Mountain	5418.68		95		30	340
Gowgaia E.	Mountain	5419.09		78	2200	8	25
Amber	Lowland	5437.08	6.3	87	46	4	72
Wright	Mountain	5519.77				25	36
Woodpile	Lowland	5560.34	4.9	61	71	2	4
Cedar	Lowland	5564.34				4	4
Marie	Plateau	5572.90	7.0	89		20	373
Dam	Lowland	5580.33	5.3	52	560	3	9
Spence	Lowland	5585.24	7.0	67		30	95
Stellata	Lowland	5622.79		76	180	3	3
Pontoon Center	Lowland	5625.43	6.7	88	85	< 1	1
Gudal	Mountain	5644.32	7.4	95		30	25
Tlell Estuary	Lowland	5664.51				1	
Coates	Plateau	5667.37	6.0	95	49	30	90
Otter N.	Lowland	5668.32	4.6	46	118	2	10
Desolate	Plateau	5396.37		82	35	5	3

Yakoun	Plateau	5676.10	6.5	90		60	679
Stiu	Mountain	5691.01	7.2	93	78	30	24
Darwin	Mountain	5699.50		90		15	14
White Swan	Mountain	5710.19	6.9	89	87	2	<1
Pontoon Tlell	Lowland	5747.85		75		< 1	
Blackwater Ck.*	Plateau	5749.16				< 1	
Elk Survey Ck.*	Plateau	5754.08				< 1	
Van Inlet	Mountain	5754.71				20	23
Escarpment	Mountain	5758.41		94	36	50	97
Kumdis Pd.	Lowland	5769.52	5.7		120	2	<1
Gold Ck.*	Lowland	5770.50		50	98	2	
Puffin	Mountain	5787.21		83	57	10	5
Survey Ck.*	Lowland	5829.38		84		< 1	
Florence Ck.*	Lowland	5837.06	6.6	76	125	< 1	
Otter S.	Lowland	5879.45	4.6	45	160	3	36
Fairfax	Mountain	6020.84		90	49	4	1
Snub	Mountain	6034.23	6.7	83		20	2
Irridens	Mountain	6034.67		87		5	18
Eden	Plateau	6069.38				50	513
Drizzle Inlet*	Lowland	6096.63		38		2	
Goski	Mountain	6097.62	7.3	88	64	5	10
Krajina	Plateau	6099.06	6.0	87	47	10	16
Vaccinium	Lowland	6100.17				4	8
Kumara	Lowland	6114.50	5.5	62		4	15
Menyanthes	Plateau	6127.57	6.3	82	17	5	6
Dawson	Mountain	6180.45		82	55	4	1
Oeanda Rv.*	Lowland	6251.47				3	
Seal Inlet	Plateau	6303.36		82	34	5	2
Mayer	Lowland	6311.60	4.9	57	90	20	373
Blue Danube	Lowland	6336.35		65		< 1	8
Drizzle	Lowland	6337.81	5.1	67	69	16	97
Anser	Lowland	6451.92	5.3	76		4	18
Sundew	Mountain	6467.30	6.3	87	46	4	5
Downtree	Lowland	6468.98	6.2	84	40	3	27
Pure	Lowland	6566.41	4.5	77	76	10	34
Skidegate	Plateau	6579.31		94		30	545
Cumshewa	Plateau	6593.93				2	8

Lower Victoria	Mountain	6668.66				25	149
Kumdis L.	Lowland	6790.50				5	28
Geikie 3*	Lowland	6810.44	4.9	63	91	< 1	
Grus	Lowland	6840.17				3	14
Mica	Lowland	6920.24				5	11
Geikie 2*	Lowland	6927.94	4.7	63	109	< 1	
Hickey	Lowland	6996.96				20	122
Anderson S.	Plateau	7085.11	7.1	91	78	4	14
New Years	Lowland	7088.25	4.7	69	70	2	7
Bruin	Lowland	7146.25				10	9
Debris	Plateau	7300.77	5.3	57	128	4	4
Anderson N.	Plateau	7370.86	7.1	88	83	4	3
Clearwater	Lowland	7441.65		76		10	53
Parkes	Lowland	7476.00	4.8	61		5	16
Skonun	Lowland	7532.00	4.5	68		15	51
Loon	Lowland	7866.25				3	11
Richter	Lowland	8369.00	4.3	52		5	12
Lumme L.	Lowland	8445.50				10	39
Gross	Lowland	8456.50		65		2	2
Wegner	Mountain	8723.00				3	3
Bigfish	Lowland	8750.82	4.5			2	2
Wiggins	Lowland	9129.25				3	24
Eriophorum	Lowland	9219.75		45		1	1