THE ROLE OF PREDATION IN THE EVOLUTION OF SYMPATRIC STICKLEBACK SPECIES

by

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ABSTRACT

The contribution of predation to the origin and divergence of sympatric species has not been examined in much detail. To determine whether there is an association between fish predators and the presence of stickleback species pairs (*Gasterosteus aculeatus*), I recorded the physical characteristics and fish communities of the six lakes containing stickleback species pairs and 47 lakes in the Strait of Georgia region of British Columbia that contained only a single stickleback species. A number of lakes were identified that had similar physical characteristics to lakes containing stickleback species pairs. These lakes, however, tended to have more fish species than lakes with two stickleback species, which had only one other fish species, the predatory cutthroat trout (*Oncorhynchus clarki*). The absence of certain fish species, thus, appeared to be important in allowing speciation of sticklebacks. Predation may have played a significant role in the morphological differentiation of sympatric stickleback species pairs. I measured the defensive armor of sticklebacks from lakes with one and two stickleback species. I provide evidence of character shifts in defense armor as a consequence of sympatry. Living in different habitats in sympatry may have been accompanied by adaptation to different predators in the two habitats. In lab predation experiments, I found that the species that lives in the pelagic zone was selectively preyed upon by predators commonly found in the littoral zone. The species from the littoral zone, in contrast, was selectively preyed upon by a piscivorous bird, the double-crested cormorant (*Phalacrocorax auritus*), which usually forages in the pelagic zone. Finally, I investigated whether the two species and their F1 hybrids are differentially vulnerable to predators. The species associated with the pelagic zone had significantly reduced survival in the presence of cutthroat trout, whereas the other species was largely unaffected by their presence. First generation hybrids had low survival regardless of treatment. Cutthroat trout predation may be a significant source of mortality for the species associated with the pelagic zone but does not contribute to reproductive isolation between the two species.
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who has always provided a shoulder to lean on
The effects of predation on the process of speciation are poorly understood. [McPhail 1969]

**GENERAL INTRODUCTION**

Adaptive radiations are characterized by the "more or less simultaneous divergence of numerous lines all from much the same ancestral adaptive type into different, also diverging adaptive zones" (Simpson 1953). An adaptive zone represents a "characteristic reaction and mutual relationship between environment and organism, a way of life and not a place where life is led" (Simpson 1953). Adaptive zones can be thought of as divergent ways of obtaining food, avoiding encounters with predators, or resisting parasites (Schluter 2000b). Classic examples of adaptive radiations include Galápagos finches (Lack 1947), East African cichlids (Fryer and Iles 1972, Greenwood 1974), and Hawaiian honeycreepers (Amadon 1950) and silverswords (Carlquist 1980).

The idea that interspecific competition between consumer species can promote morphological divergence in adaptive radiation has been addressed by numerous theoretical (e.g., Slatkin 1980; Milligan 1985; Taper and Case 1985; Abrams 1987a,b) and empirical (e.g., Brown and Wilson 1956; Grant 1975; Schluter et al. 1985; Schluter and McPhail 1992) studies. However, less attention has focused on the impact of predation by a higher trophic level on two consumer species. When interactions with members of other trophic levels, such as predators or parasites, have been recognized, they have often been invoked mainly as mechanisms that maintain potential competitors at densities so low that they do not compete (e.g., Paine 1966; Caswell 1978; Glasser 1979).

More recently, researchers have explicitly considered the contribution of predation to the morphological differentiation of prey species. This approach is concerned with how: (1) prey diverge to reduce their consumption by predators, (2) prey species that partition habitats can further diverge by adapting to the predators that co-exist with them in their habitats, or (3) reproductive isolation between sympatric prey species is strengthened by predation. In the first scenario, if two or
more prey species are shared by a predator or predator complex, this may lead to divergent selection on antipredator defences of the prey species to minimize consumption by their predators (Holt 1977, Jeffries and Lawton 1984, Brown and Vincent 1992, Seger 1992, Holt and Lawton 1994, Abrams 2000). In the second scenario, species interactions such as interspecific competition may initiate habitat segregation and accompanying morphological differentiation in trophic characters, and predation may then drive divergence in antipredator defences. This may result in a predation based trade-off: prey species may become well adapted to the predators that they co-exist with, at the cost of increased susceptibility to predators from other habitats (McPeek 1990, Kruuk and Gilchrist 1997). Finally, in the third scenario, reproductive isolation may be maintained because of increased predation on interspecific hybrids as a consequence of their intermediate morphological defences, body shapes, and antipredator behaviors (Fryer 1959, Whitham 1989). Predation may be important in driving and maintaining morphological differentiation in many ways, but in this thesis I focus primarily on the correlation between predator community and speciation, character displacement in defensive armor, survival trade-offs associated with predators from different habitats, and reduced fitness of hybrids.

I conducted this research on three-spined sticklebacks (*Gasterosteus aculeatus*) from lakes with one and two stickleback species. Marine sticklebacks have given rise to a number of lake populations in the northern hemisphere. Most lakes are occupied by a single ("solitary") population of sticklebacks. Six lakes in the Strait of Georgia region contain two sympatric species called benthics and limnetics (e.g., Larson 1976; McPhail 1984, 1992, 1993; Schluter and McPhail 1992). Limnetics are slender fish, with many long gill rakers, foraging primarily on zooplankton in open water. Benthics have larger, deeper bodies and fewer short gill rakers, spending most of their time foraging in the littoral zone and open sediments in deeper waters. Although the taxonomy of freshwater sticklebacks remains muddled, benthics and limnetics in any given lake are reproductively isolated and can be considered "good" biological species (e.g., McPhail 1984, 1992; Vamosi and
Solitary populations often have trophic morphologies and habitat preference intermediate to the two sympatric species (Schluter and McPhail 1992). Because much has been written on the natural history of sticklebacks (e.g., Bell and Foster 1994), I do not attempt a longer synthesis here. Each chapter is meant to be largely self-contained and details relevant to the research are presented as needed.

A number of features of sympatric stickleback species suggested that they would provide a good system for testing hypotheses about the role of predation in morphological divergence and speciation. First, Schluter and McPhail (1992), in their classic paper on ecological character displacement in sympatric sticklebacks, made a brief reference to the fact that cutthroat trout were native to all lakes with stickleback species pairs. A number of studies have demonstrated that cutthroat trout are significant and selective predators of sticklebacks (Moodie 1972, Moodie et al. 1973, Reimchen 1995). Second, differences in the defensive armor of benthics and limnetics from Paxton Lake have long been noted (e.g., Larson 1976), especially in reference to the striking reduction observed in benthics from that lake. Therefore, there was a hint that benthics and limnetics from other sympatric lakes may have diverged not only in trophic traits (Schluter and McPhail 1992), but also in antipredator traits. Third, the habitat segregation by benthics and limnetics appears not only to result in them encountering different prey types, but also different predators. I have observed common loons (Gavia immer) foraging on sticklebacks during spring and summer and they have concentrated their efforts in open waters (see also Reimchen 1994). Kingfishers and herons, in contrast, also forage on sticklebacks but tend to forage only in shallow waters along the edge of the lake. Benthics and limnetics are capable of producing viable, fertile F1 hybrids (McPhail 1984, 1992; Hatfield and Schluter 1996, 1999; Vamosi and Schluter 1999, Vamosi et al. 2000) and, because the incidence of hybrids does not appear to be increasing over time, ecological factors such as predation may be partially responsible for maintaining postmating isolation between sympatric benthics and limnetics.

Chapter One investigates whether the presence of sympatric stickleback...
species pairs in lakes is associated with the fish community of those lakes. I recorded the physical characteristics and fish communities of the six lakes containing stickleback species pairs and other lakes that contained only a single stickleback species. Analysis of physical characteristics revealed a number of lakes that, although they contain only a single stickleback species, were similar to lakes with two stickleback species. These lakes, however, tended to have more fish species than lakes with two stickleback species, which had only one other fish species, the predatory cutthroat trout \textit{(Oncorhynchus clarki)}. Prickly sculpin \textit{(Cottus asper)} and rainbow trout \textit{(O. clarki)}, two other significant predators of sticklebacks, were commonly found in solitary lakes but never in sympatric lakes. The results suggest that speciation can proceed under certain predation regimes but not others.

Chapter Two explores whether “character displacement” of defensive armor has accompanied ecological character displacement of foraging traits in sympatric sticklebacks. A number of theoretical papers suggest predation may favor evolutionary shifts of antipredator traits of sympatric species, but empirical studies have been lacking. I measured the defensive armor of sticklebacks from all lakes with benthics and limnetics, along with sticklebacks from lakes having similar characteristics and fish communities with only a single stickleback species. I provide evidence of character displacement in defense armor as a consequence of sympatry. The pattern is not a straightforward mutual divergence in characters, and I discuss possible reasons for this.

Chapters Three and Four focus on a single pair of stickleback species, the benthics and limnetics from Paxton Lake. In Chapter Three, I use laboratory predation experiments to determine whether living in different habitats in sympatry has been accompanied by adaptation to different predators in the two habitats. Limnetics, which live in the open waters of the pelagic zone, were selectively preyed upon by predators commonly found in the littoral zone. Benthics, in contrast, were selectively preyed upon by a piscivorous bird, the double-crested cormorant \textit{(Phalacrocorax auritus)}, which usually forages in the pelagic zone. This
pattern of differential mortality is probably a consequence of differences in body size, defensive armor and antipredator behaviors of the two species.

Finally, Chapter Four investigates the impact of the presence of cutthroat trout on the survival and growth of juveniles of sympatric stickleback species and their F₁ hybrids. This study was based on a large scale pond experiment, conducted in five divided ponds at the UBC research pond facility. The largest impact of the introduction of trout was a marked reduction in the survival of limnetics. Benthics, in contrast, were relatively unaffected by their presence. First generation hybrids had low survival regardless of treatment. Ecological factors appear important in postmating isolation, although the exact mechanisms are yet to be determined (see also Vamosi et al. 2000).

In short, I use lake surveys, morphological measurements, lab and field predation experiments to ask the following questions:

(i) Is the distribution of sympatric stickleback species associated with the presence, and absence, of certain other fish species?
(ii) Has the defensive armor of sticklebacks undergone evolutionary shifts in response to the presence of two species?
(iii) Are predator-mediated survival trade-offs associated with the habitat segregation of sympatric stickleback species?
(iv) Do hybrids suffer disproportionately high mortality due to predators?

The answers that I have obtained are far from complete, but I feel that they highlight important ways in which predation may have been critical in the evolution of these species pairs.
Chapter One

THE EFFECTS OF PREDATOR COMMUNITY ON SPECIATION
Predation has long been recognized as a mechanism that can affect the diversity of coexisting species (Darwin 1859, Gause 1934, Paine 1966, Addicott 1974, Chesson 2000). In general, predation is thought to promote coexistence by reducing the densities of superior competitors, which might otherwise outcompete inferior competitors. While this may be an oversimplification of the mechanism (Addicott 1974, Chesson 2000), a number of studies have demonstrated an increase in diversity as a consequence of predation. Paine (1966), for example, demonstrated that the removal of the predatory sea star (*Pisaster ochraceus*) caused a reduction from 15 to eight coexisting species of intertidal organisms. A notable exception is Addicott’s (1974) classic work on pitcher plant communities, which demonstrated a decrease in the diversity of protists and rotifers in the presence of predatory mosquito larvae (*Wyeomyia smithii*).

Theory suggests that predation, under certain circumstances, may spur diversification of prey species. Brown and Vincent (1992) used evolutionary game theory to investigate the influence of predators on the diversity of prey species. In their model, the evolutionary stable strategy (ESS) contained a single prey species in the absence of predators. The ESS with the addition of a predator with a broad niche width also contained a single prey species. Increasing restrictions on predator niche breadths, however, resulted in ESSs with two prey species and a single predator species and even three prey species and two predator species. Fryer (1959), in a verbal model, suggested that selective predation of “unfit intermediates” could assist in the evolution of reproductive isolation between species.

The empirical evidence for the role of predation in speciation, however, has been controversial. Worthington (1940, 1954) and Jackson (1961) proposed that the radiation of cichlid fishes was retarded in Lake Albert and Lake Rudolph by the presence of predatory fish, especially tiger fish (*Hydrocynus vittatus*) and the Nile perch (*Lates niloticus*). Worthington (1940; pg. 300) surmised that adaptation to
new niches was hampered "because predators have acted as a steadying influence: unless the variants slipped immediately into vacant and suitable niches, they had short lives." Fryer (1959, 1965; Fryer and Iles 1955) envisioned a different role for predation, arguing that predation may have actually accelerated speciation because spectacular radiations of cichlids had occurred in the presence of rich communities of predatory fish, including cichlids (*Haplochromis* spp.) and non-cichlids, such as catfish (*Bagrus meriodionalis*). McPhail (1969) documented isolated populations of three-spined stickleback (*Gasterosteus aculeatus*) in which males developed black nuptial colours rather than the usual red. He suggested that, because juveniles of black males were preyed on less by Olympic mudminnows (*Novumbra hubbsi*) than juveniles of red males, predation had contributed to the reproductive isolation of these populations. However, Hagen et al. (1980) later interpreted the pattern as convergence of threat signals rather than as an adaptation to predation. Farrell et al. (1991) demonstrated that clades of plants with latex and resin canals, which reduce herbivory by insects, tended to have more species than sister clades that lacked these structures. "Escape and radiation" in clades with canals is likely responsible for this pattern, although extinction rates may also be higher in sister clades. Clearly, more work is needed to elucidate whether and how predation contributes to speciation.

The three-spined stickleback is found in coastal waters throughout the northern hemisphere. Most lakes with sticklebacks are occupied by a single (solitary) population of sticklebacks, and these populations are thought to be descendants of marine sticklebacks that invaded freshwater environments during postglacial submergence periods. However, benthic and limnetic species pairs of sticklebacks are found in six lakes in four drainage basins in the Strait of Georgia, British Columbia, Canada (McPhail 1984, 1992, 1993; Schluter and McPhail 1992). McPhail (1993) noted that lake morphometry (surface area and depth) and altitude were correlated with their local distribution and proposed the double invasion hypothesis to explain the origin of the species pairs. In brief, lakes with stickleback species pairs are thought to have been colonized twice, about 15000 and 13500 yr
BP, by marine sticklebacks (see also Schluter and McPhail 1992, McPhail 1994). The opportunity for two invasions was permitted in the Strait of Georgia region by special conditions related to sea-level fluctuations. Mathews et al. (1970) provided evidence of a short-lived second postglacial submergence in this region which involved a 50 m change in sea level. Thus, the altitude of these lakes (50 m to 90 m) and the peculiar geological history of this region are thought to have interacted to “prime” speciation (McPhail 1993, Taylor and McPhail 2000). Here I show that another strong correlate of the origin of the species pairs is the community of predatory and competitor fish found in the lakes.

1.2 MATERIALS AND METHODS

Data were collected on the six lakes in which benthics and limnetics are present (hereafter, “sympatric lakes”) and all 47 coastal lakes (Table 1.1) in the same region (defined below) that are known to contain solitary populations of sticklebacks (hereafter, “solitary lakes”). This region, which includes the east coast of southern Vancouver Island, the Gulf Islands, and the Sechelt Peninsula (Fig. 1.1), was chosen based on Mathews et al.’s (1970) estimate of the coastal lands subject to the second postglacial submergence. I did not a priori exclude any solitary lakes in this region (i.e., selecting only those are above 50 m elevation). Therefore, instead of measuring “opportunity for double invasion” explicitly, I scored elevation and distance to sea, variables that might influence this opportunity. Comparison with sympatric lakes is how I test whether this opportunity indeed varies. I also scored surface area, perimeter, and mean depth of all lakes. Few lakes (n = 11 for solitary lakes, n = 4 for sympatric lakes) had data on pH values. These variables presumably do not influence the opportunity for double invasion, but likely influence the probability of persistence of the differentiated stickleback populations. Finally, I scored the presence of other native fish species for all lakes. Presence and absence of fish species was confirmed by field observations where possible. Introduced exotic species, such as smallmouth
bass (*Micropterus dolomieui*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*), which were found in only a few lakes, were not included in the results. The conclusions are the same when they are included in the survey. All data were gathered using published sources (Facchin and King 1980, 1981, Lavin and McPhail 1985, Foster and Ploch 1990, Balkwill 1991, McPhail 1993) and Fish Wizard, an on-line database containing bathymetric maps and information on fish distributions in British Columbia (http://www.fishwizard.com). Traits other than pH and presence of other fish species were log transformed prior to analysis.

Of the eight other native fish species encountered (Table 1.3), four of the species have been demonstrated to prey on sticklebacks. Cutthroat trout and rainbow trout prey on juvenile and adult sticklebacks in the littoral and pelagic zones of lakes (Hagen and Gilbertson 1973, Reimchen 1994). Prickly sculpin are associated with the littoral zone and they have been known to prey on eggs, juveniles and adults (Moodie 1972, Pressley 1981). Finally, the northern pikeminnow is a voracious predator of adult sticklebacks in the littoral zone (Moodie *et al.* 1973; J. McPhail, personal communication). Coho salmon fingerlings may attack juvenile sticklebacks (Larson 1976), although it is not known if they are successful predators.

I tested whether sympatric lakes are different from solitary lakes in their physical characteristics and fish communities with a multivariate discriminant function analysis (DISTCVA; Anderson and Robinson, unpublished manuscript). DISTCVA uses permutations (number used = 999) of the observations to determine whether the traits considered significantly discriminate between the two types of lakes. Use of different distance measures (Euclidean distance and Orloci's Chord distance) did not qualitatively change the results, so the results presented below, calculated using the Bray-Curtis distance, can be considered robust. pH values were not included because of missing data. Univariate comparisons of the individual traits were conducted with Wilcoxon's test. Finally, I examined the correlation between lake size and number of other fish species.
1.3 RESULTS

Sympatric lakes were well discriminated from solitary lakes when all traits were considered, but not when the number of other fish species was excluded (Fig. 1.2). With all traits included, sympatric lakes were significantly discriminated from solitary lakes \( (P = 0.004) \). Four solitary lakes overlapped with sympatric lakes in this analysis (Fig. 1.2). When number of other fish species was excluded, sympatric and solitary lakes could not be discriminated \( (P = 0.41) \), and 18 solitary lakes overlapped with sympatric lakes (Fig. 1.2). The exclusion of other traits did not have a significant impact on the discrimination between sympatric and solitary lakes (S. Vamosi, unpublished observations). Thus, about 38\% of the lakes in this region that contain a single stickleback population appear to have physical characteristics similar to lakes with two stickleback species.

The conclusions of the discriminant function analysis are supported by analyses of the individual traits. No significant differences were detected between sympatric and solitary lakes in any of the physical characteristics considered (Table 1.2). Some of these characteristics may still be important in determining whether one or two stickleback populations will be found. An examination of lake size, for example, revealed that no sympatric lake was larger than 45 ha (as previously noted by Schluter and McPhail 1992), whereas the largest solitary lake in the region was over 800 ha. A highly significant difference was detected between solitary and sympatric lakes in the number of other fish species (Table 1.2). The indigenous cutthroat trout is present in all lakes surveyed and is the only other fish species present in sympatric lakes. Only three of the 47 lakes, in contrast, have communities composed of sticklebacks, cutthroat trout and no other fish species. This difference between sympatric and solitary lakes in fish communities is highly significant (Fisher exact test, \( P < 0.0001 \)). Rainbow trout \( (Oncorhynchus mykiss) \) and prickly sculpin \( (Cottus asper) \) are the most common fish species also found with sticklebacks (Table 1.3), but they never occur with stickleback species pairs.

The data presented above clearly indicate that solitary lakes tend to have
more other fish species than do sympatric lakes. What I am most interested in, however, is determining the impact of predatory fish on either facilitating or depressing divergence and (or) persistence. Therefore, I examined the communities of the 44 lakes with fish species other than cutthroat trout and asked: how many of these lakes contain other predators? Forty one of the lakes contain at least one other predator species, and only three low elevation lakes (Greenway, Katherine, and Mixal) contain cutthroat trout and only competitor fish species. Because of their low elevations, it is likely that these lakes were not able to retain their initial populations of colonists (see McPhail 1993).

An examination of the relationship between lake size and the number of other fish species also sheds light on why benthics and limnetics are not found in large lakes (Fig. 1.3a). The number of other fish species increases significantly with lake size ($r = 0.65, P < 0.001$). Lakes above 45 ha in size tended to have three or more other fish species. This relationship is similar if one restricts the relationship to predatory fish (Fig. 1.3b).

1.4 DISCUSSION

Three-spined sticklebacks have long been used as a model system to study variation and function of antipredator traits (e.g., Heuts 1947, Hoogland et al. 1957, Moodie et al. 1973, Swain and Lindsey 1984). Previous studies have demonstrated that: (1) predation is a significant source of mortality (Hagen and Gilbertson 1973, Reimchen 1994), (2) predators exert selection on antipredator characteristics (Hagen and Gilbertson 1973, Moodie et al. 1973, Reimchen 1995), and (3) variation in antipredator traits is related to presence of predators (Hagen and Gilbertson 1972, Bell and Richkind 1981, Walker 1997, Walker and Bell 2000). Despite 50 years of studying predation on sticklebacks, its role in speciation has largely been overlooked. In this study, I sought to determine if there was a correlation between native fish predators and the presence of stickleback species pairs.

I found a striking pattern: cutthroat trout were found in all lakes surveyed,
most lakes have other fish species in addition to cutthroat trout, but benthics and limnetics were found only in lakes with cutthroat trout and no other fish species. Furthermore, a large number of solitary lakes could not be discriminated from sympatric lakes using physical characteristics alone. This pattern suggests that stickleback populations cannot persist as separate species if predation levels are too high. The two most common fish species encountered, other than cutthroat trout, are predators of sticklebacks. Prickly sculpin, which occur predominantly in the littoral zone, prey on stickleback eggs and juveniles guarded by parental males and also on adult sticklebacks (Moodie 1972, Pressley 1981). Rainbow trout, found in pelagic waters, prey on adult sticklebacks (Hagen and Gilbertson 1973). The presence of these predators in solitary lakes may have prevented the stickleback colonist populations from partitioning resources across the two habitats. Rather than holding prey densities down to a point at which they do not compete for resources (e.g., Paine 1966), these predators may prevent stickleback populations from invading, and subsequently adapting to, separate habitats.

Divergence and persistence may also be hampered in lakes with other fish species due to competition for food. Salmonids (coho salmon, Dolly Varden, and kokanee) and cyprinids (peamouth chub and northern pikeminnow) may compete with sticklebacks for food. Of these species, only the northern pikeminnow is likely to be a significant predator of sticklebacks (Moodie et al. 1973; J. McPhail, personal communication). Lakes with a large population of juvenile salmonids, which often prey on zooplankton (Jaenicke et al. 1987, O'Neill and Hyatt 1987), may not be able to support a stickleback population which lives in the pelagic zone and feeds exclusively on zooplankton. The incidence of these salmonids and cyprinids was much lower than the incidence of predatory prickly sculpins and rainbow trout, suggesting that predation has been relatively more important than competition in retarding divergence and persistence in solitary lakes.

Conclusions about the impact of the presence of certain fish species on sympatric sticklebacks must await detailed behavioral observations and large scale field and (or) pond experiments. However, the sensitivity of benthics and limnetics
to other fish species is revealed by two introductions into species pair lakes. First, Larson (1976) recorded the distribution and behaviour of benthics and limnetics prior to, and following, the temporary introduction of coho salmon into Paxton Lake. The presence of coho salmon fingerlings in the pelagic zone caused schools of limnetics to move into the littoral zone. The coho salmon did not persist and limnetics have since "reclaimed" the pelagic zone. Second, in Hadley Lake, the introduction of brown bullhead catfish (Ameiurus nebulosus), an omnivore which feeds on eggs and small fish, in the early 1990s appears to have been the cause of the recent extinction of the stickleback species pairs in this lake (E. Taylor, unpublished observations). Such observations suggest that other fish species can have significant impacts on the habitat use and fitness of sympatric stickleback species.

A number of alternative explanations for the patterns observed in this study can be considered. First, solitary lakes may not have the same proportion of littoral and pelagic habitats as do sympatric lakes. If, for example, solitary lakes are consistently dominated by pelagic habitat, then one would not expect the persistence of benthic morphs. While the largest lakes are dominated by pelagic habitat, preliminary analysis of solitary lakes similar in size to sympatric lakes reveals no systematic difference in relative littoral area between the two types of lakes (S. Vamosi, unpublished observations). Second, other fish species may have been driven extinct by sticklebacks in sympatric lakes. Studies have documented, for example, that sticklebacks compete with juvenile salmon for zooplankton (Jaenicke et al. 1987, O'Neill and Hyatt 1987). The widespread occurrence of other fish species in solitary lakes and the results of the introductions to sympatric lakes discussed above argue that the scenario of sympatric sticklebacks driving other fish species extinct is unlikely. Finally, solitary lakes may be different in biological or physical characteristics not considered in this study. It is possible, for example, that the initial divergence of benthics could have been greater in lakes with cover than in lakes with little cover, facilitating the persistence of secondary invaders (S. Foster, personal communication). Although I do not believe that such a systematic
bias exists between sympatric and solitary lakes, such alternatives can not be
discounted with the data currently available.

Mayr (1963), after reviewing Worthington’s assertion that predation had
retarded speciation and Fryer’s counter that predation had possibly accelerated it,
concluded that the impact of predation was likely between these two extreme views.
The results of this study agree with his conclusions in that I have detected: (1) some
retardation of speciation or, perhaps, increase of extinction, in lakes with a rich
community of predatory fish, and (2) divergence and persistence in lakes with a
community consisting of a single fish predator. Future experimental work should
focus on determining if, and how, interactions with other fish species affect the
probability of divergence and speciation.

Diversification in species-poor environments is common and most often
attributed to a release from competition (Robinson and Wilson 1994, Smith and
Skúlason 1996). In most of these cases, predators are also rare or absent. The
squanga whitefish (Coregonus sp.), a pelagic zooplanktivore, is known to coexist
with lake whitefish (Coregonus clupeaformis) in only four lakes in the Yukon
Territory, Canada (Bodaly 1979). The generally accepted explanation for the
limited distribution of the squanga is the distribution of a potential competitor, the
planktivorous least cisco (Coregonus sardinella), which is absent from these lakes
but common throughout the region (Lindsey 1981). Bodaly et al. (1988), however,
noted unusually low abundances of predatory fish in the four lakes with squanga
whitefish. Trophic polymorphisms in arctic charr (Salvelinus alpinus) (Malmquist
et al. 1992, Reist et al. 1995) and temperate bass (Percichthys trucha) (Ruzzante et
al. 1998) are also associated with both few competitors and few predators. Only
with continued research on the interactions between sympatric species and other
community members will we elucidate the relative roles of competition and
predation in promoting divergence and speciation.
Table 1.1 Lakes in the central Strait of Georgia region which contain a solitary population of sticklebacks.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Elevation (m)</th>
<th>Lake</th>
<th>Elevation (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrose</td>
<td>56</td>
<td>Long</td>
<td>110</td>
</tr>
<tr>
<td>Beaver</td>
<td>61</td>
<td>Mackechnie</td>
<td>27</td>
</tr>
<tr>
<td>Blackburn</td>
<td>106</td>
<td>Maxwell</td>
<td>313</td>
</tr>
<tr>
<td>Brannen</td>
<td>78</td>
<td>Michael</td>
<td>25</td>
</tr>
<tr>
<td>Brown</td>
<td>32</td>
<td>Mixal</td>
<td>24</td>
</tr>
<tr>
<td>Bullocks</td>
<td>61</td>
<td>North</td>
<td>130</td>
</tr>
<tr>
<td>Chemainus</td>
<td>91</td>
<td>Ogden</td>
<td>76</td>
</tr>
<tr>
<td>Chickadee</td>
<td>51</td>
<td>Paq</td>
<td>21</td>
</tr>
<tr>
<td>Cranby</td>
<td>88</td>
<td>Pike</td>
<td>59</td>
</tr>
<tr>
<td>Cusheon</td>
<td>91</td>
<td>Priest</td>
<td>15</td>
</tr>
<tr>
<td>Diver</td>
<td>107</td>
<td>Quamichan</td>
<td>26</td>
</tr>
<tr>
<td>Dougan</td>
<td>60</td>
<td>Ruby</td>
<td>23</td>
</tr>
<tr>
<td>Elk</td>
<td>61</td>
<td>Sakinaw</td>
<td>5</td>
</tr>
<tr>
<td>Fuller</td>
<td>46</td>
<td>Somenos</td>
<td>4</td>
</tr>
<tr>
<td>Garden Bay</td>
<td>30</td>
<td>St. Mary</td>
<td>42</td>
</tr>
<tr>
<td>Graham</td>
<td>33</td>
<td>Stowell</td>
<td>71</td>
</tr>
<tr>
<td>Green</td>
<td>94</td>
<td>Swan</td>
<td>14</td>
</tr>
<tr>
<td>Greenway</td>
<td>24</td>
<td>Thetis</td>
<td>52</td>
</tr>
<tr>
<td>Hoggan</td>
<td>60</td>
<td>Trout</td>
<td>145</td>
</tr>
<tr>
<td>Holden</td>
<td>8</td>
<td>Waugh</td>
<td>55</td>
</tr>
<tr>
<td>Horne</td>
<td>92</td>
<td>West</td>
<td>24</td>
</tr>
<tr>
<td>Hotel</td>
<td>30</td>
<td>Weston</td>
<td>60</td>
</tr>
<tr>
<td>Katherine</td>
<td>24</td>
<td>Wolf</td>
<td>214</td>
</tr>
<tr>
<td>Klein</td>
<td>135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2 Physical characteristics and number of other fish species in sympatric and solitary lakes. Traits, with the exception of pH and number of other fish species, are log transformed. Sample sizes for pH: $n = 11$ for solitary populations, $n = 4$ for sympatric populations; for all other traits: $n = 47$ for solitary populations, $n = 6$ for sympatric populations. Values are means ($\pm 1\ SE$).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Solitary</th>
<th>Sympatric</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42 ± 0.17</td>
<td>8.13 ± 0.30</td>
</tr>
<tr>
<td>Area</td>
<td>1.46 ± 0.09</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>Perimeter</td>
<td>3.47 ± 0.06</td>
<td>3.30 ± 0.10</td>
</tr>
<tr>
<td>Mean depth</td>
<td>0.80 ± 0.05</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>Elevation</td>
<td>1.68 ± 0.06</td>
<td>1.73 ± 0.08</td>
</tr>
<tr>
<td>Distance to sea</td>
<td>3.37 ± 0.05</td>
<td>3.46 ± 0.10</td>
</tr>
<tr>
<td>Number of other fish species</td>
<td>3.10 ± 0.23</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 1.3 Occurrences of other fish species in sympatric and solitary lakes. Predatory fish species are listed in bold.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Solitary (n = 47)</th>
<th>Sympatric (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coho salmon (<em>Oncorhynchus kisutch</em>)</td>
<td>13</td>
<td>—</td>
</tr>
<tr>
<td><strong>Cutthroat trout</strong></td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>Dolly Varden (<em>Salvelinus malma</em>)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Kokanee (<em>Oncorhynchus nerka</em>)</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Peamouth chub (<em>Mylocheilus caurinus</em>)</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td><strong>Prickly sculpin (Cottus asper)</strong></td>
<td>29</td>
<td>—</td>
</tr>
<tr>
<td>Northern pikeminnow (<em>Ptychocheilus oregonensis</em>)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Rainbow trout (<em>Oncorhynchus mykiss</em>)</td>
<td>31</td>
<td>—</td>
</tr>
</tbody>
</table>
Fig. 1.1 Map of study area. Sympatric lakes \((n = 6)\) are indicated by triangles. Solitary sites, and number of lakes sampled, are: Denman Island (D; 2), Gabriola Island (G; 1), Lasqueti Island (L; 1), Nelson Island (N; 2), Saltspring Island (S; 7), Sechelt Peninsula (P; 13), Texada Island (T; 1), and Vancouver Island (V; 20).
Fig. 1.2 Canonical scores obtained from multivariate discriminant function analysis of all characteristics (top) and physical characteristics only (bottom) of 47 solitary (open) and 6 sympatric (filled) lakes.
Fig. 1.3 The relationship between the number of other species (top panel) or number of predator species (bottom panel) and log transformed surface area of solitary (open) and sympatric (filled) lakes.
Chapter Two  "CHARACTER DISPLACEMENT" OF DEFENSIVE ARMOR
2.1 INTRODUCTION

Ecological character displacement refers to evolutionary shifts of morphological traits in response to the interspecific competition for resources between similar species (Brown and Wilson 1956, Grant 1972, Schluter 2000b). The observation that species are frequently more divergent in areas where they coexist than where they are found alone (Lack 1947, Brown and Wilson 1956, Schluter 2000b) has focused attention on divergence, although a number of outcomes are possible, including parallel shifts and convergence of traits (Grant 1972; Abrams 1996, 2000). Numerous studies have demonstrated evidence consistent with morphological divergence as a result of interspecific competition (e.g., Lindsey et al. 1970, Fjeldså 1983, Malmquist 1985, Schluter and McPhail 1992, Dayan and Simberloff 1994, Kawano 1995, Chiba 1999, Adams and Rohlf 2000). Adams and Rohlf (2000), for example, reported a case of character displacement between two salamander species. Sympatric *Plethodon hoffmani* populations have evolved a faster closing, albeit weaker, jaw, whereas sympatric *P. cinereus* populations have evolved a slower, stronger jaw. Furthermore, these differences were correlated with differences in prey consumption, with *P. hoffmani* individuals consuming larger prey than *P. cinereus* individuals.

Studies of character displacement have rarely considered how interactions with other trophic levels, notably predators, may contribute to morphological differentiation of prey yet there are several reasons to expect defensive traits of prey to diverge when they coexist. Predation by a single predator species may lead to divergent selection on antipredator defences between prey species to minimize their consumption (Holt 1977, Jeffries and Lawton 1984, Brown and Vincent 1992, Holt and Lawton 1994, Abrams 2000). For example, selection may favor larger body size in one species and decreased size in the other if the shared predator preys most heavily on individuals of an intermediate body size. The mechanism is

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1 I acknowledge the contribution of Dolph Schluter to fish sampling and statistical analyses by using “we” throughout this chapter.
analogous to divergence of foraging traits in response to competition for shared resources except that the interactions between the species are mediated through a higher trophic level and competition is for “enemy-free space.” Holt (1977) coined the term “apparent competition” to denote the difference between this interaction and “true competition” for resources. The introduction of a new prey species is hypothesized to increase the density of the shared predator, which, in turn, results in higher predation on the first species. Divergence is a possible outcome of such an interaction, although Abrams (2000) demonstrated that parallel increases, parallel decreases and convergence in antipredator traits of the prey species may also occur. Parallel decreases, for example, are predicted when predation risk is lower in sympatry (Abrams 2000).

Alternatively, interspecific competition may initiate habitat segregation, such that two prey species evolve to exploit different habitats, and this would secondarily favor divergence in antipredator traits if different predators are found in these habitats (e.g., Mittelbach 1984, Reimchen 1994, Gilbert et al. 1999, Pitt 1999). An increase in body size, for example, may be selected for in a habitat where predators tend to be small, whereas a decrease in body size may result in reduced consumption by large predators in a second habitat (Reznick et al. 1990). Divergence may also result if the vulnerability of a prey species to a given predator depends on habitat structure. Such a predator x habitat interaction appears to characterize the risk of predation for desert gerbils (Kotler et al. 1991, 1993): snakes are perceived as being more dangerous in the bush microhabitat, whereas owls are the main threat in the open microhabitat. In this scenario, individuals living in the bush microhabitat would be expected to evolve antipredator defences that reduce their consumption by snakes. Divergence is the most likely outcome of habitat-specific predation risk.

The first step to evaluating competing hypotheses about the role of predation in evolutionary shifts is to compare the antipredator traits of sympatric populations with those of solitary populations. Because empirical tests are currently lacking, our methods for detecting character shifts are modified from tests of ecological
character displacement (Grant 1972, Schluter and McPhail 1992, Schluter 2000b). Character states of the sympatric species may become more different from (or similar to) one another than predicted by comparing random solitary populations to one another (Schluter and McPhail 1992) (Fig. 2.1A, D). This, in effect, entails comparing the variance between sympatric species with the variance between solitary populations. The widely recognized pattern of ecological character displacement, modified for a double-invasion scenario (Grant 1972, Schluter and McPhail 1992), corresponds to Fig. 2.1D, where the distance between pairs of sympatric species is greater than the distance between the solitary populations. If sympatric species show consistent differences, but are not more variable than predicted based on comparisons with solitary populations (Fig. 2.1B, E), character displacement may be rejected but the species may still have adapted to habitat-specific predation risk because of different predators. Mean character states in sympatry may also be different from mean character states in solitary populations (Fig. 2.1D – F), as a consequence of changes in predation risk. Predation risk in sympatry may increase if the addition of the second species increases densities of shared predators and, in contrast, may decrease if predator densities do not respond to the addition of the second species. In the former case, the prey species act as apparent competitors, whereas in the latter case they are apparent mutualists. This test compares the midpoint value of sympatric species with means of the solitary populations.

We tested for character shifts in defensive armor of three-spined sticklebacks previously shown to exhibit ecological character displacement in trophic morphology and habitat use (Schluter and McPhail 1992). The defensive armor of sticklebacks consists of a number of bony structures that in concert increase the probability of post-capture escape (Reimchen 1983). Dorsal and pelvic spines, when erect, increase the effective diameter of the stickleback, which decreases the handling success of gape-limited predators (Reimchen 1991). Lateral plates have a number of functions, including buttressing the dorsal and pelvic spines (Reimchen 1983), protecting the epidermis from lacerations by toothed predators (Reimchen
1992), and possibly interfering with the pharyngeal jaw mechanism of predatory fish (Reimchen 2000). The pelvic girdle buttresses the pelvic spines and helps create a protective bony “ring” around the stickleback (Reimchen 1983). We tested whether defensive armor shifted in response to interactions with predators by comparing the defensive armor traits of sticklebacks from lakes containing both benthics and limnetics with sticklebacks from lakes containing a single population.

2.2 MATERIALS AND METHODS

The study was carried out on small lakes of the Strait of Georgia region of British Columbia, Canada. Two categories of lakes were sampled. First, we sampled sympatric benthic and limnetic species from the five lakes previously considered by Schluter and McPhail (1992) plus the benthic species from Balkwill Lake (limnetic specimens were unavailable) (Table 2.1, Fig. 2.2). These six lakes occur in four drainages. Balkwill, Priest and Emily lakes are connected as a chain by short streams. The remaining three lakes occur in separate drainages connected to the sea. Second, we included sticklebacks from five lakes containing only a single species of stickleback and a single predatory fish, cutthroat trout (Table 2.1, Fig. 2.2). These stickleback populations are most comparable to the lakes containing stickleback species pairs, which also contain cutthroat trout and no other fish species (Facchin and King 1980, 1981; see Chapter One). We also sampled a stream population of marine (anadromous) sticklebacks (Fig. 2.2), for reference to the ancestral state. Only a single marine population was used because Walker and Bell (2000) have confirmed that marines are similar throughout the Pacific Ocean.

We measured sticklebacks from collections in the University of British Columbia Fish Museum. With the exception of Tom Lake \((n = 13)\) and Hadley Lake limnetics \((n = 10)\), at least 30 fish were examined per population. In total, we measured 181 benthic, 142 limnetic, 40 marine and 181 solitary sticklebacks. Sticklebacks from sympatric populations had been classified as benthics or
limnetics based on trophic characters (Schluter and McPhail 1992). Thus, if any differences in defensive armor are found between sympatric species, it is not an artefact of our classification method.

Standard length (SL) and five defensive armor traits were measured on the left side of the body: length of first dorsal spine, length of second dorsal spine, length of pelvic spine, length of pelvic girdle, and number of lateral plates (Fig. 2.3). Standard length, the distance along the body from the anterior tip of the mouth to the end of the caudal peduncle, was used rather than total body length. The latter measure includes the caudal fin, which may be damaged prior to, or following, fixing and staining. We adjusted all defensive armor traits, other than number of lateral plates, of each population to 47.5 mm SL using the equation: \( Y_i = X_i - \beta(SL_i - 47.5) \), where \( Y_i \) is the size-adjusted trait value at 47.5 mm, \( X_i \) is the original value for the trait in individual \( i \) (\( i = 1 \) to 40 for each population), \( \beta \) is the population-specific regression coefficient of the original trait values on standard length (Hagen and Gilbertson 1972). The choice of universal SL is somewhat arbitrary (Hagen and Gilbertson 1972), but we chose 47.5 mm because it was approximately the mean of the population means (mean = 47.46 mm). Mean standard lengths (± SE) of the different population types were as follows: benthic (52.98 mm ± 0.51); limnetic (43.36 mm ± 0.41), marine (59.35 mm ± 0.63) and solitary (46.31 mm ± 0.50).

We first tested for character shifts in overall defensive armor, calculated as the first principal component in a principal components analysis on the correlation matrix of size-adjusted traits and ln-transformed number of lateral plates. We then examined patterns in individual size-adjusted traits. Tests for divergence were carried out using a variance-ratio test, using the estimated variance between sympatric species in the numerator, and the estimated variance between solitary populations in the denominator (Schluter and McPhail 1992). Tests for parallel shifts were carried out by comparing the midpoint value of sympatric species, calculated as the average of the trait values of the limnetic and the benthic species present in each lake, with the mean trait values for solitary populations using a two sample t-test or the Wilcoxon test. The latter test was used when data were not
normally distributed.

2.3 Results

Multivariate analysis of defensive armor revealed striking differentiation among populations (Fig. 2.4). The first principal component (PC1), which had an eigenvalue of 3.95 and accounted for 79% of the total variance, represented variation in all defensive armor traits (Table 2.2). This axis separated populations with short spines and girdles and few plates at one extreme from those with long spines and girdles and many plates at the other extreme. Marines, which had long spines, robust girdles and lateral plates along the entire length of their body, had the highest PC1 score. Benthics from Paxton Lake, many of which were lacking the first dorsal spine, pelvic girdle, pelvic spines, and lateral plates (see also Chapter Three), correspondingly had the lowest PC1 score.

Interestingly, although limnetics overlapped with solitary populations in defensive armor, there was a negative correlation among populations between length of spines and number of lateral plates (Fig. 2.5). Limnetics tended to have more plates but shorter spines than solitary populations.

Tests for divergence revealed differentiation between sympatric benthics and limnetics. The defensive armor, summarized by PC1, of sympatric species was significantly more divergent than expected ($F_{5,4} = 9.20, P = 0.03$), although this difference is not significant when Paxton Lake is excluded from the analysis ($F_{4,4} = 1.08, P = 0.47$). Limnetics always had more defensive armor than sympatric benthics (Wilcoxon paired-sample test, $P = 0.05$). In analyses of individual traits, sympatric species were significantly more divergent in size-adjusted length of pelvic girdle ($F_{5,4} = 48.96, P = 0.001$) and number of lateral plates ($F_{5,4} = 9.89, P = 0.02$) than expected (Fig. 2.6). When Paxton Lake is excluded, the divergence in pelvic girdle persists, while the divergence in lateral plates becomes marginally significant (Table 2.3). Limnetics had consistently longer size-adjusted spines (Wilcoxon paired-sample test, $P = 0.05$), with the exception of first dorsal spine ($P =
and more lateral plates ($P = 0.05$) (Fig. 2.6) than sympatric benthics.

Tests for parallel shifts revealed that most traits are reduced in sympatric populations compared to solitary populations. The midpoint PC1 scores of sympatric species were significantly lower than PC1 scores of solitary populations (Wilcoxon test, $\chi^2_1 = 5.77, P = 0.02$) (Fig. 2.4). Parallel decreases in sympatry were evident for lengths of all spines and length of pelvic girdle (Table 2.4). No shift in the midpoint of number of lateral plates was observed (Table 2.4, Fig. 2.6).

In summary, the lengths of spines are significantly reduced in sympatry, the length of pelvic girdle is significantly diverged and reduced in sympatry, and the number of lateral plates is diverged in sympatry, with no shift in the mean of sympatric benthics and limnetics. These results all support the conclusion that defensive armor traits have evolved as a consequence of sympatry.

### 2.4 Discussion

The three-spined stickleback system is one in which competition for resources has probably played a significant role in the divergence between species (Schluter and McPhail 1992; Schluter 1994, 2000a). However, the potential role of predation and other interactions in adaptive radiation have been inadequately addressed. Sticklebacks are subject to predation by a wide variety of invertebrate and vertebrate predators (Reimchen 1994), many of which have been demonstrated to act as selective agents on defensive armor traits. Cutthroat trout, for example, are a significant cause of mortality of sticklebacks (Reimchen 1995), and stickleback populations preyed on by trout tend to have increased defensive armor compared to populations where trout are absent (Hagen and Gilbertson 1972). Selective predation by trout has been demonstrated, such that phenotypes with more lateral plates and longer spines have a selective advantage over less armored phenotypes (Moodie et al. 1973; Reimchen 1995, 2000). The presence of cutthroat trout in all lakes containing both benthics and limnetics (Schluter and McPhail 1992; Chapter One) prompted an investigation into the defensive armor of sympatric species.
We found evidence for character shifts in defensive armor of sympatric sticklebacks. While there was a tendency for traits to be more divergent in sympathy than between random pairs of solitary populations, the most striking result was the reduction of defensive armor in sympathy. This reduction, most pronounced in benthics, suggests two ways in which predation may have caused shifts in sympathy. First, if increased predator densities have not resulted from the presence of two stickleback populations, then predation risk to individual sticklebacks may be reduced in sympathy. Although detailed population estimates of predators in lakes with one and two species of sticklebacks are lacking, observations at two sympatric (Paxton and Priest) and two solitary (Cranby and Klein) lakes indicate that only a single pair of adult loons tends to be present during the summer at sympatric and solitary lakes. Second, benthics may experience lower encounter rates with predators in the vegetated waters of the littoral zone. A number of studies have demonstrated that the littoral zone of lakes acts as a refuge from predation for small fish (Crowder and Cooper 1982, L'Abée-Lund et al. 1993).

A number of alternate mechanisms may have also produced the reductions observed in benthics. First, it is possible that the reduced defensive armor of benthics is merely the result of longer time in freshwater, as suggested by the double invasion scenario (McPhail 1993). The size and salinity tolerance of eggs, for example, are more similar between limnetic and marine sticklebacks than between benthic and marine sticklebacks (Kassen et al. 1995). However, the observation that the defensive armor of stickleback populations can shift rapidly, sometimes within a generation (Reimchen 1995), in response to changes in predation regime, argues that selection will mask any differences in the amount of time exposed to freshwater. Second, armor reduction may simply be due to low calcium levels in lakes with two species. The observation that limnetics actually tend to have more lateral plates than the solitary populations examined makes this explanation unlikely.

Potential consequences of living in sympathy are illuminated by comparing
the relative elaboration of spines and plates in limnetic and solitary populations. Robust spines are typically associated with increased risk of predation by gape-limited predatory fish (Hagen and Gilbertson 1972, Reimchen 1991; S. Vamosi, unpublished observations). Piscivorous birds, in contrast, do not appear to be deterred by spines (Reimchen 1980, Whoriskey and FitzGerald 1985). Although lateral plates have a number of functions (Reimchen 1983, 1992, 2000), perhaps the most important is preventing the body of the stickleback from being crushed when the fish is first captured (J. McPhail, personal communication). If the dominant predator is a gape-limited fish, selection should favor robust spines, which increase the probability of escape once captured (Reimchen 1991). Sticklebacks at risk of predation by piscivorous birds may have little to gain by having robust spines but may gain a survival advantage if, when given an opportunity to escape, they are reasonably intact after being in the bird’s beak (Reimchen 1980). The increased number of lateral plates and reduced spines in limnetics suggests, as a consequence of living in the open waters of the pelagic zone, a selection regime dominated by exposure to avian piscivores, such as the common loon. The opposite pattern in solitary populations, which forage in both littoral and pelagic zones (Schluter and McPhail 1992), suggests that they have adapted primarily to a regime dominated by cutthroat trout predation.

What is the evidence that reductions in defensive armor and divergence in length of pelvic girdle and number of lateral plates are due to interactions via a shared predator? Schluter (2000b) has suggested six criteria that studies of apparent competition (and mutualism) should satisfy. First, chance should be ruled out. The reduction in the defensive armor of benthics was observed in all lakes with species pairs. Similarly, the divergence between sympatric species in length of pelvic girdle and number of lateral plates was repeatable across lakes.

Second, population differences should represent evolutionary shifts in sympatry, rather than persistence in sites where the populations were already sufficiently diverged when they first came into contact. The range of number of lateral plates of benthics and limnetics was greater than that observed in solitary
populations, implying divergence occurred in, and as a consequence of, sympatry. Schluter and McPhail (1992) discuss further reasons that suggest that lakes with benthics and limnetics are not simply those in which the original colonists were already sufficiently diverged to allow coexistence.

Third, the differences in antipredator traits among populations should have a genetic basis. Benthic, limnetic, and solitary fish have been reared in the lab and characteristic differences among populations in defensive armor have been maintained (McPhail 1984, 1992; Schluter 1994, Hatfield 1997, Vamosi et al. 2000, Grand 2000). Furthermore, other studies of stickleback populations have demonstrated that differences within and among populations in number of lateral plates is heritable (reviewed by Ziuganov 1983).

Fourth, environmental conditions should be similar between sympatric and solitary sites. Physical characteristics measured (surface area, mean depth, and total dissolved solids) were not significantly different between sympatric and solitary lakes. Although solitary lakes sampled in this study tended to be at a higher elevation than sympatric lakes, an analysis of variation in defensive armor among solitary populations revealed no significant effect of elevation but a highly significant effect of predator community (S. Vamosi, unpublished observations). Accordingly, lakes in this study contain sticklebacks and only one other native fish species, the cutthroat trout. Schluter and McPhail (1992) present data suggesting that lakes with solitary populations appear to contain both benthic and planktonic prey, as do the sympatric lakes.

Fifth, shifts in defensive traits should affect their vulnerability to shared predators. Laboratory (Chapter Three) and field (Chapter Four) predation experiments confirm selective predation on benthics and limnetics consistent with their defensive armor differentiation, although these patterns may also be the result of body size (Schluter and McPhail 1992; see Chapter Three) and behavioral differences (see Chapter Three). A number of studies of solitary populations have demonstrated a relationship between predation risk and defensive armor (Hagen and Gilbertson 1972, 1973; Bell and Haglund 1978; Reimchen 1991, 1994), some of
which have been discussed earlier.

Finally, independent evidence should be gained that similar phenotypes interact as apparent competitors (or mutualists). Unfortunately, there is currently no evidence available that bears on this criterion. In short, we can address five of the criteria, which is rare in studies of evolutionary shifts in response to a shared predator (Schluter 2000b).

To the best of our knowledge, this is the first study to demonstrate parallel reductions of defensive traits in sympatry. Furthermore, this is only the second case where exaggerated divergence between sympatric species of putative defensive traits has been documented. Chiba (1999) investigated a case of character displacement between land snails (Mandarina spp.) on the Bonin Islands. Habitat segregation in sympatry was accompanied by divergence of shell coloration, such that species in wet and sheltered sites had dark shells and species in dry and exposed sites were brighter and often polymorphic. Two hypotheses have been proposed to account for the differences in shell color. First, the shell colour differences may reflect adaptation to different light environments, with dark shells warming more rapidly when exposed to sunlight and bright shells reflecting the higher levels of sunlight associated with exposed sites. The adaptation to differential light environments hypothesis, however, does not predict within-habitat polymorphism. An alternative hypothesis is that the divergence was driven by visual predators, especially birds, with colour differences evolving to match the dominant background of their preferred microhabitat. These competing hypotheses have not been tested to date.

Because sympatric species often have divergent habitat preferences (Robinson and Wilson 1994), we feel that the lack of evidence regarding the role of predation in morphological divergence partially reflects the "back seat" that predation has historically taken to competition (Schluter 2000b). The vast array of striking antipredator traits in a wide variety of plants (latex and resin canals: Farrell et al. 1991; secondary compounds: Sagers and Coley 1995; spines: Belovsky et al. 1991, Rohner and Ward 1997) and animals (aposematism: Brodie 1992,
Servedio 2000; crypsis: Sandoval 1994, Stoddard 1999; ink: Carefoot et al. 1999; shells: Donovan et al. 1999) suggests a role for predation in producing divergence in sympatry. Further studies of closely related species which have sympatric and solitary populations, including sticklebacks and other fish species pairs (see Robinson and Wilson 1994 for a review), should concentrate their efforts on teasing apart the relative contributions of shared predation and habitat-specific predation to shifts of antipredator traits.
Table 2.1 Physical characteristics of lakes with sympatric and solitary populations of sticklebacks.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Elevation (m)</th>
<th>Surface Area (ha)</th>
<th>Mean Depth (m)</th>
<th>Total Dissolved Solids (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>species pair lakes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balkwill</td>
<td>61</td>
<td>11.5</td>
<td>6.3</td>
<td>49</td>
</tr>
<tr>
<td>Emily</td>
<td>23</td>
<td>7.2</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Enos</td>
<td>55</td>
<td>17.6</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Hadley†</td>
<td>50</td>
<td>6.7</td>
<td>3.5</td>
<td>—</td>
</tr>
<tr>
<td>Paxton</td>
<td>88</td>
<td>17</td>
<td>6.2</td>
<td>30</td>
</tr>
<tr>
<td>Priest</td>
<td>75</td>
<td>44.3</td>
<td>5.4</td>
<td>20</td>
</tr>
<tr>
<td><strong>solitary lakes with only cutthroat trout</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranby</td>
<td>88</td>
<td>44.6</td>
<td>3.2</td>
<td>—</td>
</tr>
<tr>
<td>Klein</td>
<td>121</td>
<td>13.5</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>Mike</td>
<td>229</td>
<td>4.2</td>
<td>3.4</td>
<td>32</td>
</tr>
<tr>
<td>Tom</td>
<td>195</td>
<td>17</td>
<td>1.7</td>
<td>15</td>
</tr>
<tr>
<td>Trout</td>
<td>145</td>
<td>7.6</td>
<td>5.8</td>
<td>—</td>
</tr>
</tbody>
</table>

† benthics and limnetics in Hadley Lake now presumed to be extinct
Table 2.2 Principal components analysis of defensive armor morphology. Traits, with the exception of number of lateral plates, were size-adjusted before analysis. Numbers represent loadings of traits on principal component axes.

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>first dorsal spine length</td>
<td>0.439</td>
<td>0.493</td>
<td>-0.243</td>
</tr>
<tr>
<td>second dorsal spine length</td>
<td>0.419</td>
<td>0.601</td>
<td>0.050</td>
</tr>
<tr>
<td>pelvic spine length</td>
<td>0.476</td>
<td>-0.319</td>
<td>-0.343</td>
</tr>
<tr>
<td>pelvic girdle length</td>
<td>0.448</td>
<td>-0.503</td>
<td>-0.305</td>
</tr>
<tr>
<td>number of lateral plates</td>
<td>0.452</td>
<td>-0.203</td>
<td>0.853</td>
</tr>
<tr>
<td>% of total variance</td>
<td>78.91</td>
<td>14.71</td>
<td>4.47</td>
</tr>
</tbody>
</table>
Table 2.3 Tests of character displacement. Observed values are absolute differences between sympatric benthics and limnetics averaged over five lakes (Balkwill excluded). Expected values are the mean absolute differences between all pairs of solitary populations. Values are based on ln-transformed data. Traits other than number of lateral plates are size-adjusted. $P$ values are obtained from variance-ratio tests.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Observed</th>
<th>Expected</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of first dorsal spine</td>
<td>0.58</td>
<td>0.55</td>
<td>0.41</td>
</tr>
<tr>
<td>Length of second dorsal spine</td>
<td>0.25</td>
<td>0.52</td>
<td>0.88</td>
</tr>
<tr>
<td>Length of pelvic spine</td>
<td>0.69</td>
<td>0.55</td>
<td>0.8</td>
</tr>
<tr>
<td>Length of pelvic girdle</td>
<td>1.08</td>
<td>0.34</td>
<td>0.04</td>
</tr>
<tr>
<td>Number of lateral plates</td>
<td>1.13</td>
<td>0.57</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Table 2.4 Tests of parallel changes in sympatry. Sympatric values are means of sympatric benthics and limnetics averaged over five lakes. Solitary values are means of solitary populations. Traits other than number of lateral plates are size-adjusted.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sympatric</th>
<th>Solitary</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of first dorsal spine</td>
<td>1.65</td>
<td>2.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Length of second dorsal spine</td>
<td>2.71</td>
<td>3.41</td>
<td>0.04</td>
</tr>
<tr>
<td>Length of pelvic spine</td>
<td>3.64</td>
<td>5.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Length of pelvic girdle</td>
<td>6.99</td>
<td>8.36</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of lateral plates</td>
<td>4.82</td>
<td>5.22</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Fig. 2.1 Hypothetical character states in sympatric (open and filled) and solitary (shaded triangles) populations. Numbers indicate sympatric sites. Panel A illustrates a situation where sympatric species are more divergent than solitary populations (i.e., $d_{sym} > d_{sol}$, where $d$ is the distance between pairs of species); B illustrates consistent differences between sympatric species, but not more divergent than expected; C illustrates no differences in sympatry. Panels D to F are analogues to A to C, with parallel reductions of the character in sympatry (i.e., $M_{sym} < M_{sol}$, where $M$ is the midpoint of pairs of species).
Fig. 2.2  Map of the study area. Labels 1 – 6 indicate lakes containing two species of three-spined sticklebacks, whereas A – E contain one species each. The lakes are (1) Balkwill, (2) Priest, (3) Emily, (4) Paxton, (5) Hadley, (6) Enos, (A) Tom, (B) Cranby, (C) Klein, (D) Trout, and (E) Mike. Marines were collected at Lang Creek (M).
Fig. 2.3 Measured defensive armor characters of benthic, limnetic, and solitary sticklebacks. 1, Length of first dorsal spine; 2, length of second dorsal spine; 3, length of pelvic spine; 4, length of pelvic girdle; 5, number of lateral plates.
Fig. 2.4 Defensive armor morphology of benthic (filled), limnetic (open), solitary (triangle), and marine (star) populations. The axis accounts for 79% of the total variance among population means in armor traits.
Fig. 2.5 The relationship between number of lateral plates and length of spines in limnetic (open) and solitary (triangle) populations. Number of lateral plates is negatively correlated with length of spines \( (r = -0.79, P = 0.007) \).
Fig. 2.6 Size adjusted length of pelvic spine and number of lateral plates of sympatric benthics (filled) and limnetics (open). Number of lateral plates for Balkwill limnetics from McPhail (1993). Solitary (triangles) and marine (star) populations included for reference. Population codes as in Fig. 2.2.
Chapter Three  PREDATION SHARPENS THE ADAPTIVE PEAKS: SURVIVAL TRADE-OFFS
3.1 INTRODUCTION

Interspecific competition for resources has historically been the favored explanation for the observation that closely related species tend to be more divergent when they are found together than when they occur alone (Lack 1947, Brown and Wilson 1956, Schluter 2000b). Numerous theoretical (Slatkin 1980, Milligan 1985, Abrams 1986) and empirical studies (e.g., Schluter et al. 1985, Schluter and McPhail 1992, Dayan and Simberloff 1994; for review, see Schluter 2000b) confirm its role in divergence of morphology, behaviour and habitat use. More recently, the role of other interactions, such as predation (Holt 1977, Brown and Vincent 1992, Abrams 2000) and parasitism (Hudson and Greenman 1998), in the evolution of divergence has started to receive attention. Despite the wealth of empirical studies addressing character displacement, no study has considered whether divergence due to interspecific competition for resources has had any consequences for interactions between the consumer species and their predators or parasites.

One frequently observed consequence of competition for shared resources, particularly in aquatic systems, is habitat segregation (Werner et al. 1977, Schluter and McPhail 1992, Robinson and Wilson 1994, Kawano 1995, Chiba 1999). Different habitats often present different prey types for consumers, and divergence of consumers may involve adaptations to feeding on these alternate types (Schluter 2000b). These adaptations can be accompanied by trade-offs in foraging efficiency (Schluter 1993) and growth rates (Schluter 1995), such that each species outperforms the other in its native habitat. However, habitats may also contain different predators (Mittelbach 1984, Reimchen 1994, Gilbert et al. 1999, Pitt 1999), a fact that has been largely overlooked by studies of character displacement. These predators, in turn, may have different modes of detecting or capturing prey (e.g., Reimchen 1994, DeWitt et al. 2000). Analogous to the case of resources, a diversity of predator types can drive the divergence of antipredator traits in the consumer species inhabiting differing habitats. The result is a trade-off in survival when one
consumer species encounters predators associated with the habitat of the competitor species. The purpose of this study was to explore whether: (1) divergence in behavioural and morphological antipredator traits accompanies divergence in trophic traits in a pair of sympatric species and (2) trade-offs in survival exist, such that a consumer species has higher relative survival in its preferred habitat when in the presence of predators associated with the preferred habitat but lower survival in the other habitat when presented with predators from the other habitat.

To address these questions, I studied benthic and limnetic sticklebacks (*Gasterosteus aculeatus*) from Paxton Lake. These two species are divergent in their habitat use, preferred prey and trophic traits. Benthics are larger fish with fewer, shorter gill rakers that feed on macro invertebrate prey in the shallower, vegetated waters of the littoral zone, whereas limnetics are smaller fish with many, long gill rakers and forage predominantly on zooplankton in the pelagic zone (Larson 1976, McPhail 1984, 1992; Schluter and McPhail 1992). The divergence in morphology is thought to have arisen in part as a result of competition for shared resources (Schluter and McPhail 1992, McPhail 1993, Schluter 1994) and is associated with trade-offs in foraging performance (Schluter 1993) and growth rates (Schluter 1995) between habitats.

Previous studies (Foster *et al.* 1988, Foster and Ploch 1990, Reimchen 1994) and field observations (Table 3.1) suggest that different predators of sticklebacks are associated with the two habitats. Sticklebacks in the littoral zone are expected to encounter primarily invertebrate predators (Reimchen 1980, Foster *et al.* 1988) and cannibalistic adult sticklebacks (Foster *et al.* 1988), whereas sticklebacks in the pelagic zone are more likely to encounter diving birds and predatory fish (Reimchen 1994). I tested whether benthics and limnetics were divergent in defensive armor and whether this divergence was associated with trade-offs in survival between habitats, such that benthics are more vulnerable to pelagic zone predators, whereas limnetics are more vulnerable to littoral zone predators. I used insects and large benthics as predators in the littoral treatment and diving birds in the pelagic
treatment. Cutthroat trout, which may prey on sticklebacks in both habitats, will be examined in a later study (see Chapter Four).

3.2 MATERIALS AND METHODS

Defensive armor

Adult fish used for morphometric measurements were collected from Paxton Lake by Schluter and McPhail (1992). These fish had been fixed in 10% formalin for a week, stained in Alizarin red S dye, and preserved in 37% isopropyl alcohol. Standard length (SL) and five defensive armor traits were measured on the left side of 40 benthic and 40 limnetic sticklebacks following the methods of Lavin and McPhail (1985): length of the first dorsal spine, length of the second dorsal spine, number of lateral plates, pelvic girdle length, and pelvic spine length (Fig. 2.1). The five traits were chosen because they have been shown to be correlated with predation pressure in a number of studies (Hagen and Gilbertson 1973, Reimchen 1994; see Chapter Two). Lateral plates provide protection for the epidermis against attacks by toothed predators (Reimchen 1992, 2000). When erect, pelvic spines, in conjunction with dorsal spines, increase the effective diameter of an individual when they are locked erect, reducing vulnerability to gape-limited predators (Reimchen 1991). The pelvic girdle buttresses the pelvic spines and helps create a protective bony “ring” around the stickleback (Reimchen 1983).

I adjusted all defensive armor traits, other than number of lateral plates, of each population to a universal standard length of 47.5 mm using the equation: 

\[ Y_i = X_i - \beta(SL_i - 47.5) \]

where \( Y_i \) is the size-adjusted trait value at 47.5 mm, \( X_i \) is the original value for the trait in individual \( i \) (\( i = 1 \) to 40 for each species), \( \beta \) is the regression coefficient of the original trait values on standard length, and (SL\(_i - 47.5\)) is the standard length of the individual minus the universal standard length (Hagen and Gilbertson 1972). The choice of universal standard length is somewhat arbitrary (Hagen and Gilbertson 1972), but I chose 47.5 mm because it was approximately the mean length of sticklebacks measured (mean = 47.59 mm).
To determine whether benthics and limnetics were divergent in defensive armor, I first conducted an exploratory principal components analysis using the correlation matrix of ln-transformed body-size corrected traits and ln-transformed number of lateral plates. Restricting my attention to principal components with eigenvalues greater than one, I then extracted traits with significant component loadings (≥ 0.40) and compared means among benthics and limnetics using a two sample t-test.

**Schooling behaviour**

Schooling is an antipredator behavioral trait (Seghers 1974, Magurran 1990), which may differ between benthics and limnetics because of the different habitats with which the two species are associated. If schooling behavior differs between the species it should differ in the following way. Limnetics, which live mostly in open waters, are predicted to display strong schooling behavior (Bertram 1978, Pitcher 1986). Benthics, conversely, which live in a highly structured environment where aggregations may be hard to maintain, are predicted not to display strong schooling behavior (Lima and Dill 1990, Rangeley and Kramer 1998).

Schooling behavior was measured in an experimental arena, a large (200 L) aquarium divided into three sections by inserting two panels of window screen mesh 30 cm from either end. This created two end sections (each 30 cm x 30 cm x 50 cm) and a central section (75 cm x 30 cm x 50 cm). In the central section, I drew nine equally spaced, vertical lines on the front of the aquarium with a Sharpie® pen to create 10 equally sized “compartments.”

Ten conspecifics were introduced to a randomly chosen end section and one conspecific was introduced to the other end section. The compartment nearest the single conspecific was assigned a score of one, the second nearest compartment a two, etc., and the compartment closest to the group of 10 conspecifics was assigned a score of 10. These fish were acclimated for 1 hr and then a single fish (= focal individual) was introduced into the central section. I recorded from behind a blind which compartment the focal individual was in at 30 s intervals for 30 min. If the
body of the focal individual spanned two compartments, I recorded which compartment the head was in.

I calculated the mean position of each fish and compared the mean position for each species to a random expectation with a one sample $t$-test. If the mean position for a species was significantly greater than 5.5, the random expectation, I would consider this evidence for schooling behavior. Mean position significantly lower than 5.5 would indicate avoidance of schools, and a lack of a significant difference would indicate no preference. I compared means of the two species with a two sample $t$-test.

**Predation experiments – design**

To determine whether survival trade-offs have accompanied the divergence of benthics and limnetics, I measured the relative predation rates on benthics and limnetics by predators from the two habitats. Two special features of the design of the predation experiments require explanation. First, my design modifies the typical reciprocal transplant experiment (e.g., McPeek 1990) by using individuals of different age in different habitats. In the typical design, individuals of the same age from the two species are assigned at random to one of two habitats that differ only in one way (e.g., presence or absence of a predator). In my experiment, the arena representing the pelagic habitat is larger and the individuals are older than in the littoral habitat. A larger arena was chosen because of the larger size of diving birds in relation to invertebrate and fish predators. Older individuals were used because diving birds do not tend to feed on juvenile sticklebacks (Reimchen 1995).

Second, logistic constraints prevented the use of common loons (*Gavia immer*), which have been observed at Paxton Lake in summer (Table 1), in the pelagic arena experiments. Double-crested cormorants (*Phalacrocorax auritus*), which have not been observed but may be present in winter, were used instead. Both species are common in the Strait of Georgia region, forage for sticklebacks predominantly in the open water zone of lakes (Reimchen 1994, 1995), and dive
under water to pursue and capture prey.

Predation experiments—experimental fish

Fish for experimental studies of predation were obtaining by making crosses in May of the year of study for littoral arena trials and the year before for pelagic arena trials. Adults for crosses were collected from Paxton Lake using minnow traps. Crosses were made by stripping eggs from gravid females into Petri dishes using gentle abdominal pressure and adding water and macerated testes from males. A single male was used per five female clutches. After two hours had elapsed, fertilized eggs were transferred to plastic hatching containers (175 mL) with mesh bottoms suspended in 100 L aquaria above air stones. Air stones provide oxygen to the eggs through the mesh. Eggs that became infected with fungus were removed daily. After hatching, fish were transferred from the hatching containers to the aquaria. A minimum of 25 benthic and 40 limnetic crosses were made in each year.

Juveniles were fed with infusoria cultures for the first two days, then switched to diets consisting of live brine shrimp (Artemia) nauplii fed once or twice daily. After three months, diets of fish to be raised to adulthood were supplemented with frozen bloodworms and brine shrimp.

Predation experiments—littoral arena

To assess the relative predation rates of juvenile benthics and limnetics, I conducted laboratory experiments based on the design of Foster et al. (1988). The littoral zone treatment involved placing juvenile sticklebacks into arenas containing three types of predators: dragonfly nymphs (Aeshna spp.), backswimmers (Notonecta spp.), and large adult benthics. Littoral trials were conducted in July of 1995, 1996, and 1998. Invertebrate predators were obtained from Paxton Lake, Texada Island (1995) or the Experimental Research Ponds, University of British Columbia (1996 and 1998) using dip nets. Adult benthic sticklebacks were caught with minnow traps from Paxton Lake (1995) or were
taken from a lab population of fish obtained from Paxton Lake as juveniles the previous year (1996 and 1998). All predators were collected one to three days prior to trials, and maintained in an unfed state in vegetated aquaria.

Experimental containers were circular wading pools (0.75 m diameter × 0.2 m deep). Pools were filled with freshwater 24 hr before each trial. Substrate consisted of a thin layer (5 – 10 mm) of coarse granular beach sand, instead of lake sediment, to allow accurate enumeration of individuals during observation periods. Each pool contained two clumps of an aquatic macrophyte (*Chara* sp.), two lily pads, and a cattail stalk for cover.

To start a trial, ten juvenile benthics and ten juvenile limnetics were haphazardly selected from stock aquaria, added to each pool and acclimated for at least 30 min before three predators (either adult benthics or backswimmers or dragonfly larvae) were added. Predators and prey were used only once. Number of predators was chosen to match the predator densities used by Foster *et al.* (1988). Pools with sticklebacks but no predators served as controls. After addition of insect predators, pools were censused every 24 h. Pools with adult benthics were censused eight hours after trials began because the results of Foster *et al.* (1988) indicated that the rates of predation by adult sticklebacks are relatively high compared to those of insect predators. Pools were observed from behind mesh screens to minimize disturbances to juvenile sticklebacks. Dead or missing predators were replaced at each census, and dead fish were removed. Trials were terminated after four days in 1995, seven days in 1996 and 1998, or earlier if fewer than five juvenile individuals of one or both stickleback species remained.

Predator feeding bias was quantified using Manly's preference index:

$$\alpha = \frac{\ln(p_b)}{\ln(p_b p_l)} ,$$

where $p_b$ is the proportion of benthics surviving out of the initial ten and $p_l$ is the proportion of limnetics surviving (Manly 1974, Chesson 1978). Values for $\alpha$ range from 0 to 1, with $\alpha = 0.5$ representing even prey selection by the predator, $\alpha = 0$
representing only limnetics being consumed and $\alpha = 1$ only benthics.

*Predation experiments – pelagic arena*

Experiments designed to assess the relative predation rates of adult benthic and limnetic sticklebacks were conducted July to September 1998. The experimental arena was a large (9 m × 3 m × 1 m deep) unvegetated indoor pool. An aviary was built above the pool and two perches were provided for the birds.

Predators were lab-reared adult double crested cormorants (2.0 – 2.5 kg). Their regular diet consisted of a daily ration of four herring and 14 – 18 smelt. Three to five days before conducting a set of trials, I introduced a pair of cormorants to the experimental arena. During their acclimation, I released 4 – 5 adult benthics and 4 – 5 adult limnetics into the experimental arena prior to their daily feeding. I considered a pair of cormorants to be acclimated when they: (1) entered the water within 30 min to forage on sticklebacks after I stepped behind a blind, (2) left the water and flew to their perches after I stepped out from behind the blind and (3) repeated these behaviors two days in a row. Each pair was used for 10 replicates, and a total of three pairs were used.

Adult sticklebacks used in each trial were haphazardly selected from stock aquaria. Eight adult benthics and eight adult limnetics were added to the experimental arena in each trial. Benthics and limnetics were introduced simultaneously at the end of the arena furthest from the perch of the cormorants. After introduction, I would stand in full view of the cormorants for 5 min before stepping behind the blind. This acclimation period was chosen to minimize disturbance to the cormorants. Sticklebacks often swam the entire length of the pool more than once during this time.

The trial was terminated after approximately half of the fish were consumed. The remaining fish were then counted and identified. Preliminary observations indicated that entering the aviary to collect the surviving fish with a dip net would alter the behaviour of the birds in subsequent trials, so this method was not used in any trials. Instead, my assistant and I would count and identify remaining fish
separately from the edge of the pool, after which we could compare counts. Once detected, fish were easily identified to species because of the considerable length (Table 3.4), weight (Table 3.4), and shape (Schluter and McPhail 1992) differences that exist between adult benthics and limnetics. After we finished counting, cormorants were allowed to return to the pool to consume the remaining sticklebacks. In this way, individual sticklebacks were used for only a single trial. Cormorant feeding bias was calculated using Manly's preference index, as detailed for the littoral arena experiment.

3.3 Results

Defensive armor

Exploratory principal components analysis suggests that adult limnetics have more defensive armor than adult benthics. The first principal component (PC1), which had an eigenvalue of 3.82 and accounted for 76.44% of the total variance, represented variation in first dorsal spine length (component loading = 0.40), pelvic spine length (0.49), pelvic girdle length (0.48), and number of lateral plates (0.48) (Table 3.2). There was no overlap between benthics and limnetics, with limnetics having higher PC1 scores than benthics (Fig. 3.1), indicating that they have longer spines and girdles and more lateral plates. PC1 was the only principal component with an eigenvalue greater than one, so PC2 to PC5 (Table 3.2) are not considered further.

Analysis of the individual traits identified by the principal components analysis confirms that adult limnetics have more defensive armor than adult benthics. Limnetics had longer size-corrected pelvic spines ($t_{78} = 38.88, P < 0.001$) and girdles ($t_{78} = 20.10, P < 0.001$) than benthics (Table 3.3). Similarly, limnetics had longer size-corrected first dorsal spines than benthics ($t_{78} = 8.44, P < 0.001$) (Table 3.3). Finally, limnetics had more lateral plates than benthics ($t_{78} = 28.40, P < 0.001$) (Table 3.3).
Schooling behaviour

Benthics and limnetics differed in their distributions in the schooling trials (Fig. 3.2); limnetics spent more time near the group of 10 conspecifics than did benthics. The mean position of benthics in the aquarium was not significantly different from a random expectation ($t_{10} = 0.87, P = 0.41$). Mean limnetic position, conversely, was highly significantly different from random ($t_{10} = 10.44, P < 0.001$) and from the mean position of benthics ($t_{20} = 3.70, P = 0.001$).

Predation experiments

Limnetics tended to be more vulnerable than benthics to predators in the littoral arenas (Table 3.5). Relative survival of limnetics was significantly lower in the presence of adult benthics ($\alpha = 0.34 \pm 0.06; t_{13} = 2.81, P = 0.015$) and backswimmers ($\alpha = 0.16 \pm 0.06; t_{15} = 6.13, P < 0.001$) but not dragonfly larvae ($\alpha = 0.46 \pm 0.08; t_{11} = 0.52, P = 0.61$). The pattern of vulnerability was reversed in the open water arena (Table 3.5), with benthics being more vulnerable than limnetics to double-crested cormorants ($\alpha = 0.62 \pm 0.03; t_{28} = 4.05, P < 0.001$).

The results from the two experiments support the prediction that a survival trade-off is associated with adaptations to predators from the two habitats. Illustrating this relationship with a fitness set (Levins 1962) reveals a negative correlation between performance in the two experiments (Fig. 3.3): each species is more vulnerable to predators from the habitat of the other species.

3.4 Discussion

An old observation in biology is the divergence of closely related species when they occur together. Competition for food has been, and continues to be, the most widely studied mechanism as a cause of behavioral and morphological differences between sympatric species (Schluter 2000b). Predation is an important ecological phenomenon, yet we remain largely ignorant of the evolutionary consequences of the interactions between coexisting species and their predators (Abrams 2000). In
this study, I documented antipredator traits of, and differential predation on, two sympatric stickleback species.

The data clearly indicate that benthic and limnetic sticklebacks in Paxton Lake differ in defensive armor. Limnetics had longer mean size-corrected pelvic girdles, pelvic spines and first dorsal spine and more lateral plates. Benthics, in contrast, tended to lack these structures. When present in benthics, the girdle and spines were reduced in size. Although a number of studies have documented a correlation between predation pressure and defensive armor among populations from different lakes, this is only the second study to document significant differences between individuals associated with different habitats within the same lake. Reimchen (1980) documented an association between number of lateral plates and habitat use in a solitary population polymorphic for number of dorsal and pelvic spines, with individuals having higher spine number tending to be found in the pelagic zone and individuals with fewer spines being found in the littoral zone.

The defensive armor of benthics and limnetics suggests that they have adapted to different predators. Limnetics most closely resemble individuals from typical solitary populations. Individuals from these populations tend to have six to seven lateral plates, robust girdles and long spines (Bell et al. 1993, Reimchen 1994, Walker 1997). Benthics, in contrast, most closely resemble solitary populations in lakes where fish predators are uncommon or absent and invertebrate predators are common. Sticklebacks in these lakes tend to have reduced spines, both in number and length, and often fewer than five lateral plates (Hagen and Gilbertson 1972, Reimchen 1980). The reduction in the defensive armor of benthics is not likely due to low calcium levels, as has been suggested for some cases of armor reduction (Giles 1983), because of the levels of defensive armor observed in limnetics and the presence of calcium-rich marl deposits in Paxton Lake (Larson 1976; S. Vamosi, unpublished observations). A comparison of benthics and limnetics from lakes in independent drainages reveals a similar, although less marked, reduction of the defensive armor of benthics (see Chapter Two), implying that the divergence is adaptive.
Benthics and limnetics also differ in antipredator behavioral traits. Limnetics displayed a stronger tendency than benthics to associate with a larger group of conspecifics. Aggregating with conspecifics has been associated with animals living in open habitats (Bertram 1978, Pitcher 1986) and avoidance of conspecifics with those living in structurally complex habitats (Sih 1987, Lima and Dill 1990, Rangeley and Kramer 1998). Limnetics may school to dilute predation risk and to increase vigilance. In contrast, benthics may remain solitary to avoid detection by ambush predators. In a previous study of antipredator behaviour, Law and Blake (1996) demonstrated that the two species differed in the direction of their fast-start escape response. When startled, benthics moved their centre of mass primarily in a horizontal plane and their path of escape tended to be in a downward direction. Limnetics, in contrast, moved their bodies in three dimensions after being startled and had a variable escape path. These differences were interpreted as adaptations to their different habitats, with benthics using downward pitches to dart into vegetation or the substrate and limnetics using less predictable escape responses to confuse predators in open waters (Law and Blake 1996).

The results of the predation experiments were consistent with the prediction of predator-mediated survival trade-offs across habitats. Both species tended to have higher relative survival when in their preferred habitat. These survival trade-offs are in addition to the ones in foraging efficiency and growth associated with divergence in foraging traits (Schluter 1993, 1995). Schluter (1995) argued that the sharp trade-off in foraging performance and growth rates may be responsible for the diversification across the two habitats. Habitat-specific predation may, thus, "sharpen" the adaptive peaks and contribute to the observed divergence between benthic and limnetic sticklebacks.

What traits contributed to the differential predation observed across habitats and predators? Body size differences are likely an important factor contributing to differential predation. Smaller individuals tend to be most susceptible to adult sticklebacks (Foster et al. 1988) and invertebrate predators (Hay 1974, Foster et al. 1988), with the possible exception of dragonfly larvae (Foster et al. 1988), which
would result in higher predation rates on limnetics in the littoral experiments. Diving birds, such as cormorants and loons, tend to prefer larger sticklebacks (≥ 50 mm; Reimchen 1995). Adult benthics in predation trials tended to be larger than 50 mm whereas limnetics tended to be smaller than 50 mm (Table 3.4). Other traits may have also contributed to the observed pattern. The presence of well developed defensive armor may have contributed to the vulnerability of limnetics to backswimmers (Reimchen 1980, Reist 1980). Reimchen (1980) suggested that invertebrates, such as backswimmers, which grapple their prey may be better able to capture sticklebacks with defensive armor than those lacking such structures. Finally, limnetics often formed tight schools in the pelagic arena (S. Vamosi, unpublished observations). Rangeley and Kramer (1998) suggested that fish form tight schools as an adaptive response to the presence of avian piscivores.

A small, but growing, number of empirical studies suggest that differential predation may aid in the divergence of sister species. Kruuk and Gilchrist (1997) studied the role of predation in the differentiation of two hybridizing taxa of *Bombina*. Fire-bellied toads (*B. bombina*) tended to be found in semi-permanent ponds which had higher predator densities than the temporary puddles used by yellow-bellied toads (*Bombina variegata*). Yellow-bellied toads were more active, in the presence and absence of predators, and suffered higher mortality than fire-bellied toads when presented with predators characteristic of *B. bombina* habitats. Chiba (1999) reported a case of habitat segregation between land snails (*Mandarina* spp.) when in sympatry. Interestingly, this habitat divergence was accompanied by genetically based differences in shell colour. Species in wet and sheltered sites had dark shells, whereas species in dry and exposed sites were brighter and often polymorphic. It remains to be investigated whether differential predation by birds, which use visual cues when foraging, is responsible for the divergence in colour patterns.

In conclusion, benthic and limnetic sticklebacks differ not only in trophic traits, but also in defensive armor and antipredator behaviors. These traits appear to be adaptations to different habitat-specific predation regimes and are
accompanied by survival trade-offs across habitats. What is not clear at this time is whether the habitat divergence was initiated in response to competition for food with subsequent adaptation to different predators or in response to shared predators with subsequent adaptation to different prey. The stickleback species pairs may prove to be an ideal system for determining the relative contributions of competition and predation to divergence of sympatric species.
Table 3.1  Predators observed at Paxton Lake (1994 – 1997). Invertebrate predators in the littoral zone were collected with minnow traps or sweep nets; their absence from the pelagic zone was determined by snorkelling along transect lines. Observations of the other predators were made as follows: birds: spotting scope; snakes: from shore or boat; trout: from boat. With the exception of determining that a single pair of adult loons was present during the summer, reliable population estimates for predator populations are not available.

<table>
<thead>
<tr>
<th>Invertebrate</th>
<th>Littoral Zone</th>
<th>Pelagic Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>back swimmer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>belastomatid bug (<em>Bela</em>stoma sp.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dragonfly larvae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>water scorpion (<em>Ranatra</em> sp.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>belted kingfisher (<em>Ceryle alcyon</em>)</td>
<td>common loon</td>
<td></td>
</tr>
<tr>
<td>cutthroat trout</td>
<td>cutthroat trout</td>
<td></td>
</tr>
<tr>
<td>garter snake (<em>Thamnophis</em> sp.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>great blue heron (<em>Ardea herodias</em>)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2 Principal components analysis of the defensive armor of adult benthics and limnetics. Numbers represent loadings of traits on principal component axes.

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>first dorsal spine length</td>
<td>0.402</td>
<td>-0.375</td>
<td>0.834</td>
<td>0.031</td>
<td>-0.036</td>
</tr>
<tr>
<td>second dorsal spine length</td>
<td>0.360</td>
<td>0.898</td>
<td>0.234</td>
<td>0.022</td>
<td>0.098</td>
</tr>
<tr>
<td>pelvic spine length</td>
<td>0.492</td>
<td>-0.035</td>
<td>-0.293</td>
<td>0.123</td>
<td>-0.810</td>
</tr>
<tr>
<td>pelvic girdle length</td>
<td>0.482</td>
<td>-0.182</td>
<td>-0.315</td>
<td>0.615</td>
<td>0.508</td>
</tr>
<tr>
<td>number of lateral plates</td>
<td>0.484</td>
<td>-0.138</td>
<td>-0.255</td>
<td>-0.778</td>
<td>0.275</td>
</tr>
<tr>
<td>% of total variance</td>
<td>76.44</td>
<td>11.95</td>
<td>8.58</td>
<td>1.99</td>
<td>1.04</td>
</tr>
</tbody>
</table>
Table 3.3  Summary statistics for the five defensive armor traits of adult benthic \((n = 40)\) and limnetic \((n = 40)\) sticklebacks. Values for traits, with the exception of number of lateral plates, are adjusted to a body length of 47.5 mm.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Species</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>first dorsal spine length</td>
<td>benthic</td>
<td>0.90</td>
<td>(0 – 2.28)</td>
</tr>
<tr>
<td></td>
<td>limnetic</td>
<td>2.39</td>
<td>(1.34 – 3.09)</td>
</tr>
<tr>
<td>second dorsal spine length</td>
<td>benthic</td>
<td>2.50</td>
<td>(1.69 – 3.43)</td>
</tr>
<tr>
<td></td>
<td>limnetic</td>
<td>3.14</td>
<td>(2.16 – 4.02)</td>
</tr>
<tr>
<td>pelvic spine length</td>
<td>benthic</td>
<td>0.1</td>
<td>(0 – 3.21)</td>
</tr>
<tr>
<td></td>
<td>limnetic</td>
<td>4.52</td>
<td>(2.91 – 5.75)</td>
</tr>
<tr>
<td>pelvic girdle length</td>
<td>benthic</td>
<td>0.69</td>
<td>(0 – 4.97)</td>
</tr>
<tr>
<td></td>
<td>limnetic</td>
<td>7.87</td>
<td>(6.13 – 9.49)</td>
</tr>
<tr>
<td>number of lateral plates</td>
<td>benthic</td>
<td>0.30</td>
<td>(0 – 3)</td>
</tr>
<tr>
<td></td>
<td>limnetic</td>
<td>5.58</td>
<td>(4 – 7)</td>
</tr>
</tbody>
</table>
Table 3.4 Mean (± 1 SE) standard length and mass (for pelagic experiment only) of benthics and limnetics used in predation experiments. Standard length of fish reported for the littoral experiment based on individuals retrieved from control pools only, whereas standard length and mass of all fish in the pelagic experiment were recorded before their introduction to the pool.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Species</th>
<th>SL (mm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littoral</td>
<td>Benthic</td>
<td>11.86 ± 0.28</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Limnetic</td>
<td>11.26 ± 0.30</td>
<td>—</td>
</tr>
<tr>
<td>Pelagic</td>
<td>Benthic</td>
<td>55.80 ± 0.31</td>
<td>2.54 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Limnetic</td>
<td>48.13 ± 0.19</td>
<td>1.42 ± 0.02</td>
</tr>
</tbody>
</table>
Table 3.5 Selective predation on benthic and limnetic sticklebacks. Values given for Manly's index are means (± 1 SE) of trials for each predator. \( N \) is the number of replicates for each predator.

<table>
<thead>
<tr>
<th>Arena</th>
<th>( n )</th>
<th>Predator</th>
<th>Manly's index (( \alpha ))</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littoral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>adult benthic</td>
<td>0.34 ± 0.06</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>backswimmer</td>
<td>0.16 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>dragonfly larva</td>
<td>0.46 ± 0.08</td>
<td>0.61</td>
</tr>
<tr>
<td>Pelagic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>cormorant</td>
<td>0.62 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: \( \alpha > 0.5 \): higher relative survival of limnetics; \( \alpha < 0.5 \): higher relative survival of benthics
Fig. 3.1 Plot of the relationship between standard length and PC1 in adult benthic and limnetic sticklebacks. Symbols indicate individuals. PC1 accounted for 76.44% of the total morphological variance. $n = 40$ for each species.
Fig 3.2 Mean position of adult benthic and limnetic sticklebacks in the schooling behavior experiment. A value of 1 represents the focal fish being beside the single individual during all observations, a value of 10 beside the group of 10 individuals, and 5.5 (dashed line) is the random expectation. $n = 11$ for both species.
Fig. 3.3 Trade-off in survival between habitats. Symbols indicate means (± 1 SE) for benthics and limnetics. Survival in littoral arenas is an average across the three predators (adult benthics, back swimmers, and dragonfly larvae).
Chapter Four

IMPACTS OF TROUT PREDATION ON FITNESS OF SYMPATRIC STICKLEBACKS AND THEIR HYBRIDS
Adaptive radiations are characterized by the "more or less simultaneous divergence of numerous lines all from much the same ancestral adaptive type into different, also diverging adaptive zones" (Simpson 1953). Adaptive zones can be thought of as divergent ways of obtaining food, avoiding encounters with predators, or reducing the virulence of parasites. The idea that interspecific competition between consumer species can promote morphological divergence, and subsequent speciation, in adaptive radiation has been addressed by numerous theories (Slatkin 1980; Milligan 1985; Taper and Case 1985; Abrams 1987a,b) and empirical studies (Brown and Wilson 1956; Grant 1975; Schluter et al. 1985; Schluter and McPhail 1992).

Predation may be critical to the origin and maintenance of divergence in sympatry, yet it has received less attention than competition (Schluter 2000b). When considered, it has primarily been viewed as an interaction that may alleviate the effects of competition (Paine 1966, Werner 1991). However, predation may influence divergence via a number of other mechanisms. First, if two or more consumer species share a predator, they may diverge as a result of "competition for enemy-free space" (Ricklefs and O'Rourke 1975, Jeffries and Lawton 1984, Holt and Lawton 1994, Abrams 2000). Second, predation may favor further divergence after character displacement stemming from competition for food (Schluter 1994) if they encounter different suites of predators in the new habitats. Predation may even facilitate character displacement by creating steeper fitness trade-offs between habitats. Finally, hybrids between two consumer species may suffer higher levels of predation if their intermediate defences are maladaptive in the habitats of their parents. This would result in disruptive selection that would also facilitate divergence.

In this paper we study some of the effects of a major predator, cutthroat trout.
(Oncorhynchus clarki), on the survival and growth of two species of sympatric sticklebacks and their first generation (F₁) hybrids. This study was prompted by two considerations. First, a recent analysis indicates that cutthroat trout are present in all lakes containing species pairs of benthic and limnetic sticklebacks (Chapter One; see also Schluter and McPhail 1992). Interactions between cutthroat trout and sympatric sticklebacks, however, have not been addressed to date. This predator occurs in both littoral and pelagic habitats (Nilsson and Northcote 1981, Reimchen 1994) and potentially preys on both species. The well-developed defensive armour of limnetics (Chapter Three) and their exclusion from the littoral zone, a habitat which can provide protection against predators for small fish (Mittelbach 1984), suggest they have higher encounter rates with cutthroat trout. For this reason, we predict similar survival of the parent species in the absence of predators and a reduction in limnetic survival in the presence of cutthroat trout. The alternative hypothesis is that the strategies adopted by limnetics and benthics (defensive armor and habitat refuge, respectively) are equally effective and the presence of trout will not affect the relative survival of the parent species. Growth rate of benthics is predicted to be higher than limnetics, and this difference is expected to increase in the presence of cutthroat trout.

Second, F₁ hybrids are found in low frequencies in lakes with benthic and limnetic species (McPhail 1992). A component of reproductive isolation between sympatric species itself may involve ecological selection pressures against hybrids (McMillan et al. 1997, Hatfield and Schluter 1999, Vamosi and Schluter 1999, Vamosi et al. 2000) and predation may play a part. The morphology and behaviour of the parent species may represent adaptive peaks between which hybrids may be more susceptible to predators, herbivores, and parasites (Fryer 1959, Whitham 1989). The empirical evidence, however, has been mixed on this issue. The view that hybrids are more susceptible to attack than their parent species has received support from a number of studies (Floate et al. 1993, Hjalten 1998, Cummings et al. 1999). However, other studies have found that hybrid fitness was not different from the mean fitness of the parent species (Aguilar and Boecklen 1992) or was
similar to the fitness of the more susceptible parent species (Gange 1995, Hjalten 1998, Hjalten et al. 2000). Hybrid survival is predicted to be lower than the parent species in the absence of cutthroat trout and further reduced in their presence. Hybrid growth rate is predicted to be intermediate in the absence of cutthroat trout and reduced in their presence.

4.2 MATERIALS AND METHODS

*Sympatric sticklebacks*

Benthic and limnetic species of stickleback are found in Paxton Lake (49°42', 124°31'), British Columbia, Canada. Paxton Lake is a small lake, with a surface area of 17 ha and a mean depth of 6 m. The species are named for their morphological and habitat use differences: benthics are larger, deeper bodied fish that forage on invertebrates associated with vegetation and substrate, whereas limnetics are smaller, streamlined fish that forage on zooplankton in the open water zone (Larson 1976, McPhail 1992). First generation hybrids tend to have trophic morphologies intermediate to those of benthics and limnetics, resulting in intermediate growth rates and the consumption of both benthos and zooplankton in experimental ponds (Vamosi et al. 2000). There is evidence that premating isolation is based on habitat segregation (Hatfield and Schluter 1996, Vamosi and Schluter 1998) and body size differences (Nagel and Schluter 1998). Postmating isolation exists in the form of ecological selection (Hatfield and Schluter 1999, Vamosi et al. 2000) and sexual selection (Vamosi and Schluter 1998) against $F_1$ hybrids. Consequently, benthics and limnetics from Paxton Lake are reproductively isolated and are considered good biological species.

In addition to their differences in trophic morphology, limnetics have more defensive armour than benthics (Table 4.1). Limnetics have more lateral plates and tend to have all three dorsal spines and a pelvic girdle. In Paxton Lake, benthics have fewer plates and tend to have two dorsal spines and no pelvic girdle. First generation hybrids have an intermediate number of plates and more closely
resemble limnetics for number of spines and presence of the pelvic girdle (Table 4.1).

Wild benthic and limnetic individuals in reproductive condition were used as parents to make crosses. We made approximately 50 benthic, 35 limnetic, and 80 reciprocal F₁ hybrid crosses for the experimental ponds. To supplement numbers of limnetics, owing to low trapping success and clutch sizes of limnetic females, we collected eggs from nests guarded by wild limnetic males. For the same reasons, F₁ juveniles are ~90% (B♀ × L♂) and ~10% (L♀ × B♂). The morphology, hatching success and growth rates of the reciprocal hybrid crosses do not differ in the laboratory (Hatfield and Schluter 1999). We made crosses in May by stripping eggs from gravid females into Petri dishes using gentle abdominal pressure and adding water and macerated testes from males. A single male was used per five female clutches. After two hours had elapsed, fertilized eggs were transferred to plastic hatching containers (175 mL) with mesh bottoms suspended in 100 L aquaria above air stones. The air stones provide oxygen to the eggs through the mesh. Eggs that became infected with fungus were removed daily. After hatching, fish were transferred from the hatching containers to the aquaria.

Fish were fed with infusoria cultures for the first two days, then switched to diets consisting of live Artemia nauplii fed once or twice daily. Fish were reared for four weeks in aquaria before introducing them to the ponds.

Cutthroat trout

Cutthroat trout are native to all lakes with both benthic and limnetic sticklebacks (Schluter and McPhail 1992; Chapter One). Cutthroat trout in small lakes forage in both littoral and pelagic zones (Nilsson and Northcote 1981). Cutthroat trout larger than 100 mm standard length (SL) are known to forage on sticklebacks (Reimchen 1990) and piscivory can reach high levels in trout larger than 180 mm (Moodie 1972). Sticklebacks of all size classes (10 to 80 mm) have been found in the diets of cutthroat trout (Moodie 1972, Reimchen 1990).

We obtained cutthroat trout (range in SL 170 – 250 mm) by angling in Placid
Lake (49° 19’, 122° 34’), a small (1.6 ha) lake in the UBC Research Forest in June and August 1997. Trout were held in flow-through holding tanks for two to five days prior to their introduction to experimental ponds. Sticklebacks do not occur in Placid Lake, and the diet of cutthroat trout there consists of insects gleaned from the surface, Chaoborus and other large zooplankton from deeper offshore areas, and larval dragonflies, mayflies, and other invertebrates from the littoral zone (Nilsson and Northcote 1981; S. Vamosi, unpublished observations). Nevertheless, cutthroat trout readily consumed sticklebacks in holding tanks, and analyses of stomach contents of trout retrieved at the end of the experiment revealed a diet that included sticklebacks (Fig. 4.1). Logistic considerations, namely the difficulty in obtaining sufficient numbers of cutthroat trout from large lakes frequented by anglers and mortality associated with transport of live cutthroat trout from more distant lakes, encouraged the use of Placid Lake as the source for trout. The absence of sticklebacks from this lake, while preventing the coevolution of Placid Lake cutthroat trout with sticklebacks, ensures that the results of our study would not be solely the result of prior experience with a specific phenotype.

**Experimental Design**

The experiment was conducted in 1997 at the University of British Columbia experimental pond facility. We used an incomplete block design having three treatments, with two treatments per divided pond. The design was unbalanced because only five ponds, rather than six, were available for the experiment. In the Trout treatment, we introduced high densities of sticklebacks and added cutthroat trout (Table 4.2). The High density treatment, paired with the Trout treatment in two ponds (Table 4.2), was included as a control for the presence of trout. The Low density treatment, paired with the Trout treatment in two ponds (Table 4.2), was a control for the effects of lower density, hypothesized to occur as a consequence of predation by trout, on the growth of sticklebacks. We paired the Low and High density treatments in a fifth pond (Table 4.2).
**Pond Experiment**

Five ponds (23 m x 23 m, 3 m max. depth) were each divided in two with UV-resistant polyethylene plastic barriers to prevent the movement of fish. From June 20 to 22, juvenile sticklebacks were stocked to each side of every pond. Fish from each cross type were added in batches of 100 sampled haphazardly from a larger pool, which was created by combining fish from all aquaria. Batches were assigned randomly to a specific pond and side. We added 700 individuals of each cross type to randomly assigned Trout and High density pond-sides, and 300 were added to Low density pond-sides (Table 4.2).

At the time of counting fish for introduction, 100 randomly selected individuals of each cross type were preserved to provide an estimate of the starting size of individuals of each cross type. These fish were narcotized with tricaine methanesulfonate (MS-222; Syndel Laboratories, Vancouver, BC), fixed in 10% formalin for a week, stained in Alizarin red S and then preserved in 37% isopropyl alcohol. Additionally, at least 400 fish of each cross type were raised in the laboratory for the duration of the experiment. A random sample of these fish was later used to discriminate between benthics, limnetics and F$_1$ hybrids retrieved from the ponds using the methods detailed in Vamosi et al. (2000).

Three trout were added to Trout pond-sides a week after introduction of sticklebacks. Because the viability of cutthroat trout in the experimental ponds had not previously been assessed, we placed gill nets into the ponds at eight weeks to confirm that trout were still present in Trout pond-sides and absent from other pond-sides. Gill nets were removed after we had retrieved two trout from each Trout pond-side, after which we introduced two new trout from the holding tanks. Cutthroat trout were retrieved from only Trout pond-sides at this time; however, at the end of the experiment, we retrieved a single trout from the High pond-side of Pond 3 (Table 4.2). We assume it got there by leaping the barrier between pond sides, since the barrier itself was still intact and without holes. This pond was excluded from the statistical analyses, although we include the Trout pond-side in the figures for comparison.
The experiment was terminated after three months following the methods of Vamosi et al. (2000) and Pritchard and Schluter (2001). We added 0.5 kg of 5% rotenone \((C_{23}H_{22}O_6;\) Syndel Laboratories, Vancouver\) to each pond-side and retrieved fish with dip nets as they swam to the surface. Sticklebacks were narcotized with MS-222 and fixed with 10% formalin. Trout were narcotized with MS-222 before their stomachs were dissected, fixed with 10% formalin for at least a week and transferred to 37% isopropyl alcohol. The following day, we searched the bottom of each pond-side for dead sticklebacks overlooked the first day. These fish were also fixed with formalin but were used only to calculate the total number of fish retrieved from each pond-side. Fish were removed from formalin after a week, stained with Alizarin red S and preserved in 37% isopropyl alcohol. All fish recovered from ponds were counted and absolute survival and growth rates of the cross types were determined in a random sample of 100 fish from each pond-side.

Statistical Analyses

We analyzed results from the incomplete block design (blocks = ponds) using multiple regression. This is the recommended approach when designs are unbalanced (Hicks 1964, Lindman 1992) as in the present experiment. With this analysis, the effect of treatment is calculated simultaneously with block effects. The significance of treatment effects is tested after block effects are accounted for. The whole pond-side, rather than the individual fish, was the unit of replication. For this reason the interaction between block and treatment was not included in the regression model.

Because all three cross types were stocked together, their separate survival and growth rates are not independent (Werner and McPeek 1994). Thus, we analyzed the mean response of each type separately or we analyzed differences between the mean of types in their responses. We were interested in two differences: that between mean benthic and limnetic responses; and that between mean hybrid and the mean of the parental species responses. Survival is the number of individuals retrieved from a pond-side divided by the number introduced.
at the start of the experiment. Survival proportions were arcsin transformed prior to analysis. Growth rates were calculated as: growth rate (mm·day\(^{-1}\)) = \((L_e - L_s)/D\), where \(L_e\) is the standard length of an individual at the end of the experiment, \(L_s\) is the mean standard length of its cross type at the start of the experiment, and \(D\) is the duration of the experiment in days (\(D = 45\) days). Growth rates were In-transformed prior to analysis.

4.3 RESULTS

**Limnetics and Benthics**

Survival of benthics and limnetics was highly variable among replicates and did not vary significantly with treatment (Table 4.3; Fig. 4.2). There was, however, a significant treatment effect on the difference between benthic and limnetic survival (Table 4.3). Tukey's HSD test revealed significant differences between Trout and Low density treatments and between Trout and High density treatments. The relative survival of limnetics was higher than benthics in the absence of trout and lower in their presence (Fig. 4.3). Pond effects were absent in all three cases (Table 4.3). We wished to determine which of the two species was most affected by the addition of trout, thus contributing to the observed pattern in relative survival. To do this, we analyzed benthic and limnetic survival using a one way ANOVA, ignoring block effects. The results of this post hoc analysis suggest that limnetics (\(F_{1,7} = 5.85, P = 0.05\)) contributed more strongly to the observed pattern in relative survival (Fig. 4.3) than did benthics (\(F_{1,7} = 0.13, P = 0.73\)) (also see Fig. 4.2).

We detected a significant treatment effect on growth of both benthics and limnetics (Table 4.4; Fig. 4.4). Growth rates of both species were higher in the Low density treatment than in Trout and High density treatments (Tukey's HSD test). There was no effect of treatment on the difference in mean growth between benthics and limnetics (Table 4.4). Significant pond effects were lacking in all three cases (Table 4.4).
First generation hybrids survived poorly overall (Fig. 4.2). Their survival was significantly below the average survival of limnetics and benthics (paired t-test; $t_7 = 5.56, P < 0.001$). First generation hybrids constituted $23.7\% \pm 1.5$ of the fish retrieved at the end of the experiment, which is $9.6\%$ lower than their frequency at introduction. However, there was no treatment effect on the survival of $F_1$ hybrids (Table 4.5). Neither was there a treatment effect on the difference between the survival of $F_1$ hybrids and the mean of benthic and limnetic survival (Table 4.5). Adding trout did not reduce relative $F_1$ hybrid survival below the low value already present in the absence of trout. No significant pond effects were detected on hybrid survival (Table 4.5).

We detected a highly significant effect of treatment on the growth of $F_1$ hybrids (Table 4.6; Fig. 4.4). Tukey’s HSD test revealed that their growth rates were higher in the Low density treatment than in the Trout and High density treatments. There was also a significant pond effect on $F_1$ hybrid growth (Table 4.6). There was neither an effect of treatment nor of pond on the difference between hybrid growth rates and the mean of benthic and limnetic growth rates (Table 4.6).

### 4.4 Discussion

Benthics and limnetics are sympatric species that are divergent in trophic morphology, defensive morphology, and habitat use (Larson 1984; McPhail 1992, 1994). Previous studies have demonstrated that divergence in trophic morphology has been accompanied by adaptation to resources in their preferred habitat and, furthermore, by a reduction in the performance of $F_1$ hybrids (Schluter 1993, 1995; Hatfield and Schluter 1999). Surveys of the fish communities have revealed that cutthroat trout, and no other fish species, occur in all lakes that contain sympatric stickleback species (Schluter and McPhail 1992; Chapter One). In this experiment we measured the survival and growth of benthics, limnetics, and their $F_1$ hybrids in the presence and absence of trout.
The two parent species were differentially affected by the presence of trout. Limnetics survived better than benthics in the absence of trout but worse in their presence. Limnetics are predicted to be more vulnerable to gape-limited predators, such as cutthroat trout, because of their smaller body size and narrower body depth (e.g., Brönmark and Miner 1992). The vulnerability of limnetics based on their morphology may have been enhanced by their greater use of the open water habitat, inferred from analyses that revealed a diet high in zooplankton even in Trout pond-sides (S. Vamosi, unpublished observations). Use of the open water habitat in the ponds would result in higher encounter rates with cutthroat trout. Two factors may reduce predation of limnetics by cutthroat trout in the wild compared to the experimental ponds. First, schooling with conspecifics in surface waters far from shore may provide some protection. The size of the experimental ponds, especially when divided as in the current experiment, likely reduced the ability of limnetics to use this behavioral defence against predatory fish. Second, the presence of diving birds, such as common loons and double crested cormorants (Phalacrocorax auritus), may restrict the habitat use of small and medium sized cutthroat trout. Although these birds do forage on sticklebacks (Reimchen 1995) they prefer larger fish, such as cutthroat trout (Schullery and Varley 1995). Diving birds were not observed in the ponds during the experiment.

Survival of F₁ hybrids was low overall but, contrary to our predictions, not significantly affected by the presence of trout. Reproductive isolation between sympatric benthics and limnetics does not appear to be strengthened by increased trout predation on F₁ hybrids. Despite the lack of a treatment effect on hybrid survival, ecological factors are nevertheless implicated in their poor performance. Hybrids may suffer because they do not have a unique feeding niche (Vamosi et al. 2000; S. Vamosi, unpublished observations) and their foraging efficiency on benthic and planktonic prey is reduced compared with benthics and limnetics, respectively (Schluter 1993). However, their growth rates, which are expected to reflect foraging success, were not depressed below that expected from the mean of the parent species.
Alternately, invertebrate predators (e.g., backswimmers, dragonfly larvae, and dytiscid beetles), which are present in the ponds and are important predators of young-of-the-year sticklebacks in the wild (Reimchen 1980, Reist 1980, Foster et al. 1988), may have reduced hybrid survival. These invertebrates are found in shallow, vegetated waters where hybrids and benthics concentrate their foraging activities in these ponds, as inferred from gut content analyses (Vamosi et al. 2000; S. Vamosi, unpublished observations). Evidence from other studies, which demonstrates selective predation by invertebrate predators on smaller (Hay 1974) and (or) more armored (Ziuganov and Zotin 1995) sticklebacks, suggests that hybrids may be more susceptible than benthics to invertebrate predators.

Generalizations have yet to emerge on the effects of predation on the relative fitness of parent species and their hybrids. This is partially due to the paucity of studies considering predation on hybrids and partially to the diversity of results obtained in the few studies conducted to date. Semlitsch (1993), for example, measured the survival of hybridogenetic Rana esculenta and one of the parent species (R. lessonae) and demonstrated higher survival of the hybrid in the presence of dragonfly larvae and no difference between the two taxa in the presence of fish and newt predators. Spaak and Hoekstra (1997) showed that Daphnia cucullata × D. galeata hybrids were less vulnerable to fish predation than D. galeata but more vulnerable than D. cucullata. Finally, Wahl and Stein (1989) demonstrated that Esox masquinongy × E. lucius hybrids were more vulnerable to predation by largemouth bass (Micropterus salmoides) than either of the parent species.

In conclusion, we demonstrate that the presence of a major predator altered the relative survival of sympatric species but had little effect on the survival of their F₁ hybrids, which was low throughout. Studies of the effects of predation on the fitness of sympatric species and their hybrids are in their infancy, but promise to yield important insights into the processes which initiate and maintain divergence. Postglacial fishes, which frequently partition their habitats (Smith and Skúlason 1996) and hybridize when in sympathy (Schwartz 1972), are particularly suited to such investigations.
Table 4.1. Meristic comparison of benthic, limnetic and F$_1$ hybrid sticklebacks. Data for dorsal spines and pelvic girdle are given as proportions of individuals. Number of lateral plates (mean ± SE) refers to counts on left side of body.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>$n$</th>
<th>2</th>
<th>3</th>
<th>No. of lateral plates</th>
<th>Pelvic girdle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benthic</td>
<td>382</td>
<td>0.678</td>
<td>0.322</td>
<td>0.64 ± 0.05</td>
<td>0.016</td>
<td>0.984</td>
<td></td>
</tr>
<tr>
<td>Hybrid</td>
<td>239</td>
<td>0.113</td>
<td>0.887</td>
<td>3.85 ± 0.06</td>
<td>0.921</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>Limnetic</td>
<td>382</td>
<td>0.005</td>
<td>0.995</td>
<td>5.22 ± 0.05</td>
<td>1.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 Design of the pond experiments, showing the treatments and the number of sticklebacks retrieved from the pond replicates. The total number of sticklebacks introduced was 900 in Low replicates and 2100 for High and Trout replicates. The ratio of benthic to $F_1$ hybrid to limnetic sticklebacks at introduction was 1:1:1 in all replicates.

<table>
<thead>
<tr>
<th>Pond</th>
<th>Low Density</th>
<th>Trout</th>
<th>High Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>228</td>
<td>395</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>291</td>
<td>385</td>
<td>—</td>
</tr>
<tr>
<td>3$^*$</td>
<td>—</td>
<td>440</td>
<td>471</td>
</tr>
<tr>
<td>4</td>
<td>576</td>
<td>—</td>
<td>534</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>738</td>
<td>808</td>
</tr>
</tbody>
</table>

$^*$ Note: a trout was retrieved from the High density pond-side of Pond 3 at the end of the experiment, thus this replicate is not included in the analyses.
Table 4.3 Effects of treatment (Low density, Trout, High density) and pond (= block) on survival of benthics, survival of limnetics, and the difference between benthic and limnetic survival.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>129.84</td>
<td>6.40</td>
<td>0.14</td>
<td>180.14</td>
<td>3.52</td>
<td>0.22</td>
<td>1015.69</td>
<td>40.17</td>
<td>0.02</td>
</tr>
<tr>
<td>Pond</td>
<td>3</td>
<td>208.11</td>
<td>10.26</td>
<td>0.09</td>
<td>102.38</td>
<td>2.00</td>
<td>0.35</td>
<td>106.94</td>
<td>4.23</td>
<td>0.20</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>20.29</td>
<td>—</td>
<td>—</td>
<td>51.13</td>
<td>—</td>
<td>—</td>
<td>25.29</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 4.4 Effects of treatment (Low density, Trout, High density) and pond (= block) on growth rate of benthics, growth rate of limnetics, and the difference between benthic and limnetic growth rates.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.00131</td>
<td>38.51</td>
<td>0.03</td>
<td>0.00110</td>
<td>54.95</td>
<td>0.02</td>
<td>0.00006</td>
<td>0.39</td>
<td>0.72</td>
</tr>
<tr>
<td>Pond</td>
<td>3</td>
<td>0.00011</td>
<td>3.70</td>
<td>0.22</td>
<td>0.00016</td>
<td>8.14</td>
<td>0.11</td>
<td>0.00004</td>
<td>0.28</td>
<td>0.84</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.00003</td>
<td>—</td>
<td>—</td>
<td>0.00002</td>
<td>—</td>
<td>—</td>
<td>0.00016</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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Table 4.5 Effects of treatment (Low density, Trout, High density) and pond (= block) on survival of hybrids and the difference between the survival of hybrids and the mean survival of parent species.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>148.64</td>
<td>2.94</td>
<td>0.25</td>
<td>0.51</td>
<td>0.11</td>
<td>0.90</td>
</tr>
<tr>
<td>Pond</td>
<td>3</td>
<td>63.17</td>
<td>1.25</td>
<td>0.47</td>
<td>65.63</td>
<td>14.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>50.64</td>
<td>—</td>
<td>—</td>
<td>4.63</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 4.6 Effects of treatment (Low density, Trout, High density) and pond (= block) on growth rate of hybrids and the difference between growth rates of hybrids and the mean growth rate of parent species.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.001559</td>
<td>519.77</td>
<td>0.002</td>
<td>0.000008</td>
<td>0.54</td>
<td>0.65</td>
</tr>
<tr>
<td>Pond</td>
<td>3</td>
<td>0.000069</td>
<td>22.89</td>
<td>0.04</td>
<td>0.000076</td>
<td>5.41</td>
<td>0.16</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.000003</td>
<td>—</td>
<td>—</td>
<td>0.000014</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Fig. 4.1 Diet of cutthroat trout (n = 12) in ponds. Vertical axis indicates percentage of guts containing the item. Prey categories: sticklebacks (G), chironomid larvae (C), dragonfly larvae (A), Daphnia (D), terrestrial insects gleaned from surface (T).
Fig. 4.2 Survival of benthic, F₁ hybrid, and limnetic sticklebacks in ponds. The dashed line indicates the overall mean survival (all cross types combined). Symbols indicate individual ponds.
Fig. 4.3 Difference in the absolute survival of benthic and limnetic sticklebacks in ponds. Relative survival of benthics was higher in Trout pond-sides (filled symbols) whereas relative survival of limnetics was higher in Low and High density pond-sides (open symbols). Symbols indicate individual ponds, as in Fig. 2.
Fig 4.4 Mean (± 1 SE) growth rates of benthic, F$_1$ hybrid, and limnetic sticklebacks in ponds. Symbols indicate individual ponds, as in Fig. 4.2.
GENERAL DISCUSSION

In Chapter One, I examined the association between predators and the presence of sympatric stickleback species pairs. I compared the physical characteristics and number of other fish species of lakes containing sympatric species with those lakes with only a solitary stickleback population. Sympatric lakes were poorly discriminated from solitary lakes when only physical characteristics were considered, but well discriminated when the number of other fish species was also considered. When other fish species were considered, one striking observation emerged: benthics and limnetics were found only in lakes having exactly one other fish species, the cutthroat trout. Furthermore, the analysis revealed that such lakes were relatively rare and the incidence of diversification under the right conditions was high, with benthics and limnetics being found in six of these nine lakes. Other lakes in this region typically had greater numbers of other fish species, suggesting that interactions with predatory and (or) competing fish species have prevented the origin or persistence of the species pairs in these lakes.

In Chapter Two, I investigated patterns of defensive armor in sympatric species of three-spined sticklebacks previously shown to exhibit ecological character displacement. I scored five defensive armor traits in benthic, limnetic, and solitary populations from southwestern British Columbia. Limnetics tended to have longer spines and more lateral plates than sympatric benthics. Benthics and limnetics were more divergent in length of pelvic girdle and number of lateral plates than expected, based on comparisons with solitary populations. Furthermore, sympatric species exhibited parallel reductions in all defensive armor traits measured, with the exception of number of lateral plates. Benthics, by living in the littoral zone or by virtue of the presence of limnetics, appear to experience a release from predation. Limnetics, in contrast, may be more susceptible to predation by piscivorous birds and fish as a consequence of living in the pelagic zone. Shifts in sympathy appear to be the result of both adaptation to habitat-specific predation

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risk and interactions through shared predators.

In Chapter Three, I tested whether adaptation to the littoral and pelagic zones by benthics and limnetics, respectively, had resulted in divergence of morphological and behavioral antipredator traits and the evolution of trade-offs in survival between the two habitats. Benthics are larger than limnetics, have poorly developed defensive armor and show no tendency to school with conspecifics. Limnetics, in contrast, are smaller, have well developed defensive armor and demonstrate strong schooling behavior. In littoral arenas, juvenile limnetics were significantly more vulnerable than juvenile benthics to predation by adult benthics and backswimmers but not dragonflies. This pattern was reversed in open water arenas when adult benthics and limnetics were exposed to diving avian predators, double-crested cormorants. Divergence in antipredator traits between benthics and limnetics has accompanied divergence in foraging traits, resulting in survival trade-offs in addition to those previously observed for foraging success and growth rates across habitats. By exposing consumer species to different habitat-specific predation risks, character displacement may be more important to the divergence of sympatric species than previously recognized.

In Chapter Four, I examined the consequences of predation for the fitness of benthics, limnetics and their F1 hybrids. Benthic sticklebacks are found in the littoral zone of lakes associated with vegetation and bare sediments, whereas limnetics spend most of their lives in the pelagic zone. In pond experiments I found that the addition of these predatory cutthroat trout primarily impacted the survival of limnetics. In contrast, benthic survival was unaffected by trout addition. The result was that relative survival of benthics and limnetics was reversed in the presence of trout. The presence of trout had no effect on the rank order of parent species growth rates, with benthics always growing faster than limnetics. F1 hybrids survived poorly relative to benthics and limnetics and their growth rates were intermediate regardless of treatment. The results suggest that the two species are differentially vulnerable to cutthroat trout, although reproductive isolation is not strengthened by increased predation by trout on F1 hybrids. I discussed other
ecological factors which may have reduced the survival of F₁ hybrids overall.

To summarize, I believe that predation has played a role in the adaptive radiation of sympatric stickleback species pairs. Species pairs are never found with a diverse fish community, but they all coexist with cutthroat trout. This suggests that speciation may have been facilitated by low levels of predation, but hampered by higher levels of predation. Sympatric benthics and limnetics displayed consistent differences in their defensive armor, presumably as a response to differential encounters with cutthroat trout, diving and wading birds, and a variety of invertebrate predators. Of the two species, benthics displayed greater reductions in their defensive armor compared to solitary populations. The differences in antipredator morphological and behavioral traits have been accompanied by adaptations to predators typically associated with their habitat and increased vulnerability to predators from the habitat of the other species. Benthics may be less vulnerable to invertebrate and fish predators as a consequence of their larger size and more sheltered habitat, although their size and reduced armor make them choice prey items for diving birds. Limnetics, in contrast appear to be more vulnerable to invertebrates and cutthroat trout and less vulnerable to diving birds. Increased predation by cutthroat trout on F₁ hybrids was not observed, although predation by invertebrates may contribute to their low survival.

Furthermore, I feel that other aspects of the adaptive radiation of sticklebacks might be better understood if predation is kept in mind. For instance, salinity tolerance experiments (Kassen et al. 1995) and molecular genetic analyses (Taylor and McPhail 2000) suggest that the first wave of marine colonists gave rise to modern day benthics and that limnetics are descendants of the second invasion. But, why should this be so? One possible explanation is that the littoral zone is a safer habitat for sticklebacks and was occupied by the descendants of the first wave following the second invasion. The longer spines and more numerous plates of limnetics (Chapter Two) certainly suggest that predation risk is higher in the pelagic zone, although detailed information on predation risk in the two habitats of sympatric lakes is currently lacking.
The most promising avenues for future research, I believe, involve studying the mechanisms that (1) prevent speciation in lakes with diverse fish communities and (2) cause evolutionary shifts in antipredator traits in sympatry. To answer the first question, the interactions between sticklebacks and fish predators other than cutthroat trout will need to be investigated. With regard to the predators likely to retard speciation of sticklebacks in this region, prickly sculpins and rainbow trout are the primary suspects at this point. Detailed behavioral observations, along with large scale enclosure or pond experiments, may shed light on why speciation does not appear to be favored in the presence of these predators. For the second question, it might be enlightening to measure whether competition for enemy-free space is more intense between individuals similar in defensive armor than those more different in defensive armor — basically, the apparent competition version of Schluter's (1994) pond experiment on interspecific competition for resources. Predation risk faced by sympatric populations should also be compared to predation risk in solitary populations. The generation times of sticklebacks and (especially) their dominant predators complicate experiments which rely on the hypothesized mechanism of evolutionary shifts because of shared predation — that of the increased recruitment of the shared predator in response to the presence of both prey species (e.g., Holt 1977). Thus, doing similar experiments with smaller, faster reproducing organisms, such as dytiscid beetles and cladocerans, may be more feasible.

Whichever study organisms are chosen, the time has come for other researchers to take McPhail's (1969) lament (see General Introduction) as a challenge. Although interspecific competition has a richer history of inquiry, sufficient models (Holt 1977, Jeffries and Lawton 1984, Brown and Vincent 1992, Abrams 2000) and potential study systems (e.g., dytiscid beetles: Juliano and Lawton 1990a,b; passion flowers: Gilbert 1975; three-spined sticklebacks: Schluter and McPhail 1992; this thesis) are available to more fully evaluate the role of predation in adaptive radiation. I hope that my work will, in some small way, contribute to our understanding and generate enthusiasm for further studies.
LITERATURE CITED


Gause, G. F. 1934. The struggle for existence. Williams & Wilkins, Baltimore.


Jaenicke, H. W., M. S. Hoffman, and M. L. Dahlberg. 1987. Food habits of


Reimchen, T. E. 1990. Size-structured mortality in a threespine stickleback (Gasterosteus aculeatus) - cutthroat trout (Oncorhynchus clarki) community. Canadian Journal of Fisheries and Aquatic Sciences 47: 1194-1205.


Reist, J. D., E. Gyselman, J. A. Babaluk, J. D. Johnson, and R. Wissink. 1995. Evidence for two morphotypes of Arctic char (Salvelinus alpinus (L.)) from Lake Hazen, Northwest Territories, Canada. Nordic Journal


Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and


Schwartz, F. J. 1972. World literature to fish hybrids with an analysis by family, species, and hybrid. Gulf Coast Research Laboratory, Ocean Springs.


Spaak, P., and J. R. Hoekstra. 1997. Fish predation on a \textit{Daphnia} hybrid species complex: a factor explaining species coexistence? Limnology and


Werner, E. E. 1991. Nonlethal effects of a predator on competitive interactions...


