The presence of other fish species affects speciation in threespine sticklebacks

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ABSTRACT

Competition for shared resources is typically invoked as the most important ecological inter-action promoting diversification and speciation, whereas the roles of abiotic variables and natural enemies have been largely neglected. To improve our understanding of the ecological factors involved in the evolution of sympatric stickleback species pairs (*Gasterosteus aculeatus*), I examined whether lakes in the same region with only a solitary population of sticklebacks had unique features not shared with lakes containing stickleback species pairs. Sixteen lakes with a solitary population of sticklebacks were identified as potential candidates for two invasions of marine ancestors. Several physical and chemical characteristics of these candidate lakes were found to be not significantly different from those of lakes with sympatric species pairs. How-ever, when the community of other fish species was considered, a striking pattern emerged: all but one of the 16 candidate lakes contained other fish species in addition to cutthroat trout (*Oncorhynchus clarki*), whereas sympatric species pairs were found only in lakes with cutthroat trout and no other fish species. The two other fish species most often encountered, prickly sculpin (*Cottus asper*) and rainbow trout (*Oncorhynchus mykiss*), are potential competitors and predators of threespine sticklebacks. The results of this survey suggest that our understanding of the role of ecology in diversification and speciation will be furthered by a consideration of interactions other than interspecific competition between focal populations.

Keywords: competition, diversification, predation, speciation, sticklebacks, sympatry.

INTRODUCTION

One of the goals of evolutionary ecology is to determine how ecological factors contribute to diversification and speciation of closely related populations. The role of competition for resources between the focal populations has been most emphasized (Rosenzweig, 1978; Schluter, 2000), although interactions with other aspects of the environment (e.g. abiotic factors, distantly related competitors, natural enemies) may also be important (Buckling and Rainey, 2002; Vamosi and Schluter, 2002). For example, the opportunity for diversifi-cation may be minimized in areas where suitable alternative prey types are scarce or absent.

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In such circumstances, the superior competitor on the shared resource may exclude the weaker competitor (Kirk, 2002; Schellhorn *et al.*, 2002), and there would be no evolutionary shifts in either species. The presence of other competitor species may prevent adaptation to alternative prey types even if these resources are apparently available to the focal species (Benkman *et al.*, 2001). Finally, interactions with natural enemies (e.g. predators) may have several consequences. Increases in predator densities in response to the presence of two consumer species may increase mortality, thereby reducing the strength of divergent natural selection (Abrams, 1986). Alternatively, the two species may not compete for resources in sympatry but diverge nonetheless as a result of 'apparent competition' if they are preyed upon by shared predators (Holt, 1977; Abrams, 2000).

Here I examine whether previously uncharacterized features of the environment have played a role in the repeated evolution of sympatric pairs of stickleback species (*Gasterosteus aculeatus* complex). Marine sticklebacks are thought to have given rise to single (hereafter, solitary) populations of sticklebacks in thousands of lakes throughout the Holarctic during post-glacial submergence periods (Bell and Andrews, 1997). In the Strait of Georgia, British Columbia, six lakes in four drainage basins contain two sympatric species (Schluter and McPhail, 1992; McPhail, 1993, 1994). Benthic and limnetic stickle-backs have divergent habitat and resource use preferences, feeding on benthos in the littoral zone and plankton in the open water zone, respectively (Larson, 1976; Schluter and McPhail, 1992; Vamosi *et al.*, 2000). Because they meet the criteria of the biological species concept (McPhail, 1984, 1992), I follow previous studies in referring to them as species. Solitary populations that inhabit similar sized lakes tend to be intermediate in their habitat and resource use (Schluter and McPhail, 1992) but not in their defensive armour (Vamosi, 2001). Where benthics and limnetics occur together, diversification is thought to be the result of ecological character displacement as a result of interspecific competition for resources.

The existence of only six sites in such a restricted geographical region suggests that, at least at some level, lakes with sympatric species pairs are unique in some feature(s). To explain the limited distribution of benthic and limnetic sticklebacks, McPhail (1993, 1994; Schluter and McPhail, 1992) proposed the double invasion scenario: lakes with two species are those that were colonized twice, about 15,000 and 13,500 years BP, by marine stickle-backs. The opportunity for two invasions, rather than the usual single invasion, is thought to have been provided by the existence of a short-lived second post-glacial submergence, which involved a 50 m change in sea level, that was restricted to this region (Mathews *et al.*, 1970). The weight of the genetic (Withler and McPhail, 1985; McPhail, 1994; Taylor and McPhail, 2000) and physiological data (Kassen *et al.*, 1995) collected thus far, in addition to the geological data, strongly supports a double invasion hypothesis (with limnetics being more recent) over other hypotheses (e.g. sympatric speciation in each lake). Although the double invasion scenario remains the best-supported explanation for the origin of the species pairs, some questions remain:

1. Are there lakes with solitary populations in the Strait of Georgia region located within the range of elevation and distance to sea characteristic of lakes with sympatric species pairs?
2. Do these lakes consistently differ from lakes with sympatric species pairs in certain physical, chemical or biological characteristics?
3. If the answer to (2) is yes, then how might these differences have prevented the origin and (or) persistence of sympatric species pairs?

If other lakes are identified by this survey, and a consistent difference is found in some characteristic(s) of these lakes, then this would suggest that a model that considers only how competition between the descendants of temporally distinct invasions by marine sticklebacks leads to diversification and speciation (e.g. Schluter and McPhail, 1992) has captured only a portion of the evolutionary dynamics. Using the double invasion scenario as a guide, I identify a number of lakes that were putatively subject to two colonization events but contain only a solitary population of sticklebacks, and show that a strong correlate of the absence of sympatric species pairs is the community of other fish found in these lakes.

MATERIALS AND METHODS

Lake surveys

Data on elevation and distance to sea were collected on the six lakes in which sympatric populations of benthics and limnetics are present (hereafter, sympatric lakes) and all lakes in the region that are known to contain solitary populations of sticklebacks. Data were gathered from several published sources (Facchin and King, 1980, 1981; Lavin and McPhail, 1985; Foster and Ploch, 1990; Balkwill, 1991; McPhail, 1993) and Fish Wizard (http://www.fishwizard.com; an on-line database containing bathymetric maps and information on fish distributions in British Columbia). The region, which includes the east coast of southern Vancouver Island, the Gulf Islands and the Sechelt Peninsula (Fig. 1), was chosen based on Mathews and colleagues' (1970) estimate of the coastal lands subject to the second post-glacial submergence.

Elevation and distance to sea of sympatric lakes were used as surrogate measures of 'opportunity for double invasion'. A subset of lakes with a solitary population of sticklebacks (hereafter, candidate lakes) were chosen that had elevations between 50 and 100 m and, secondarily, were ≤5000 m from the sea. These values were chosen based on the values for sympatric lakes (Table 1). The benthics and limnetics in Emily Lake (23 m elevation) appear to be the result of downstream migration from Priest Lake (Taylor and McPhail, 2000); thus, I follow McPhail (1993) in considering 50 m as the lower limit. An alternative method, using a multivariate discriminant analysis (see below for details), did not differ significantly in the lakes identified (Vamosi, 2001).

I then scored several variables that measure the 'opportunity for persistence and divergence' in sympatric and candidate lakes: surface area, relative littoral area, perimeter, mean depth, Secchi depth, pH and presence of other native fish species. These variables were considered for two reasons. First, data for these variables (other than pH and Secchi depth) were readily available for most lakes. Second, these variables are expected to have important consequences for the ability of two morphologically distinct species to co-exist in a lake. Limnetic sticklebacks, for example, would probably not persist in a lake that had a relative littoral area score of 100% (i.e. no open water zone), which is expected to favour an abundance of littoral prey and a scarcity of zooplankton. Surface area, perimeter and mean depth were considered because Schluter and McPhail (1992, p. 89) commented that 'the lakes [with benthics and limnetics] are small' and I wished to determine whether they were unique in this feature. Schluter and McPhail also stated that 'one other fish species is present in these lakes, the indigenous cutthroat trout'; as with lake size, I wished to determine how rare this condition was in this region. Secchi depth is a measure of the trophic
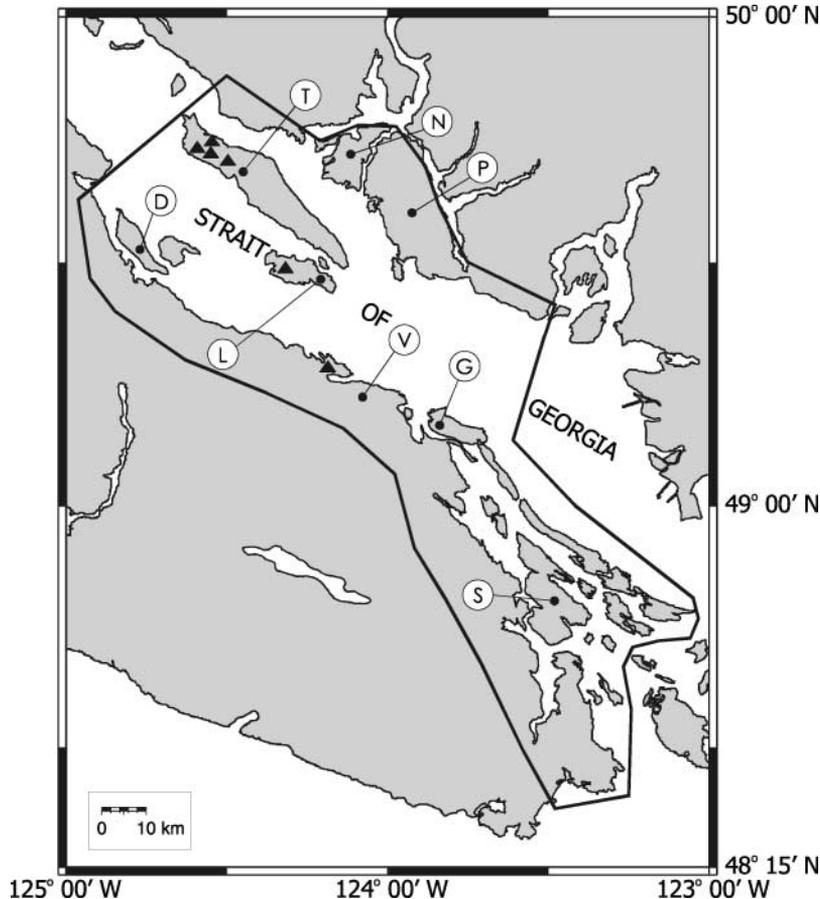
Fig. 1. Map of the study area. Survey area delimited by thick solid line. Sympatric lakes (*n* = 6) are indicated by triangles. Sites, and number of solitary stickleback populations, are: Denman Island (D; 2), Gabriola Island (G; 1), Lasqueti Island (L; 1), Nelson Island (N; 2), Saltspring Island (S; 7), Sechelt Peninsula (P; 13), Texada Island (T; 1) and Vancouver Island (V; 20).

status, or productivity, of a lake and may be correlated with encounter rates between sticklebacks and piscivorous fish. Finally, pH may indicate the solubility and availability of dissolved calcium for the defensive armour of sticklebacks.

Data for these variables were obtained by referring to the sources listed above for elevation and distance to sea. Character states of variables other than pH and Secchi depth for sympatric and candidate lakes are given in Table 1. For some lakes, I was able to confirm the presence (absence) of fish species (especially prickly sculpin) with the use of minnow traps. Introduced exotic species [i.e. 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 analyses. My conclusions were not affected when they were included. Data other than number of other fish species were log-transformed (base 10) before analysis.

To determine whether candidate lakes could be discriminated from sympatric lakes, I used DISTCVA (Anderson, 2000; see Acknowledgments), a computer program that

Table 1. Characteristics of coastal British Columbia sympatric and candidate lakes

Lake	Elevation (m)	Distance to sea (m)	Surface area (ha)	RLA (%)	Perimeter (m)	Mean depth (m)	CT	RB	CAS	CO	KO	NSC	PCC
Sympatric lakes													
Balkwill	61	5000	11.5	49.6	2268	6.3	✓						
Emily	23	3150	7.2	—	1091	3.0	✓						
Enos	55	1500	17.6	61.8	1440	4.0	✓						
Hadley	50	1330	6.7	—	1130	3.5	✓						
Paxton	88	3840	17.0	53.5	3500	6.2	✓						
Priest	75	4720	44.3	58.5	4400	5.4	✓						
Candidate lakes													
Ambrose	56	940	29.8	40.7	3200	13.3	✓		✓				
Brannen	78	4160	108.7	24.2	4298	11.6	✓	✓	✓	✓	✓		
Bullocks	61	3310	9.4	92.3	1300	4.0	✓	✓	✓				
Chemainus	91	2650	5.0	84.5	856	4.0	✓	✓					
Chicadee	51	3350	14.2	40.8	930	8.7	✓		✓	✓			
Cranby	69	2530	44.6	—	3280	3.2	✓						
Cusheon	91	1570	26.9	65.6	3950	4.5	✓	✓	✓				
Dougan	60	3000	10.0	36.0	1500	8.5	✓	✓					
Green	94	2370	13.4	44.0	1400	5.7	✓	✓	✓				
Hoggan	60	310	19.7	—	2219	3.0	✓	✓	✓				
Ogden	76	2750	10.9	—	1424	4.0	✓		✓				
Pike	59	5000	7.4	71.1	1436	3.7	✓	✓					
Stowell	71	1400	5.6	52.0	983	4.6	✓	✓	✓	✓			
Thetis	52	1030	35.5	86.0	7870	2.8	✓	✓	✓	✓			
Waugh	55	1080	29.8	19.8	3200	16.7	✓		✓	✓	✓	✓	
Weston	60	1890	18.0	—	2091	6.0		✓	✓	✓			✓

Abbreviations: RLA, relative littoral area; CT, cutthroat trout (*Oncorhynchus clarki*); RB, rainbow trout (*O. mykiss*); CAS, prickly sculpin (*Cottus asper*); CO, coho salmon (*O. kisutch*); KO, kokanee (*O. nerka*); NSC, northern pikeminnow (*Ptychocheilus oregonensis*); PCC, peamouth chub (*Mylocheilus caurinus*).

calculates a generalized discriminant analysis based on any symmetric distance matrix. The purpose of DISTCVA is to perform a non-parametric canonical analysis for the effect of **X**, if any, on **Y** on the basis of any distance measure of choice, using permutations of the observations, where **X** is a matrix of interest for a multivariate hypothesis and **Y** is a matrix of response variables. For analyses, I utilized the Bray-Curtis distance measure and $n = 999$ permutations. The use of different distance measures (i.e. Euclidean and Orloci's Chord) did not qualitatively change the results. Because of missing values for relative littoral area ($n = 12$ for candidate lakes, $n = 4$ for sympatric lakes), Secchi depth ($n = 4$ for both lake types) and pH ($n = 11$ for candidate lakes, $n = 4$ for sympatric lakes), these variables were not included in the multivariate discriminant analysis. To determine which variables might differ between candidate and sympatric lakes, I analysed the data in two ways. First, I performed a series of discriminant analyses with DISTCVA, omitting one variable at a time to determine which variable(s) had the most influence on discrimination. Second, univariate comparisons were conducted with Wilcoxon tests for all variables.

Effects of other fish species

Because the results strongly indicated that the greatest difference between candidate and sympatric lakes in the factors measured was the community of other fish (see Results), I explored whether the foraging and anti-predator traits of solitary populations that co-exist with only cutthroat trout differ from those that co-exist with cutthroat trout and other fish species. I chose lateral plates and gill rakers as focal anti-predator and foraging traits, respectively; other traits that are involved in these functions exhibit patterns similar to the ones presented (S.M. Vamosi, unpublished observation). Lateral plates have several functions, including protecting the epidermis from lacerations by toothed predators (Reimchen, 1992), and the number of lateral plates tends to be higher in lakes with piscivorous fish than in those where they are absent or rare (Hagen and Gilbertson, 1972). Gill rakers are bony projections on the respiratory gill arches that function in retaining food particles and preventing them from entering the gills. Sticklebacks with few, short gill rakers tend to feed on invertebrates associated with vegetation and sediments, whereas those with many, long gill rakers prey on zooplankton in the open water zone (Lavin and McPhail, 1985; Schluter and McPhail, 1992).

Data on solitary populations were derived from two sources (Schluter and McPhail, 1992; Vamosi, 2001). Schluter and McPhail (1992) measured the foraging traits of 10 solitary populations from small lakes in the Strait of Georgia. Although it was supposed that all solitary stickleback populations considered co-existed with only cutthroat trout (*Oncorhynchus clarki*), subsequent investigation revealed that this is true for only Cranby and Trout Lakes (Vamosi, 2001). As part of the latter study, I measured the defensive armour of the same individuals from the 10 original solitary populations, and defensive armour and number of gill rakers from individuals from three lakes that contain only sticklebacks and cutthroat trout and from two lakes with sticklebacks, cutthroat trout and other species. Lake names, sample sizes and types of other fish present can be found in Table 2.

Number of lateral plates was the number of staining plates, regardless of size, on the left side of the body of individuals. Number of gill rakers was the total number counted on the first gill arch. Differences between lake types were assessed with *t*-tests conducted using population means of log-transformed (base 10) counts.

Table 2. Number of fish measured (*n*) and types of other fish species present in solitary lakes with only cutthroat trout and with cutthroat trout and other fish species

Lake	*n*	Other fish species						
		CT	RB	CAS	CM	CO	DV	KO
Solitary lakes with only cutthroat trout								
Cranby	68	√						
Klein	40	√						
Mike	30	√						
Tom	13	√						
Trout	30	√						
Solitary lakes with cutthroat trout and other species								
Bear	40	√	√	√		√		
Blackjack	20	√	√			√	√	
Brannen	40	√	√	√		√		√
Fairy	30	√	√	√	√	√	√	
Mesachie	40	√	√	√		√	√	√

Abbreviations: CT, cutthroat trout (*Oncorhynchus clarki*); RB, rainbow trout (*O. mykiss*); CAS, prickly sculpin (*Cottus asper*); CM, chum salmon (*O. keta*); CO, coho salmon (*O. kisutch*); DV, Dolly Varden (*Salvelinus malma*); KO, kokanee (*O. nerka*).

RESULTS

Forty-seven lakes in the study area were identified that contain a solitary population of sticklebacks (Vamosi, 2001). Of these, 20 lakes occurred at an elevation lower than 50 m and would presumably have had their first wave of colonists flushed out during the second submergence. Eight lakes were at an elevation above 100 m and, thus, are thought to have been inaccessible during the second submergence (McPhail, 1993). Nineteen lakes were found at an elevation within the range at which sympatric lakes are found (i.e. 50–100 m); three of these lakes were >5000 m from the coast and are not considered further. Thus, 16 solitary lakes were candidates for two invasions of marine sticklebacks but apparently failed to produce species pairs (Table 1).

Sympatric lakes were well discriminated from candidate lakes when all traits were considered (multivariate discriminant analysis; $\delta_1^2 = 0.055$, $P = 0.001$). To determine whether a single trait was largely associated with this difference between sympatric and candidate lakes, I conducted the analyses again, excluding one variable at a time. The two categories of lakes could not be discriminated from each other when number of other fish species was excluded from the analysis ($\delta_1^2 = 0.002$, $P = 0.59$). Excluding other traits had little effect on the discrimination between sympatric and candidate lakes ($\delta_1^2 \geq 0.044$, $P \leq 0.005$ in all cases). In other words, sympatric and candidate lakes were easily discriminated provided that number of other fish species was included. The results of the discriminant analyses suggest that: (1) the two categories of lakes are remarkably similar in the physical characteristics considered and (2) the most significant difference between sympatric and candidate lakes is the community of other fish species found in lakes.

Univariate analyses of the individual traits confirm the patterns suggested by the exploratory multivariate discriminant analyses. No significant differences were detected between sympatric and candidate lakes in the traits considered in the discriminant analyses (Wilcoxon tests; all $P > 0.55$). Relative littoral area ($\chi^2_1 = 0.13$, $P = 0.72$) and Secchi depth ($\chi^2_1 = 0.08$, $P = 0.77$) also did not differ significantly among lake types. Values for pH were marginally different between sympatric and candidate lakes ($\chi^2_1 = 3.80$, $P = 0.051$), although this was the result of region-specific differences in pH, with those from Texada Island tending to have higher pH values than those from Vancouver Island. The pH of Enos Lake (7.4), for example, was comparable to that of six nearby candidate lakes (mean ± standard deviation: 7.4 ± 0.3).

Most striking was the significant difference in the number of other fish species that were found in candidate and sympatric lakes ($\chi^2_1 = 11.99$, $P < 0.001$). Solitary stickleback populations in candidate lakes co-existed with an average of approximately three other fish species, whereas only one other fish species was found with sympatric stickleback populations (Table 1). Cutthroat trout were present in all lakes. Rainbow trout (*Oncorhynchus mykiss*) and prickly sculpin (*Cottus asper*) were the most common other fish species, present in 12 and 9 of the 16 lakes, respectively, but they were never found co-existing with stickleback species pairs (Table 1). This difference between sympatric (six lakes with only cutthroat trout and no lakes with more fish species) and candidate (one lake with only cutthroat trout and 15 lakes with more fish species) lakes was highly significant (Fisher exact test: $\chi^2_1 = 17.68$, $P < 0.001$). To summarize, stickleback co-existed with only cutthroat trout in seven of the 22 lakes (sympatric and candidate combined), six of which contained sympatric benthic and limnetic populations.

Analyses of number of lateral plates and number of gill rakers of solitary populations revealed consistent differences between population types. Sticklebacks from solitary populations that co-exist with cutthroat trout and other fish species had a significantly higher number of lateral plates than those that co-exist with only cutthroat trout ($t_8 = 3.51$, $P = 0.008$). Other armour characters, including dorsal and pelvic spines, were also longer and more robust in individuals from populations that co-exist with cutthroat trout and other fish species (S.M. Vamosi, unpublished observation). Individuals from solitary populations that co-exist with cutthroat trout and other fish species had fewer gill rakers ($t_8 = 4.03$, $P = 0.004$). Thus, sticklebacks from lakes with cutthroat trout and other fish species had robust defensive armour and were benthic-like in their feeding morphology, whereas those from lakes with only cutthroat trout had moderate amounts of defensive armour and intermediate feeding morphology (Fig. 2).

DISCUSSION

In this study, I sought to elucidate the role of environmental variables in the repeated evolution of sympatric stickleback species pairs. In the region where lakes had the opportunity to be colonized twice by marine ancestors, 16 lakes with a solitary population of sticklebacks were identified that were located within the range of elevation and distance to sea characteristic of the six lakes with stickleback species pairs (McPhail, 1993). The current hypothesis for the diversification of threespine sticklebacks, which stresses temporally separated invasions by marine sticklebacks and subsequent diversification of their descendants as a consequence of competition for resources, cannot account for the absence of benthics and limnetics from these lakes.
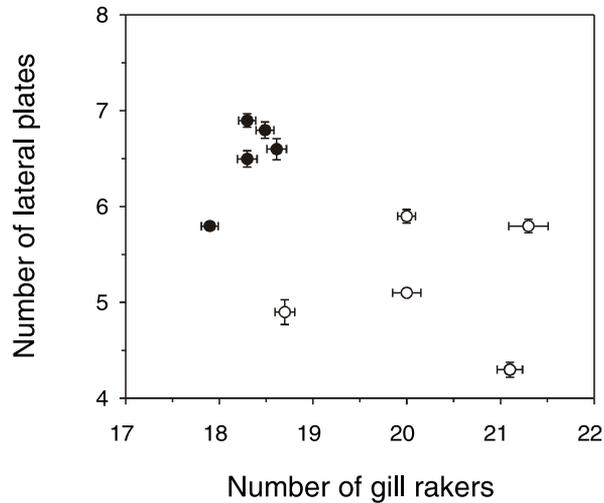
Fig. 2. Relationship between number of lateral plates and number of gill rakers for solitary populations that co-exist with only cutthroat trout (open symbols) or with cutthroat trout and other fish species (closed symbols) (mean ± standard error).

Candidate lakes were found to have several physical and chemical features remarkably similar to those of sympatric lakes. Perhaps most important was the observation that not only were these lakes indistinguishable from sympatric lakes in their overall size and volume, as indicated by surface area and mean depth measures, but that the relative amounts of the two habitats, as measured by relative littoral area, were also comparable between the two classes of lakes. If candidate lakes were consistently dominated by littoral habitat, for example, then the absence of stickleback species pairs from these lakes might have been best interpreted as resulting from an insufficient prey base (i.e. poorly developed zooplankton communities) on which the descendants of the second invasion could specialize on. The patterns in these variables, especially relative littoral area, are consistent with the inference made by Schluter and McPhail (1992) that resource differences are unlikely to explain the limited distribution of sympatric sticklebacks.

Sympatric lakes thus appear to differ from candidate lakes primarily in the community of other fish species co-existing with sticklebacks. How might the presence of these other fish species have hampered the divergence and persistence of benthic and limnetic morphs? One possibility is through their effects as predators of sticklebacks. Four of the seven native fish species encountered (Table 1) are known to prey on sticklebacks. Adult cutthroat trout and rainbow trout prey on juvenile and adult individuals in the littoral and open water zones of lakes (Hagen and Gilbertson, 1973; Reimchen, 1994). Prickly sculpin are associated with the littoral zone and are known to prey on eggs, juveniles and adults (Moodie, 1972; Pressley, 1981). Finally, the northern pikeminnow (*Ptychocheilus oregonensis*) is a voracious predator of adult sticklebacks in the littoral zone (Moodie *et al.*, 1973). In lakes with these fish species, mortality on stickleback populations may depress population sizes sufficiently to reduce the strength of selection for divergence (Abrams, 1986). Alternatively, the presence of these predators may prevent the colonization of one of the habitats even in the face of interspecific competition. Based on the data collected on lateral plates and gill rakers, it appears that predation intensity is higher in lakes with cutthroat trout and other

fish species and, furthermore, that the littoral zone is the safer and, hence, preferred habitat in such cases. Several studies have demonstrated that small fish tend to obtain refuge from predation in the shallow, vegetated waters of the littoral zone (e.g. Post *et al.*, 1998). Under this hypothesis, the evolution of a species that lives and forages exclusively in the open water zone (i.e. limnetics) is possible in lakes with only cutthroat trout but hampered in lakes with cutthroat trout and other fish species.

Curiously, the observation that the armour of sticklebacks from solitary populations that co-exist with many other fish species may be more robust than that of those that co-exist with fewer other fish species has not, to the best of my knowledge, previously been remarked on. Typically, studies of variation in the defensive armour of sticklebacks have considered two categories: with or without piscivorous fish (e.g. Hagen and Gilbertson, 1972; Walker, 1997). In the presence of piscivorous fish, selection is thought to favour the elaboration of defensive armour, whereas in their absence, it has been suggested that selection is either relaxed or actively favours reductions in defensive armour as a consequence of increased predation by large invertebrate predators (Reimchen, 1980; Vamosi, 2002). Observations such as those in Fig. 2 suggest that the amount of armour of sticklebacks may depend not only on whether fish are present, but also on the types of fish present. Freshwater populations living with the northern pikeminnow, for example, may have numbers of lateral plates approaching those of marine sticklebacks (i.e. 30–35 lateral plates on each side of the body; Hagen and Gilbertson, 1972; S.M. Vamosi, personal observation), presumably to protect them from the rasping pharyngeal teeth of these predators (J. McPhail, personal communication). It would be interesting to document whether variation in the armour of solitary populations correlates with differences in fish community across a broader geographical range.

Second, competition between sticklebacks and other fish species for shared resources may also reduce the strength of divergent selection. Most of the fish species encountered are potential competitors with sticklebacks for shared resources. Peamouth chub (*Mylocheilus caurinus*) (Aspinwall *et al.*, 1993) and juvenile salmonids (O'Neill and Hyatt, 1987) are known to forage in the open water on zooplankton. O'Neill and Hyatt (1987), for example, demonstrated that juvenile sticklebacks and sockeye salmon (*Oncorhynchus nerka*) greatly reduced the size and abundance of zooplankton in large enclosures in Kennedy Lake, British Columbia; unfortunately, effects on stickleback growth were not presented. Prickly sculpin feed on aquatic insect larvae and other benthic invertebrates (Rosenfeld, 2000) in addition to small fish.

Of course, the effects of competition and predation may interact with one another. Such an interaction may be a consequence of a change in the foraging behaviour of the different members of the fish community in candidate lakes over their ontogeny. Younger (smaller) individuals of the other populations are more likely to forage on prey utilized by stickle-backs, whereas older individuals above a certain size threshold are more likely to prey on sticklebacks (Nilsson and Northcote, 1981). To the best of my knowledge, the effects of the addition of a population whose ecological role depends on ontogeny on the evolutionary trajectories of focal species remain to be investigated.

Finally, the impacts of cutthroat trout on diversification may depend on the community of other fish species present. Nilsson and Northcote (1981) examined the habitat use and foraging patterns of rainbow trout and cutthroat trout in coastal British Columbia lakes, several of which also contained sticklebacks. One finding that is relevant to the present study was that cutthroat trout sympatric with rainbow trout and other fish species (notably

prickly sculpin) showed a marked tendency to be larger and exhibit increased piscivory. Thus, although cutthroat trout were present in all the lakes considered in the present study, they may be a more significant agent of mortality in lakes with other fish species than in those lakes with only cutthroat trout and sticklebacks. This observation may provide a partial explanation for why such a dramatic difference was observed in the probability of diversification and speciation with the addition of only a single other community member.

Conclusions about the nature of the impact of certain community members, especially rainbow trout and prickly sculpin, on the probability of diversification must await detailed behavioural observations and large-scale field experiments. However, the sensitivity of benthics and limnetics to other fish species is revealed by two independent introduction events into lakes with sympatric species pairs. First, Larson (1976) recorded the distribution and behaviour of benthics and limnetics before and after the temporary introduction of coho salmon into Paxton Lake. The presence of coho salmon fingerlings in the open water zone caused schools of limnetics to move into the littoral zone. Consistent with the notion that the littoral zone provides sticklebacks with protection from predation by piscivorous fish (Vamosi and Schluter, 2002), no effect on the benthic population was observed. The coho salmon did not persist in Paxton Lake and limnetics have since 'reclaimed' the open water zone. Second, the introduction of brown bullhead catfish (*Ameiurus nebulosus*), an omnivore which feeds on eggs and small fish, to Hadley Lake in the early 1990s, appears to have been the cause of the recent extinction of both stickleback species in this lake (Hatfield, 2001).

Considering the results of this survey, I propose a modified explanation for the evolution of sympatric stickleback species pairs. First, it should be noted that the data support the interpretation that two temporally separated invasions are fundamental to diversification and, ultimately, speciation. Thus elevation and distance to sea are still to be considered important factors determining whether a given lake was available for two colonization events. However, 'ecological determinism' (i.e. ecological speciation driving divergence) *sensu* Taylor and McPhail (2000) is probable only in lakes that were colonized by sticklebacks and cutthroat trout. Indeed, armed with the knowledge that the presence of other fish species may be important, the repeated evolution of sympatric species pairs is all the more remarkable: seven of the 22 lakes available for double invasion contained cutthroat trout as the only other fish species, six of which contained sympatric species pairs. In lakes that were colonized by sticklebacks, cutthroat trout and other fish species, the presence of these other fish species apparently retarded ecological speciation, possibly by reducing the strength of divergent selection, excluding a limnetic-like morph from the open water zone, or through complex interactions between their effects as competitors and predators.

The effects of interactions other than competition between focal populations on diversification and speciation need to be examined explicitly in other systems. The squanga whitefish (*Coregonus* sp.), for example, is an open water species that co-exists with lake whitefish (*Coregonus clupeaformis*) in only four lakes in the Yukon Territory (Bodaly, 1979). The generally accepted explanation for its limited distribution is that a potential competitor, the planktivorous least cisco (*Coregonus sardinella*), is absent from these lakes but common throughout the region (Lindsey, 1981). Bodaly *et al.* (1988), however, also noted that lakes with squanga whitefish had unusually low abundances of piscivorous fish. Gilbert (1975) suggested that high levels of diversity in leaf shape among sympatric passion vines (*Passiflora* spp.) may be largely the result of interactions between passion vine species mediated via shared herbivores, especially *Heliconius* butterflies, rather than competition for

limiting resources. Finally, sympatric land snails on the Bonín Islands have undergone not only habitat segregation but also shifts in putative defensive traits (Chiba, 1999). Species in wet and sheltered sites had dark shells, whereas those in dry and exposed sites were brighter and often polymorphic. Differences in shell colour between habitats may reflect divergent selection for protective colouration in response to bird predation and contrasting background colours between habitats, although this has yet to be demonstrated. Studies of interactions between multiple factors are still in their infancy (Smith and Skúlason, 1996; Buckling and Rainey, 2002; H. Rundle, S. Vamosi and D. Schluter, unpublished manuscript), but promise to yield useful insights into the role of ecology in diversification and speciation.

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