Environmental determinants of threespine stickleback species pair evolution and persistence

Chad I. Ormond, Jordan S. Rosenfeld, and Eric B. Taylor

Abstract: Threespine stickleback (Gasterosteus aculeatus) species pairs are found in four watersheds in southwestern British Columbia, Canada, and are listed as Endangered under the federal Species at Risk Act. Their origin is thought to be through a double-invasion process followed by character displacement; however, this hypothesis does not consider whether speciation is dependent on unique environmental factors necessary to support two species with separate habitat and resource requirements, which may be essential both for their evolution and persistence. To test whether species pair lakes have unique attributes, we compared abiotic and biotic factors of species pair lakes to lakes with only a single population of stickleback. There were no clear environmental differences between species pair and non-species pair lakes, but species pairs were only present in lakes with low fish species diversity, suggesting that evolution and persistence of species pairs requires a simplified fish community. Our study suggests that colonization history rather than unique lake attributes (related to either physical habitat or trophic resources) facilitated the evolution of stickleback species pairs and that the fish assemblage in lakes may affect resource availability and speciation potential as strongly as the limnological attributes of the lakes themselves.

Résumé : Les paires d’espèces d’épinoches à trois épines (Gasterosteus aculeatus) se retrouvent dans quatre bassins versants du sud-ouest de la Colombie-Britannique, Canada, et figurent sur la liste des espèces menacées d’après la loi fédérale sur les espèces en péril. On pense que leur origine s’explique par un processus de double invasion suivi par une divergence phénotypique; cependant, cette hypothèse ne considère pas si la spéciation dépend de caractéristiques uniques du milieu nécessaires pour le maintien de deux espèces avec des besoins différents d’habitat et de ressources, qui peuvent être essentielles tant pour leur évolution que pour leur persistance. Afin de tester si les lacs qui possèdent des paires d’espèces ont des caractéristiques uniques, nous avons comparé les facteurs abiotiques et biotiques des lacs à paire d’espèces avec ceux des lacs à une seule population d’épinoches. Il n’y a pas de différence environnementale claire entre les lacs avec ou sans paires d’espèces, mais les paires d’espèces ne sont présentes que dans les lacs à faible diversité de poissons, ce qui laisse croire que l’évolution et la persistance de paires d’espèces exigent une communauté simplifiée de poissons. Notre étude fait penser que c’est l’histoire de la colonisation plutôt que les caractéristiques uniques du lac (relées à l’habitat physique ou aux ressources trophiques) qui a facilité l’évolution des paires d’espèces d’épinoches et que le peuplement des poissons dans les lacs peut affecter la disponibilité des ressources et le potentiel de spéciation aussi fortement que les caractéristiques limnologiques des lacs eux-mêmes.

[Traduit par la Rédaction]

Introduction

The evolution of closely related, but trophically differentiated, pairs of species of freshwater fishes is a repeated occurrence in north temperate lakes and is usually attributed to ecological radiation within depauperate fish faunas (Robinson and Wilson 1994; Taylor 1999). Recently evolved taxa are of great value to understanding the processes of evolution and are often endemics with restricted distributions and therefore are of particular conservation concern (Schluter 2000). Understanding the processes that lead to species differentiation is a central goal of evolutionary biology, but may also provide essential information for species management. From a conservation standpoint, the limnological conditions that are required for species differentiation may also be essential for continued persistence, and alteration of limnological attributes has been associated with the irreversible collapse of several recently evolved species into hybrid swarms (Todd and Stedman 1989; Seehausen et al. 1997; Taylor et al. 2006).

A possible precondition for the evolution of trophically differentiated lacustrine species is their occurrence in adaptive landscapes with unusual limnological attributes (e.g., an unusual type of habitat(s) with a unique range of water chemistry or trophic conditions, referred to here as the "environmental constraints hypothesis"). Alternatively, there may be nothing intrinsically unique about the characteristics of water bodies where species pairs evolved, and their presence in particular lakes may be due solely to random colonization events or landscape filters that limited colonization and fish faunal diversity (McPhail 1993; Poff 1997; Jackson et al. 2001: the "his-
torical contingency hypothesis’"). These contrasting scenarios have different conservation implications, with the first suggesting that maintaining unique limnological attributes (e.g., water quality, habitat, or specific trophic resources) is paramount for species persistence, whereas the second suggests that maintaining low fish faunal diversity and preventing the introduction of invasive taxa are higher priorities.

Several studies have demonstrated that the environment can facilitate differentiation of species. Well-known examples include the cichlids of East African lakes (Seehausen et al. 2008), Darwin’s finches (Grant and Grant 2002), and Anolis lizards from the Caribbean (Losos et al. 1997). In the context of north temperate fish diversity, it was recently suggested that limnological attributes of lakes can influence the evolution of lake whitefish (Coregonus clupeaformis) species pairs, which show a higher degree of differentiation into limnetic and benthic ecotypes in lakes with reduced habitat and prey availability, presumably as a consequence of greater intraspecific competition for resources (Landry et al. 2007; Landry and Bernatchez 2010).

Sympatric limnetic and benthic threespine stickleback (Gasterosteus aculeatus) species pairs are of major conservation concern; they are listed as Endangered under Canada’s federal Species at Risk Act and are an ideal system for exploring the conservation implications of speciation processes (McPhail 1994; Schluter 2000). Sympatric pairs of threespine stickleback at one time occurred in seven lakes in five watersheds in the Strait of Georgia, southwestern British Columbia (Schluter and McPhail 1992; Gow et al. 2008). Over the last 10 years, species pairs in two lakes have gone extinct probably owing to interactions with invasive species (Hatfield 2001; Taylor et al. 2006). In each of these lakes, stickleback occur (or once occurred) in both benthic and limnetic forms and are adapted to different microhabitats (reviewed in McPhail 1994). Benthic sticklebacks have robust bodies with few, short gill rakers and feed on benthic macroinvertebrates in the littoral zone, while limnetic sticklebacks have a streamlined body form with many, long gill rakers and feed on zooplankton in the limnetic or pelagic zone (Larson 1976; Schluter and McPhail 1992).

The species pairs are thought to have originated through the double invasion of fresh water by a marine ancestor stickleback, with the first wave of invaders becoming the benthic form and the second wave of invaders the limnetic form (Schluter and McPhail 1992; McPhail 1993; Taylor and McPhail 2000). Although a double invasion scenario may have contributed to the evolution of sympatric species pairs, many other lakes in the region that were susceptible to more than one marine transgression contain only a single species of stickleback (non-species pair lakes). The fact that only seven lakes contained species pairs out of the multitude of lakes in the region suggests that these lakes are physically or biologically unique in some way that promoted the evolution of sympatric species. Given that benthic and limnetic sticklebacks show clear differences in habitat selection and resource use, any lake with species pairs will be required to meet the habitat needs of both species (i.e., contain both littoral and pelagic zones) as well as contain sufficient resources to support them (i.e., benthic macroinvertebrates and zooplankton). As such, under the environmental constraints hypothesis, there should be a clear difference in habitat types between species pair and non-species pair lakes, with large relative littoral and pelagic areas in species pair lakes, as well as high macrophyte abundance and a wider range of resource availability (i.e., higher plankton and benthic invertebrate biomass) than non-species pair lakes.

Alternatively, the existing biota of a habitat may play a more dominant role in subsequent evolution of communities (e.g., Shmida and Wilson 1985; Buckling and Rainey 2002; Johnson and Stinchcombe 2007). In fact, Vamosi (2003) compared six physical attributes of species pair and non-species pairs lakes, and while observing no significant difference in lake attributes, he found that they differed in their fish community. Stickleback species pair lakes contained only stickleback and coastal cutthroat trout (Oncorhynchus clarkii clarkii), while most non-species pair lakes contained both of these fishes in addition to other fish species. Other fish species may act as predators and (or) competitors with stickleback, and resource depletion by other species could reduce the scope for specialization leading to speciation. Although Vamosi’s (2003) study was important in highlighting the role of the fish community in stickleback species pair ecology, he characterized lakes largely in terms of physical habitat attributes, which represent only a subset of the axes that constitute the Grinnellian niche of an organism (Guisan and Thuiller 2005; Soberón 2007). Species can also differentiate strongly along nutrient and resource gradients (Leibold 1989), and biological resources (e.g., abundance of benthic or planktonic invertebrates) represent an additional suite of adaptive axes (Svanback and Bolnick 2007; Landry and Bernatchez 2010) that were unexplored in earlier analyses. In particular, the chemical or nutrient characteristics of lakes, extent of aquatic macrophytes, and trophic resource availability are three key factors that have recently been implicated in evolution and persistence of species pairs in sticklebacks and other taxa (e.g., Landry et al. 2007; Landry and Bernatchez 2010). These additional lake attributes are critical to examine, as they are directly relevant to the biology of sticklebacks, their prey base, and the identification and delineation of habitat features potentially crucial to the persistence of species pairs.

In this study, we include all known species pair lakes, including an additional lake (Little Quarry Lake; Gow et al. 2008) discovered since Vamosi’s (2003) study, and test the idea that limnetic and benthic stickleback species pairs have evolved because of unique abiotic and biotic factors that are shared amongst species pair lakes, but are absent in non-species pair lakes (i.e., the environmental constraints hypothesis). To test this hypothesis, physical, chemical, and biological variables were compared between lakes with and without species pairs. If species pair lakes group separately from all other lakes, this would support the hypothesis that a unique combination of environmental variables in these lakes were important determinants of stickleback species pair divergence. No difference in environmental attributes between species pair and non-species pair lakes would be consistent with the idea that the evolution of species pairs was driven more by historical factors including, but not necessarily limited to, double invasion of a random subset of lakes, and (or) that differences in fish community structure are more important than biophysical–chemical properties of lakes in influencing the evolution of species pairs of threespine stickleback.
and lakes sampled were between 45 and 100 m in elevation to other fish species. All known SP lakes were sampled; NSP spine stickleback (non-species pair, NSP; Table 1) in addition the other seven lakes contain a solitary population of threespine stickleback species pairs (SP) and (Table 1). Seven of the sampled lakes contain, or have historically colonized repeatedly by a marine ancestor as the SP lakes). Thus, it would have been ideal to sample SP lakes that contain only stickleback and coastal cutthroat trout; however, this was not possible because of the limited number of lakes with this specific, simplified fish community. Therefore, the group of NSP lakes sampled contained the following fish assemblages: (i) Cranby Lake — stickleback + coastal cutthroat trout, (ii) Ambrose, Brown, and North lakes — stickleback + coastal cutthroat trout + prickly sculpin (Cottus asper), (iii) Stowell and Weston lakes — stickleback + coastal cutthroat trout + rainbow trout (Oncorhynchus mykiss), and (iv) Chemainus Lake — stickleback + coastal cutthroat + rainbow trout + (introduced, non-native) smallmouth bass (Micropterus dolomieu) (Table 1).

All lakes were sampled for water chemistry, trophic resources, and macrophyte abundance once between 6 July and 28 August 2008 and again at Paxton and Cranby lakes between 29 November and 1 December 2008 and 20–21 April 2009. Bathymetric surveys were completed at each lake between 6 July and 28 August 2008. Logistic constraints prevented replicate sampling at the same lake, but seasonal sampling at one SP and NSP lake (Paxton and Cranby lakes) showed minimal changes in water chemistry and other attributes between spring, summer, and fall (Ormond 2010). Although the primary goal of our study was to compare attributes of SP and NSP lakes, an ancillary objective was to collect baseline habitat data for future environmental monitoring for these endangered species.

### Materials and methods

#### Study lakes

Fourteen lakes in southwestern British Columbia containing threespine stickleback were sampled during this study (Table 1). Seven of the sampled lakes contain, or have historically contained, threespine stickleback species pairs (SP) and the other seven lakes contain a solitary population of threespine stickleback (non-species pair, NSP; Table 1) in addition to other fish species. All known SP lakes were sampled; NSP lakes sampled were between 45 and 100 m in elevation and ≤5000 m distant from the sea because this is the range of elevations and distances encompassed by SP lakes (and therefore should have the same historical probability of being colonized repeatedly by a marine ancestor as the SP lakes). Elevation and distance to sea were chosen following McPhail (1993) and Vamosi (2003), with an estimated maximum 10% error in measuring these parameters. It has been found that all SP lakes contain only one other species of fish, coastal cutthroat trout (with the exception of Little Quarry Lake where construction of an outlet dam may have blocked access to spawning habitat). Thus, it would have been ideal to sample NSP lakes that contain only stickleback and coastal cutthroat trout; however, this was not possible because of the limited number of lakes with this specific, simplified fish community. Therefore, the group of NSP lakes sampled contained the following fish assemblages: (i) Cranby Lake — stickleback + coastal cutthroat trout, (ii) Ambrose, Brown, and North lakes — stickleback + coastal cutthroat trout + prickly sculpin (Cottus asper), (iii) Stowell and Weston lakes — stickleback + coastal cutthroat trout + rainbow trout (Oncorhynchus mykiss), and (iv) Chemainus Lake — stickleback + coastal cutthroat + rainbow trout + (introduced, non-native) smallmouth bass (Micropterus dolomieu) (Table 1).

### Water chemistry attributes

We sampled water chemistry parameters that were related to nutrient and trophic status, including pH, conductivity, turbidity, dissolved oxygen, temperature, water transparency, chlorophyll a, dissolved organic carbon, total organic carbon, coloured dissolved organic matter, dissolved inorganic carbon, total nitrogen, total phosphorous, alkalinity (total as CaCO₃), total suspended solids, and total dissolved solids. Turbidity samples were taken at a depth of 0.5 m and measured using a Lamotte 2020ε turbidity meter. Surface readings of pH and conductivity were measured using a YSI WTW 340i meter. Dissolved oxygen and temperature were measured using a YSI model 58 oxygen meter every 0.5 m of depth to create dissolved oxygen and temperature profiles of each lake. Water transparency was measured using a LI-COR model LI-250 light meter at 0.12, 0.5, 1.5, 2.5, and 3.5 m, and light extinction coefficient (k) values were calculated for each lake. For all other variables, water samples were taken within 100 m of the geographic centre of each lake at a depth of 0.5 m and analysed by Maxxam Analytics (Burnaby, British Columbia).

#### Zooplankton and benthic invertebrate abundance

At each lake, three vertical zooplankton tows were collected at the deepest area of each lake using a 30 cm wide Wisconsin plankton net with an 80 µm mesh. Depth of the tows varied with each lake to ensure they included the thermocline so as...
to maximize the collection of vertically migrating zooplankton (Wetzel 2001). Samples were filtered through a 250 μm mesh and preserved in 5% formalin. Volume ($V$) of water filtered for each tow was calculated using the formula

$$V = \pi r^2 h$$

where $r$ is the radius of the plankton net mouth, and $h$ is the depth of the vertical tow. Sampling methodology followed Tonolli (1971). We did not include a correction for net filtering efficiency, as this was consistent across lakes and should not alter relative plankton estimates.

Benthic invertebrates were collected from both sediment and rock substrates to determine relative differences in benthic invertebrate resource availability between lakes. Three sediment and five rock samples were collected at each lake; fewer sediment samples were collected because sediment substrate was more homogeneous. Substrate sampling stations were chosen systematically to include multiple areas of each lake; sampling stations were chosen first by locating the appropriate substrate zone within each lake and then randomly choosing the location of samples within each zone. Sediment samples ($n = 3$) were collected by carefully scooping sediment from a known area ($0.0075 \text{ m}^2$) into a 250 μm mesh net attached to the end of a pole and held against the lake bottom, filtering the sediment through the 250 μm mesh, and preserving the sediment in 5% formalin. Littoral rocks ($n = 5$; after Rosenfeld and Hudson 1997) were collected by carefully placing them into a 250 μm mesh net held next to the substrate before lifting the net out of the water and scrubbing the rock with a brush to wash off all invertebrates. Invertebrates were rinsed from the rock and net and preserved in 5% formalin. To express invertebrate abundance as a density, the surface area ($\text{m}^2$) of each rock was calculated based on rock length and width.

In the lab, zooplankton samples were divided into quarters or eighths depending on zooplankton density using a splitter so that each counted sample had not more than 2000 individuals. Accuracy of the split samples was assessed by splitting a sample and counting each subsample. The lengths of 30 individuals of each taxon in the subsamples were measured using a digitizing system (10× magnification; Roff and Hopcroft 1986), and all individuals in subsamples were counted. Cladocerans were identified to family or genus; copepods were identified as order Calanoida, order Cyclopoida, or as copepod nauplii. Chaoborus spp. were identified to genus, and mites were identified as subclass Acarina. Biomass for zooplankton was determined using length–weight regressions as published by Smock (1980), Sample et al. (1993), and Benke et al. (1999).

Macrophytes

Emergent and submerged macrophyte coverage was surveyed systematically throughout each lake. The area of macrophyte beds were measured using a range finder while canoeing around the perimeter of each lake. The range finder was used to estimate the distance from several points along each macrophyte bed to the lake shore. These distances were used to calculate the area of emergent and submerged macrophytes, which was expressed as a percentage of total lake surface area.

Bathymetry

The littoral area is often defined as either the portion of a lake that is less than 6 m deep or the threshold depth where sufficient light for plant growth reaches the lake bottom (e.g., Wetzel 2001; British Columbia Ministry of Environment 2009). For this study, we defined the littoral area as the portion of the lake that is less than 3 m in depth, because the majority of macrophytes are within the 3 m depth contour, and stickleback (both benthic and limnetic) typically breed at depths of less than 2 m (McPhail 1994; Hatfield and Schluter 1996). Therefore, the area of depth less than 3 m will contain the habitat structure that is most important for benthic sticklebacks and for reproduction of both species.

To determine relative littoral area, bathymetric data were collected along multiple transects of each lake using a Lowrance LMS-525C DF GPS Fishfinder. Relative littoral area (depth < 3 m) was extracted from the bathymetric data by uploading the UTM coordinates and corresponding depths onto georeferenced maps in ArcGIS (ESRI 2009) and fitting depth contours with the 3D Analyst tool of ArcGIS. Maximum depth was extracted from bathymetric data, while lake perimeter and surface area were extracted from georeferenced maps in ArcGIS.

Fish community

The fish communities of each lake were determined using archived data from the BC FishWizard (http://www.fishwizard.com), an online database that contains information on fish distributions in British Columbia, and from Vamosi (2003). For analysis of the fish community, exotic fish species (e.g., smallmouth bass, present in one NSP lake; and brown bullhead, Amia nebulosus, present in one extirpated SP lake) were excluded, and therefore only stickleback, coastal cutthroat trout, rainbow trout, and prickly sculpin were used, since the objective was to determine whether there was a distinct historical fish community associated with the evolution of species pairs. Rainbow trout have been stocked in both Weston and Chemainus lakes (NSP lakes; British Columbia Ministry of Environment 2010) and, therefore, may not be native to these lakes; however, the fish community previous to this stocking is unknown, and as rainbow trout are native to the region (Scott and Crossman 1973), it is possible that they are native to these lakes. As such, we considered rainbow trout to be native fish in this study.

Data analyses

Student’s $t$ tests were used on each limnological variable to test for statistical differences between the mean values for
Table 2. Physical and chemical environmental variables used in statistical analyses (PCA–DFA).

<table>
<thead>
<tr>
<th>Species pair lakes</th>
<th>Non-species pair lakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Balkwill</td>
</tr>
<tr>
<td>Physical</td>
<td></td>
</tr>
<tr>
<td>Perimeter (m)</td>
<td>2268</td>
</tr>
<tr>
<td>Relative littoral area (%)</td>
<td>27.8</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>10.4</td>
</tr>
<tr>
<td>Max. depth (m)</td>
<td>15.8</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a (µg·L⁻¹)</td>
<td>0.6</td>
</tr>
<tr>
<td>Dissolved organic carbon (mg·L⁻¹)</td>
<td>6.9</td>
</tr>
<tr>
<td>Alkalinity (total as CaCO₃; mg·L⁻¹)</td>
<td>35</td>
</tr>
<tr>
<td>Total organic carbon (mg·L⁻¹)</td>
<td>5.7</td>
</tr>
<tr>
<td>Coloured dissolved organic matter (colour unit)</td>
<td>10</td>
</tr>
<tr>
<td>Dissolved inorganic carbon (mg·L⁻¹)</td>
<td>8.3</td>
</tr>
<tr>
<td>Total nitrogen (mg·L⁻¹)</td>
<td>0.2</td>
</tr>
<tr>
<td>Total phosphorus (mg·L⁻¹)</td>
<td>0.006</td>
</tr>
<tr>
<td>Total dissolved solids (mg·L⁻¹)</td>
<td>44</td>
</tr>
<tr>
<td>pH</td>
<td>7.91</td>
</tr>
<tr>
<td>Conductivity (µS·cm⁻¹)</td>
<td>83</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>-0.02</td>
</tr>
<tr>
<td>Water transparency (k value)</td>
<td>-0.73</td>
</tr>
<tr>
<td>Dissolved oxygen epilimnion (%) sat.)</td>
<td>96.5</td>
</tr>
<tr>
<td>Dissolved oxygen hypolimnion (%) sat.)</td>
<td>33.8</td>
</tr>
<tr>
<td>Dissolved oxygen mean (% sat.)</td>
<td>59.2</td>
</tr>
<tr>
<td>Temperature epilimnion (°C)</td>
<td>21.6</td>
</tr>
<tr>
<td>Temperature hypolimnion (°C)</td>
<td>6.3</td>
</tr>
<tr>
<td>Temperature mean (°C)</td>
<td>11.1</td>
</tr>
</tbody>
</table>
Table 3. Biological environmental variables used in statistical analyses (PCA–DFA).

<table>
<thead>
<tr>
<th>Species pair lakes</th>
<th>Balkwill</th>
<th>Emily</th>
<th>Little Quarry</th>
<th>Paxton</th>
<th>Priest</th>
<th>Enos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zooplankton biomass (mg·m⁻²)</td>
<td>67.7</td>
<td>3168.7</td>
<td>1.0</td>
<td>2.5</td>
<td>175.9</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>(17.1)</td>
<td>(244.0)</td>
<td>(0.2)</td>
<td>(0.8)</td>
<td>(77.2)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>Invertebrate biomass in sediment (mg·m⁻²)</td>
<td>3030.1</td>
<td>7204.0</td>
<td>949.0</td>
<td>3650.7</td>
<td>8279.1</td>
<td>1514.5</td>
</tr>
<tr>
<td></td>
<td>(757.7)</td>
<td>(3099.2)</td>
<td>(122.1)</td>
<td>(864.2)</td>
<td>(2860.1)</td>
<td>(393.3)</td>
</tr>
<tr>
<td>Invertebrate biomass on rock (mg·m⁻²)</td>
<td>739.3</td>
<td>1600.6</td>
<td>291.4</td>
<td>1385.5</td>
<td>2105.6</td>
<td>538.8</td>
</tr>
<tr>
<td></td>
<td>(429.6)</td>
<td>(104.5)</td>
<td>(307.9)</td>
<td>(959.4)</td>
<td>(98.2)</td>
<td></td>
</tr>
<tr>
<td>Emergent macrophyte coverage (% of lake surface area)</td>
<td>0.6</td>
<td>6.7</td>
<td>1.5</td>
<td>22.9</td>
<td>16.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Submerged macrophyte coverage (% of lake surface area)</td>
<td>0.6</td>
<td>20.6</td>
<td>0.5</td>
<td>7.6</td>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Total macrophyte coverage (% of lake surface area)</td>
<td>1.2</td>
<td>27.4</td>
<td>2</td>
<td>30.5</td>
<td>17.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Note: Where applicable, standard error is given in parentheses.

Results

Physical comparisons among lakes

PC1 and PC2 accounted for 55.7% and 26.9%, respectively, of the total variance of physical variables. Perimeter (−0.62), surface area (−0.58), and maximum depth (−0.49) showed strong negative correlations with PC1.
which contrasted large, deep lakes with smaller, shallow ones. Relative littoral area (−0.83) had a strong negative correlation with PC2, which contrasted lakes with high versus low littoral area; lakes with intermediate PC2 scores had a good mix of both littoral and pelagic habitat.

Both SP and NSP lake values occurred across the range of lake sizes (PC1) and amount of relative littoral area (PC2) with no obvious differentiation between SP and NSP lakes (Fig. 1a). Using the Jolliffe stopping rule, the first two PCs were retained. There were no significant differences in mean scores for SP and NSP lakes along PC1 ($t_{12} = 0.972, p = 0.34$) or PC2 ($t_{12} = 0.015, p = 0.99$).

The Hotelling’s $T^2$ test for differences in group centroids on PC axes (PC1 and PC2) was not significant ($p = 0.67$ and $p = 0.99$, respectively), so no DFA was attempted.

### Chemical comparisons among lakes

Species pair and NSP lakes showed near significant differences in conductivity ($t_{12} = 2.30, p = 0.052$; Fig. 2a), alkalinity ($t_{12} = 2.07, p = 0.077$; Fig. 2b), dissolved inorganic carbon ($t_{12} = 2.24, p = 0.062$; Fig. 2c), and total dissolved solids ($t_{12} = 2.25, p = 0.056$; Fig. 2d). While SP lakes as a group had higher mean values for these variables than NSP lakes, it was not a definitive factor for discriminating SP lakes because Little Quarry Lake (a species pair lake) had one of the lowest values for all four of these water chemistry variables (Fig. 2).

PC1 and PC2 accounted for 43.5% and 24.7%, respectively, of the total variance in the water chemistry data set. PC1 showed a negative correlation with dissolved oxygen in the hypolimnion (−0.32) and positive correlations with total organic carbon (0.32), total nitrogen (0.30), and dissolved organic carbon (0.30). PC2 had a negative correlation with coloured dissolved organic matter (−0.32) and positive correlations with water transparency (0.35), alkalinity (0.33), dissolved inorganic carbon (0.32), conductivity (0.32), and total dissolved solids (0.32). While SP lakes cover a larger spread on PC2, they are not obviously differentiated from NSP lakes or restricted to any part of the ordination (Fig. 1b). PC3 was most strongly correlated with mean temperature (0.51), mean dissolved oxygen (0.45), temperature in the epilimnion (0.33), and temperature in the hypolimnion (0.31).

The Jolliffe stopping rule resulted in the first five PCs being retained. Across all five PCs, there was a significant difference in the mean scores between SP and NSP lakes for only PC3 ($t_{12} = -2.52, p = 0.027$). The Hotelling’s $T^2$ test using the PC axes (PC1 to PC5) was marginally significant ($p = 0.054$), and the subsequent DFA classified the lakes correctly as SP or NSP lakes with an accuracy of 85.7% after using the leave-one-out method.

### Biological comparisons among lakes

There was no significant difference in total macrophyte abundance between SP and NSP lakes ($t_{12} = -0.39, p = 0.70$). Macrophytes showed a range from 0.2% to 30.5% for SP lakes and 0.02% to 77.9% for NSP lakes (Table 3). SP and NSP lakes were similar in biomass (mg·m$^{-2}$) for both invertebrates in sediment ($t_{12} = 0.98, p = 0.35$) and invertebrates on rock ($t_{12} = 0.62, p = 0.55$).

For the PCA, PC1 and PC2 accounted for 52.9% and 28.6%, respectively, of the total variance. PC1 was correlated with submergent macrophytes (0.47), invertebrates on rock (0.45), total macrophytes (0.41), invertebrates in sediment (0.39), zooplankton (0.38), and emergent macrophytes (0.32). PC2 showed a strong positive correlation with emergent (0.62) and total macrophytes (0.51) and a negative correlation to zooplankton (−0.43) and invertebrates in sediment (−0.38). No obvious patterns were resolved between SP and NSP lakes (Fig. 1c). Following the Jolliffe stopping rule, $t$ tests were performed on the first three PCs, and all were shown to be nonsignificant (all $p > 0.05$). Hotelling’s $T^2$ test was not significant using the PC axes (PC1 to PC3) ($p = 0.66$), so no DFA was attempted.

### Comparison among lakes with all variables combined

PC1 and PC2 accounted for 35.5% and 20.5%, respectively, of the total variance in the total data set of variables. PC1 showed a positive correlation with dissolved oxygen in the hypolimnion (0.27) and a negative correlation with total nitrogen (−0.27), total organic carbon (−0.26), and dissolved organic carbon (−0.25). PC2 was most strongly correlated with water transparency (0.32), chlorophyll $a$ (−0.30), and coloured dissolved organic matter (−0.28). PC2 was also correlated with zooplankton (0.25), conductivity (0.24), dissolved inorganic carbon (0.25), alkalinity (0.24), and total dissolved solids (0.24). Again, no obvious patterns were apparent between SP and NSP lakes (Fig. 1d). Following the Jolliffe stopping rule, the first eight PCs were kept, but no significant differences between SP and NSP lakes were observed (all $p > 0.05$). Hotelling’s $T^2$ test using PC axes...

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**Non-species pair lakes**

<table>
<thead>
<tr>
<th></th>
<th>Hadley</th>
<th>Ambrose</th>
<th>Brown</th>
<th>Chemainus</th>
<th>Cranby</th>
<th>North</th>
<th>Stowell</th>
<th>Weston</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity</td>
<td>58.4 (3.8)</td>
<td>2.9 (0.2)</td>
<td>15.7 (1.3)</td>
<td>21.2 (1.6)</td>
<td>22.8 (4.2)</td>
<td>15.4 (1.9)</td>
<td>100.6 (21.5)</td>
<td>346.3 (51.9)</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>3465.4 (388.5)</td>
<td>1193.8 (2368.2)</td>
<td>2639.3 (427.9)</td>
<td>1929.3 (616.9)</td>
<td>4192 (876.2)</td>
<td>1955.5 (1708.3)</td>
<td>4069.2 (888.1)</td>
<td>4472.8</td>
</tr>
<tr>
<td>Transparency</td>
<td>185.0 (29.3)</td>
<td>47.3 (15.5)</td>
<td>135.3 (275.3)</td>
<td>1157.4 (128.6)</td>
<td>871.0 (111)</td>
<td>557.7 (1482.7)</td>
<td>1111</td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>3.1 (0.003)</td>
<td>0.8 (0.2)</td>
<td>68.8 (9.1)</td>
<td>17.2 (1.1)</td>
<td>2.2 (0.9)</td>
<td>6.2 (1.9)</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
<td>7.4 (0.01)</td>
<td>0.2 (0.2)</td>
<td>9.1 (0.32)</td>
<td>18.3 (0.33)</td>
<td>3.1 (0.45)</td>
<td>8.1 (0.66)</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Scatterplots of means scores along PC1 and PC2 for the principal components analysis (PCA) of physical (a), chemical (b), biological (c), and all variables combined (d). For the physical PCA (a), PC1 and PC2 account for 55.7% and 26.9% of the total variation, respectively. For the chemical PCA (b), PC1 and PC2 account for 43.5% and 24.7% of the total variation, respectively. For the biological PCA (c), PC1 and PC2 account for 52.9% and 28.6% of the total variation, respectively. For the PCA of all variables combined (d), PC1 and PC2 account for 35.5% and 20.5% of the total variation, respectively. Open squares denote species pair lakes, and filled circles denote non-species pair lakes.
Fig. 2. Bar plots of conductivity (a), alkalinity (b), dissolved inorganic carbon (c), and total dissolved solids (d) for all species pair (open bars) and non-species pair lakes (filled bars).
(PC1 to PC8) was also not significant ($p = 0.49$), and no DFA was attempted.

**MCLUST analysis**

The Jolliffe stopping rule was used to determine the number of PCs to retain ($n = 8$) for the combined data. A MCLUST analysis was performed on these first eight sets of PC scores from the combined data set of all physical, chemical, and biological variables (Fig. 1d). This analysis showed that the best model to explain the data included six components (i.e., groups of lakes) with equal ellipsoidal shapes and with equal variance within groups of lakes (i.e., lake groups are of equal volume, shape, and orientation). These six groups consisted of (1) Emily Lake; (2) Priest Lake; (3) Paxton Lake; (4) Chemainus Lake; (5) North, Brown, and Little Quarry lakes; and (6) Balkwill, Enos, Hadley, Cranby, Weston, Stowell, and Ambrose lakes (Fig. 3). Lakes did not appear to group according to SP vs. NSP lakes (Table 1), geography (Table 1), underlying geology (British Columbia Ministry of Energy Mines and Petroleum Resources 2004; Table 4), other measured limnological attributes (Tables 2 and 3), or fish community (Table 1, Fig. 4).

**Fish community comparisons among lakes**

The single linkage dendrogram formed four clusters of lakes according to fish species composition; cluster 1 includes the six SP lakes (Paxton, Emily, Balkwill, Priest, Enos, and Hadley lakes) and one NSP lake (Cranby Lake); cluster 2 includes the NSP lakes containing rainbow trout (Chemainus, Stowell, and Weston lakes); cluster 3 includes NSP lakes containing prickly sculpin (North, Brown, and Ambrose lakes), and cluster 4 includes the SP lake with no fish other than stickleback (Little Quarry Lake) (Fig. 4). Clusters 1, 2, and 3 were 66.7% similar to each other, while cluster 4 was 50% similar to all other clusters (Fig. 4).

**Discussion**

**Differentiation between lake types**

One of the key factors thought to be involved in rapid divergence of a lineage into different morphs is low competition from trophically similar species, which presumably allows a diverging population to expand into new and underutilized microhabitats (i.e., open niches; Schluter 2000). For example, Icelandic Arctic char (*Salvelinus alpinus*) can be found in lakes as either a single morph or with up to four different ecomorphs. The factors allowing driving this divergence are thought to be a combination of resource heterogeneity and low species diversity and therefore low interspecific competition (Skúlason and Smith 1995; Knudsen et al. 2007). Indeed, the lake supporting four ecomorphs of Arctic char has a very heterogeneous environment and a depauperate fish community containing only Arctic char, threespine stickleback, and a small population of brown trout (*Salmo trutta*; Sandlund et al. 1987; Smith and Skúlason 1996). The importance of fish community diversity in the evolution of such species pairs can be seen in other fish taxa (Taylor 1999) as well as in other vertebrates such as lizards (Losos et al. 1997) and some birds (Grant and Grant 2002).

The repeated parallel evolution of benthic and limnetic pairs of stickleback species and their geographically clustered location suggests that there are ecological or historical factors...
specific to this geographic area that are necessary for stickleback trophic divergence (e.g., Schluter and McPhail 1992). As such, the goal of our study was to determine if species pair lakes are characterized by a specific suite of abiotic and (or) biotic lake attributes that are important determinants of divergence of the stickleback species pairs and, by inference, important for their persistence (the environmental constraints hypothesis). While specific historical events appear to be important in providing the opportunity for species pair formation (e.g., multiple invasions from the sea by marine stickleback, absence of barriers on inlet streams; Taylor and McPhail 2000), such historical factors (the historical contingency hypothesis) cannot be the only processes involved, as many more lakes were susceptible to multiple marine transgressions than those that support extant pairs of stickleback species.

Under the environmental constraints hypothesis, we expected that SP lakes would have a broader spectrum of prey resources than NSP lakes, thus providing more opportunity for sticklebacks to diverge into limnetic and benthic forms. Our results, however, showed a similar range of benthic and pelagic resources in SP and NSP lakes. The second expectation that the amount of littoral and pelagic habitat types would differ between SP and NSP lakes was also not supported, as both lake types had comparable amounts of littoral and pelagic habitat as measured by relative littoral area and macrophyte abundance. Our analysis, therefore, suggests that SP and NSP lakes have similar ranges in resource abundance, habitat structure, and other environmental attributes. By contrast, our data confirm Vamosi’s (2003) observation that fish communities differed strongly between SP and NSP lakes; the former were found to contain stickleback only or a combination of stickleback and coastal cutthroat trout. Consequently, it appears that fish community is a major ecological determinant of whether species pairs are found in lakes in coastal British Columbia.

A greater abundance and diversity of fish species may increase both predation and competition, limiting the scope for stickleback diversification. While coastal cutthroat trout are both piscivorous and benthiavorous as adults when they occur without other large predators, they can become more piscivorous when they coexist with rainbow trout (Nilsson and Northcote 1981). Consequentially, in lakes where adult coastal cutthroat and rainbow trout are both present, sticklebacks may be preyed upon heavily in the pelagic zone by coastal cutthroat trout. To reduce the risk of predation it is common for sticklebacks to inhabit areas with more habitat structure, such as the littoral zone (e.g., Walters and Juanes 1993). This coupled with competition for zooplankton with juvenile coastal cutthroat trout and rainbow trout may have prevented stickleback from becoming fully pelagic in some lakes. In the littoral zone of NSP lakes, stickleback are susceptible to competition and predation from adult coastal cutthroat trout and rainbow trout, as well as adult prickly sculpin, which prey both on adult stickleback and their eggs (Pressley 1981). In addition, if competitors for either zooplankton (e.g., juvenile salmonids) or benthos (e.g., prickly sculpin) are more efficient at exploiting these resources, this would reduce resource abundance and decrease per capita resource availability to either benthic or limnetic stickleback, thereby lessening the intensity of any divergent selection. These factors may have prevented stickleback from becoming fully benthic and driven selection for a form that is intermediate in resource and habitat use in NSP lakes. As such, our results are in agreement with Vamosi (2003) and suggest that the relatively low levels to absence of interspe-

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**Table 4.** Underlying rock types of all species pair and non-species pair lakes.

<table>
<thead>
<tr>
<th>Underlying rock type</th>
<th>Basaltic volcanic</th>
<th>Calcareous–alkaline volcanic</th>
<th>Granodioritic intrusive</th>
<th>Quartzdioritic intrusive</th>
<th>Undivided sedimentary</th>
<th>Limestone, marble, calcareous sedimentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balkwill (6)</td>
<td>Chemainus (4)</td>
<td>Ambrose (6)</td>
<td>Brown (5)</td>
<td>Enos (6)</td>
<td>Emily (1)</td>
<td></td>
</tr>
<tr>
<td>Cranby (6)</td>
<td></td>
<td>Hadley (6)</td>
<td>North (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paxton (3)</td>
<td></td>
<td>Little Quarry (5)</td>
<td>Stowell (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Priest (2)</td>
<td></td>
<td>Weston (6)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Note:** Data indicates the lake name with MCLUST group number in parentheses. Rock type data were collected from British Columbia Ministry of Energy Mines and Petroleum Resources (2004).
specific competition and predation were key factors that may have promoted diversification through character displacement by intraspecific resource competition. A similar case was presented by Landry et al. (2007), who compared environmental factors of highly differentiated lake whitefish species pair lakes with those of less differentiated species pair lakes as determined by differences in gill raker counts. Lake whitefish also differentiate as species pairs only in the absence of the lake cisco (Coregonus artedi, a direct pelagic competitor), while in our system stickleback species pairs coexist only with coastal cutthroat trout or when no other fish species are present. Thus, both in the lake whitefish and stickleback species pair systems, the fish community is depauperate and the lack of heterospecific competitors could promote expansion and persistence into new and open niches. Indeed, Lindsey (1981) originally suggested that the presence of sympatric benthic and limnetic forms of lake whitefish may be due to, and change in response to, the presence of other fish species such as ciscoes (whitefishes of the subgenus Leucichthys).

Landry et al. (2007) found that lakes with more highly differentiated lake whitefish species pairs had reduced habitat (lower hypolimnetic O2) and zooplankton availability (lower density and length) and suggested that this would increase intraspecific competition for resources. Our study, however, found similar zooplankton availability (biomass) across SP and NSP lakes. It appears, therefore, that while differences in resource abundance may promote divergence in some lineages and areas, it does not appear to be a general factor determining where species pairs can occur, at least in stickleback. It is also possible that differences between lake groups seen by Landry et al. (2007) and Landry and Bernatchez (2010) could be a result of fish species altering attributes of the environment rather than the environment driving the divergences of fishes (e.g., Harmon et al. 2009; Bassar et al. 2010); that is, greater species differentiation could increase predation on zooplankton and benthos leading to increased production of phytoplankton and therefore increased transfer of detritus to benthic sediments, decreasing hypolimnetic oxygen.

Differences that were observed between attributes of stickleback SP and NSP lakes involved conductivity, total dissolved solids, dissolved inorganic carbon, and alkalinity, all of which were nearly significantly higher in SP lakes. Despite this apparent differentiation, both the highest and lowest, or next to lowest, values measured for each of these variables occurred in an SP lake (because of very low values in newly discovered Little Quarry Lake), thus making water chemistry unlikely to be the primary driver of species pair occurrence (i.e., while these data suggest that species pairs may be more likely to occur in more productive lakes based on water chemistry, it is not a necessary precondition for their evolution).

Our analysis assumes that the conditions necessary for species pair persistence (i.e. current conditions) are similar to those required for their initial speciation (i.e., historic conditions following deglaciation) and that present environmental conditions broadly reflect historic conditions. Although impossible to verify, this assumption seems reasonable; while it is likely that there has been some level of change over time (e.g., associated with terrestrial and aquatic vegetational succession with a watershed), limnological attributes like water chemistry are determined, in large part, by underlying bedrock. Recent increased levels of hybridization events within one species pair lake (Taylor et al. 2006) that has experienced environmental changes associated with invasive crayfish indicate that persistence of sympatric stickleback species is sensitive to environmental change. The continuing presence of species pairs in the other lakes, however, suggests that these lakes have not experienced rapid or severe change relative to historic conditions.

The observation of no detectable difference in planktonic or benthic invertebrate prey abundance between SP and NSP lakes also needs to be tempered by the limitations of our analysis. While we can be confident that there are no consistent major differences between lake types, it remains possible that subtle differences in invertebrate community structure may exist between SP pair and NSP lakes (e.g., Landry and Bernatchez 2010). Cause and effect, however, become increasingly confounded as effects become more subtle (i.e., small differences in invertebrate community structure could be generated by greater predation efficiency between ecotype assemblages in different lakes; e.g., Harmon et al. 2009), rather than differences in invertebrate communities reflecting divergent adaptive landscape that facilitated speciation.

Origin and persistence of species pairs

Our results suggest that although there are no obvious limnological features among those we measured that appear to be a precondition for species pair evolution, low species diversity of potential competitors and predators appears to be a key factor that can promote rapid population divergence (Schluter 2000). Again, however, not all lakes with low species diversity contain species pairs of stickleback. These results and observations imply that some aspect of the history of the lakes must be important to species pair formation. One such possibility is the notion that multiple invasions of the lakes (and how colonization is influenced by local geology, elevation, etc.) by different groups of sticklebacks has been an important historical factor influencing the distribution of species pairs (McPhail 1993; Taylor and McPhail 2000). In addition, the geographic history of the lakes is important when considering what other species have been able to access the lakes, which, as our results and those of Vamosi (2003) suggest, probably has been an important ecological factor in species pair formation.

Our results have important implications for the conservation of stickleback species pairs, all of which are listed as Endangered under Canada’s Species at Risk Act. For instance, a critical factor in the persistence of species pairs would appear to be conserving the current species composition (i.e., keeping invasive species out and conserving coastal cutthroat trout), rather than forming specific abiotic environmental management criteria. The collapse of the Enos Lake species pair into a hybrid swarm following the appearance of the American signal crayfish, Pacifastacus leniusculus (Kraak et al. 2001; Taylor et al. 2006), and extinction of the Hadley Lake species pair following the introduction of the brown bullhead (Hatfield 2001) are examples of the devastating impact that invasive species can have on stickleback species pairs. That being said, habitat loss and degradation is the number one threat to freshwater fishes in Canada, and the effect of invasive species is often compounded by habitat
change (Dextrase and Mandrak 2006). In addition, in Enos Lake, the appearance of the American signal crayfish has been associated with the loss of aquatic macrophytes in the lake that provide important nesting habitat for benthic sticklebacks and whose loss is thought to be a major factor that drove the formation of the hybrid swarm (Taylor et al. 2006; Rosenfeld et al. 2008). Consequently, although we cannot point to one key habitat feature or combination of habitat features that sustain species pairs across all lakes, it is likely that major degradation or loss of habitat in existing SP lakes would compromise the persistence of benthic and limnetic sticklebacks. In addition, although SP lakes may not have unique limnological attributes relative to NSP lakes, water quality (e.g., dissolved oxygen, nutrients, turbidity) needs to be maintained within an appropriate range for the tolerance of the species (National Recovery Team for Stickleback Species Pairs 2007).

Key habitat with regard to stickleback species pairs includes not only the habitat needed to ensure survival of both benthic and limnetic species, but also the features of the environment that promote segregation in mating (Rosenfeld and Hatfield 2006; National Recovery Team for Stickleback Species Pairs 2007; Hatfield 2009). As SP lakes were not significantly different from NSP lakes with regard to their suite of environmental characteristics, the main management goal should be to maintain stickleback species pair lakes within their current values of all environmental parameters measured, particularly those that contribute to reproductive isolation (McPhail 1994; Gow et al. 2006), such as macrophyte abundance, which contributes to habitat heterogeneity and also affects nutrient load, water clarity, and availability of food resources. Substantial alterations to water quality or nutrients from their current levels could potentially be detrimental to the species pairs by changing the abundance and (or) type of food resources available or mate recognition cues (Boughman 2001), thereby increasing hybridization or altering selection pressures on limnetic and benthic species as well as their hybrids (Futuyma and Moreno 1988; Behm et al. 2010). Urbanization and human activities should also be regulated, as they can lead to sedimentation, eutrophication, and fluctuations of water levels, which alter the amounts of littoral and pelagic habitats available to stickleback and their resources. Finally, our study now provides baseline data for all known SP lakes to facilitate environmental monitoring of lake conditions and provides detailed environmental data for lakes that support healthy species pairs as well as for lakes that appear to favour an intermediate form (Enos Lake).

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