Repeatability of adaptive radiation depends on spatial scale: regional versus global replicates of stickleback in lake versus stream habitats

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Running title: (Non)parallel evolution in replicate stickleback adaptive radiations

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ABSTRACT: The repeatability of adaptive radiation is expected to be scale dependent, with determinism decreasing as greater spatial separation among "replicates" leads to their increased genetic and ecological independence. Threespine stickleback (*Gasterosteus aculeatus*) provide an opportunity to test whether this expectation holds for the early stages of adaptive radiation - their diversification in freshwater ecosystems has been replicated many times. To better understand the repeatability of that adaptive radiation, we examined the influence of geographic scale on levels of parallel evolution by quantifying phenotypic and genetic divergence between lake and stream stickleback pairs sampled at regional (Vancouver Island) and global (North America and Europe) scales. We measured phenotypes known to show lake-stream divergence and used reduced representation genome-wide sequencing to estimate genetic divergence. We assessed the scale-dependence of parallel evolution by comparing effect sizes from multivariate models and also the direction and magnitude of lake-stream divergence vectors. At the phenotypic level, parallelism was greater at the regional than the global scale. At the genetic level, putative selected loci showed greater lake-stream parallelism at the regional than the global scale. Generally, the level of parallel evolution was low at both scales, except for some key univariate traits. Divergence vectors were often orthogonal, highlighting possible ecological and genetic constraints on parallel evolution at both scales. Overall, our results confirm that the repeatability of adaptive radiation decreases at increasing spatial scales. We suggest that greater environmental heterogeneity at larger scales imposes different selection regimes, thus generating lower repeatability of adaptive radiation at larger spatial scales.

Keywords: adaptive radiation, convergent evolution, *Gasterosteus aculeatus*, geographic scale, parallel evolution

INTRODUCTION

An unanswered question surrounding adaptive radiation is the extent to which the process is deterministic versus stochastic. That is, if the "tape of life" were rewound and started over multiple independent times, to what extent would a similar set of species evolve when presented with a similar set of environmental niches (Gould 1990; Blount *et al.*, 2018)? This thought experiment cannot be made real outside of the laboratory (MacLean, 2005), and so evolutionary biologists have tended to substitute geographical space for evolutionary time by asking: To what extent do related species evolve in parallel in similar sets of environments that are spatially isolated and evolutionarily independent? A difficulty in addressing this question is that relatively few adaptive radiations are replicated in space; many are instead unique, such as Darwin's finches in the Galapagos Islands (Grant 1981) and the Hawaii honeycreepers (James 2004); or are relatively rare, such as the radiations of cichlids in the three Great Lakes of Africa (Kocher 2004). A few adaptive radiations are sufficiently replicated to make inferences about repeatability; and these cases generally indicate that adaptive radiations have substantial repeatable components, such as the same ecomorphs of *Anolis* lizards evolving on multiple islands in the Greater Antilles (Losos 2009) or the same ecomophs of spiders evolving on multiple Hawaiian Islands (Gillespie 2004). Yet these same radiations often show substantial non-deterministic components, such as "missing" ecomorphs of lizards or spiders on some islands (Gillespie 2004; Losos 2009) or strong contingency depending on physical properties of the system (Brawand *et al.* 2014).

A remaining complication is that repeated adaptive radiations with a given group of organisms are not entirely independent – genetically or ecologically. With respect to genetic non-independence, post-colonization gene flow occurs among islands for *Anolis* lizards (Losos 2009)

47 and Hawaiian spiders (Gillespie 2004), and independent radiations of African cichlids appear to
48 be using some of the same ancestral polymorphisms (Loh *et al.* 2012). With respect to ecological
49 non-independence, adaptive radiations are often replicated on only regional spatial scales (e.g.,
50 among islands in Caribbean or Hawaii), where overall ecological conditions are relatively
51 similar. Hence, the resulting genetic or ecological non-independence could be primary factors
52 shaping deterministic responses. Stated in the same counterfactual sense as "replaying the tape of
53 life", if a few ancestral species had colonized very different geographic locations, to what extent
54 would a similar adaptive radiation take place? Intuition would suggest that such "replicate"
55 adaptive radiations separated by large geographical scale would yield lower repeatability than
56 would replicate adaptive radiations at a more regional scale – as a result of greater genetic and
57 ecological independence.

58 The above-postulated multi-scale adaptive radiation is, in fact, not inevitably
59 counterfactual. Threespine stickleback (*Gasterosteus aculeatus*) are found throughout much of
60 the northern hemisphere, where they show many replicate adaptive radiations in freshwater
61 ecosystems arising from independent colonization events from a common marine ancestor
62 (Colosimo *et al.* 2005). Although the resulting populations are all placed under the same Latin
63 binomial, many of these populations show large genetic differences known to be shaped by
64 adaptation to different environments (Schluter *et al.* 2010; Peichel & Marques 2017). For
65 example, the F_{ST} between parapatric lake-stream stickleback is greater than 0.10 in each of many
66 watersheds colonized postglacially by marine stickleback (Kaeuffer *et al.*, 2012; Roesti *et al.*,
67 2012; Stuart *et al.*, 2017). Hence, different freshwater populations of stickleback can be thought
68 of as representing the early stages of adaptive radiation (Schluter & McPhail 1992; Schluter
69 1996; Taylor & McPhail 1999). With this stickleback system, one can compare regional replicate

70 adaptive radiations (stickleback in similar habitats in different watersheds in the same
71 geographical area) with global replicate adaptive radiations (stickleback in similar habitats in
72 different watersheds in distant geographical areas). We therefore use this system to ask whether
73 genetic and phenotypic divergence is more repeatable (similar magnitude and direction across
74 replicates) at regional than at global scales.
75
76 **Study system**
77 Parapatric pairs of lake and stream stickleback have been used frequently to study the
78 repeatability/predictability/parallelism/convergence of adaptive divergence (e.g., Reimchen *et al.*
79 1985; Lavin & McPhail 1993; Deagle *et al.* 1996; Thompson *et al.* 1997; Reusch *et al.* 2001;
80 Hendry & Taylor 2004; Aguirre 2009; Lucek *et al.* 2010; Eizaguirre *et al.* 2011; Kaeuffer *et al.*
81 2012; Roesti *et al.* 2012; Ravinet *et al.* 2013; Lucek *et al.* 2013; Weber *et al.* 2017; Stuart *et al.*
82 2017). In this study system, stickleback in a lake and its adjoining stream (hereafter, lake-stream
83 population pairs) often evolve similar patterns of phenotypic divergence in independent
84 watersheds. Most notably, lake stickleback generally evolve shallower bodies and more gill
85 rakers than do stream stickleback, a pattern driven at least in part by consistent differences in diet
86 between habitats (Berner *et al.* 2008; Kaeuffer *et al.* 2012). These phenotypic differences tend to
87 be genetically based (Sharpe *et al.* 2008; Hendry *et al.* 2011; Berner *et al.* 2011; Oke *et al.* 2016;
88 Moser *et al.* 2016) and are likely adaptive, given their close association with variation in prey
89 availability (Berner *et al.* 2008; Kaeuffer *et al.* 2012). However, non-parallel aspects of
90 divergence are known where some traits show low levels of parallelism (Stuart *et al.* 2017).
91 Most analyses of parallel evolution in lake-stream stickleback have taken place on
92 regional geographic scales, where we expect generally similar selective regimes and genetic

93 backgrounds: northern Vancouver Island (Lavin & McPhail 1993; Hendry & Taylor 2004;
94 Berner *et al.* 2008; 2009; Kaeuffer *et al.* 2012; Stuart *et al.* 2017), Haida Gwaii (Reimchen *et al.*
95 1985), Ireland (Ravinet *et al.* 2013), and northern Germany (Reusch *et al.* 2001). Several studies,
96 however, have analyzed sets of lake-stream pairs within and between regions: Vancouver Island
97 vs. Switzerland (Berner *et al.* 2010), Vancouver Island vs. artificial habitats in California
98 (Hendry *et al.* 2013) , Iceland vs. Switzerland (Lucek *et al.* 2014b), and Vancouver Island vs.
99 Switzerland vs. Ireland (Lucek *et al.* 2013). These studies revealed important morphological and
100 genetic variation in the direction and magnitude of lake-stream divergence between regions, but
101 they did not formally compare the extent of parallel evolution across different geographic scales.
102 In the present study, we conduct such a comparison by assessing parallel evolution of lake-
103 stream divergence at two very different scales: six pairs within Vancouver Island (hereafter,
104 "regional" comparisons) and six pairs from worldwide localities that encompass the majority of
105 the threespine stickleback range (hereafter, "global" comparisons).
106 We estimate phenotypic (non)parallelism by first comparing the effect sizes of multiple
107 univariate and multivariate models. We then evaluate the degree of phenotypic and genetic
108 (non)parallelism by calculating multivariate phenotypic and genetic (neutral and outlier) lake-to-
109 stream divergence vectors. Then, we quantify the differences among vectors in direction (i.e., the
110 angle between any two divergence vectors) and in magnitude (i.e., the difference in length
111 between any two divergence vectors) (Collyer & Adams 2007; Stuart *et al.* 2017). Small angles
112 and small differences in length imply highly parallel evolution. With these summary statistics for
113 (non)parallel evolution, we ask the following two questions:

114 1. What is the relative extent of phenotypic and genetic (non)parallelism at global versus
115 regional scales?

116 2. What are the potential ecological and genetic drivers of phenotypic and genetic
117 (non)parallelism at each scale?
118
119 MATERIALS AND METHODS
120
121 **Field collections and environmental data**
122 We used minnow traps to collect 39-40 lake and 40 stream stickleback, between May 21 and
123 June 28, 2013, from each of six independent population pairs on Vancouver Island (the
124 "regional" samples: Table S1A, Fig. S1A). We also used minnow traps or nets to collect 40 lake
125 and 40 stream stickleback, between April 10 and August 8, 2014, from each of six other
126 population pairs in North America and Europe (global samples: Table S1B, Fig. S1B). In all
127 collections, we trapped the lake fish at least 100 m away from the junction with any stream (apart
128 from Lake Constance, where the lake fish were captured at the mouth of the stream), and we
129 trapped stream fish at least 100 m away from the junction with any lake. We did not retain any
130 fish younger than one year or any obviously gravid females.
131 We euthanized the fish with an overdose of MS-222 fish anesthetic (Argent Chemical
132 Laboratories, Redmond, WA), and removed the right pectoral fin, which was preserved in 95%
133 ethanol for genetic analysis. We then fixed the fish in 10% neutral buffered formalin (VWR,
134 Radnor, Pennsylvania). To facilitate gill raker counts, we stained the fish using alizarin red dye.
135 To do so, we first soaked the fish in water for 24 hours, then stained the fish in a solution of
136 alizarin red and 0.5% KOH for 24 hours, and then performed a second soak in water for 24 hours
137 to remove excess stain. We then stored the fish in 40% isopropyl alcohol until they were
138 processed further (see below), approximately four weeks later.

To characterize the environment at each site, we measured several habitat variables. Using the Google Earth Pro V7.3 software, we measured total lake area (m^2), lake perimeter (m), and mean lake-stream elevation for each pair (m). Using measuring sticks and a tape meter, we also measured stream depth (cm), lake depth (cm), and stream width (cm) at each trap as well as stream flow (cm/s) using a flow meter.

Morphological measurements

In the laboratory, we photographed the left and ventral surfaces of each fish against a grid marked at 1 cm intervals. The grid was placed at a constant distance from the lens of a Canon G11 digital camera, which was positioned in the same plane (using a surface level) as the grid. Before the photograph was taken, we removed the left pectoral fin of each fish and pinned it at maximum extension onto the same grid (for measurements of fin area, perimeter, and the length of fin rays). We also inserted small pins into the fish to help indicate otherwise cryptic homologous anatomical points for subsequent geometric morphometrics (Berner *et al.* 2009). After photographing, we measured several morphological traits (described below) with a focus on body shape and gill raker traits because repeated patterns of phenotypic divergence between lake and stream fish often involve these traits (Sharpe *et al.* 2008; Berner *et al.* 2008; Hendry *et al.* 2011; Berner *et al.* 2011; Kaeuffer *et al.* 2012; Oke *et al.* 2016; Moser *et al.* 2016). In addition, we dissected each fish to determine sex by gonad inspection.

From the photographs, we measured 25 linear trait distances (Table S2), as well as the area and perimeter of the pectoral fin using the image processing software *Fiji* (Schindelin *et al.* 2012). We standardized these 27 univariate measurements to a common standard body length with the allometric formula $M_S = M_0 (L_S / L_0)^b$, where M_S is the standardized trait

measurement, M_0 is the unstandardized trait measurement, L_S is the overall mean body size of all fish in a given analysis, and L_0 is the standard length of the individual (Elliott *et al.* 1994; Lleonart *et al.* 2000). Standard length is defined as the distance from the anterior tip of the upper jaw to the posterior end of the hyperal plate. The exponent b was calculated as the common within-group slope (Reist 1986) from a linear mixed-effect model regressing $\log_{10}(M_0)$ on $\log_{10}(L_0)$ with pair as the random factor.

We counted the number of gill rakers on the first right gill arch *in situ*, then removed the right gill arch and photographed it under a dissecting scope using an ocular micrometer and a Canon EOS Rebel T5 digital camera. To quantify gill raker length, we measured the curved distance along the centre of the three longest gill rakers. We then calculated raker spacing as $5/l$, where l is the mm distance along the gill arch spanned by the five longest rakers. These four measurements (lengths of the three longest gill rakers, and gill raker spacing) were standardized to a common standard body length as described above.

To quantify overall geometric morphometric shape differences, we placed 19 homologous landmarks on the lateral photographs using the *TPSdig* software (Rohlf 2006) (Fig. S2). We then superimposed the 19 landmarks using a generalized Procrustes analysis (GPA) with *R* package *geomorph* (Adams & Otárola Castillo 2013). We performed this analysis for the global samples and the regional samples combined so that all fish would be positioned in the same shape space. These alignments resulted in 38 Procrustes residuals that described the shape differences between specimens unrelated to scale, rotation, or translation. The 38 principle components (PCs) derived from the 38 Procrustes residuals were then allometrically adjusted for centroid size and body depth using the above common within-slope approach (Reist 1986; Lleonart *et al.* 2000; Rolshausen *et al.* 2015). The first PC was found to describe shape differences due to

185 vertical bending of the fish produced during storage. We therefore excluded this artefactual PC
186 from further analyses.
187
188 **DNA extraction and restriction site associated DNA sequencing library construction**
189 DNA was extracted from stickleback fin clips using the Wizard® SV Genomic Purification kit
190 (Promega® Corp., Madison, USA) and quantified using Picogreen® ds DNA assay (Thermo
191 Fisher Scientific, Waltham, USA) on an Infinite® 200 Nanoquant (Tecan Group Ltd. Männedorf,
192 Switzerland). All samples were normalized to a dsDNA concentration of 15ng/µl, re-quantified,
193 and pooled according to sampling location. Thus, we created 24 pools of 40 individuals each,
194 consisting of 12 pools for each geographic scale (at each scale, six lake and six stream pools).
195 ddRAD PoolSeq libraries were prepared following a modified version of the Peterson *et*
196 *al*. (2012) protocol. Briefly, pooled samples were first digested using the NLAIII and MluCI
197 enzymes (New England Biolabs Inc., Frankfurt, Germany), individually barcoded using unique
198 P1 flex-adapters (Peterson *et al.* 2012), and pooled together into a single library. Fragments of
199 371-416bp were extracted using a Pippen Prep® 2% MarkerB 100-600bp cassette (Sage Science
200 Inc., Beverly, USA). In post-size selection, we isolated fragments including at least one biotin-
201 tagged P2 adapter, using streptavidin-couple beads (Dynabeads® M-270, Thermo Fisher
202 Scientific, Waltham, USA). The library was then PCR amplified in five separate aliquots (12
203 cycles, Phusion High Fidelity® PCR kit, New England Biolabs Inc., Frankfurt, Germany). We
204 used Agencourt® AMPure XP beads (Beckman Coulter, Indianapolis, USA) to clean samples
205 after each enzymatic reaction.
206
207 **Sequencing and ddRAD data processing**

208 Barcoded ddRAD samples were sequenced on four lanes of an Illumina Hi-Seq 2500 for 100 bp
209 paired-end, and demultiplexed using *STACKS* (Catchen *et al.* 2013) v. 1.13 (*process_radtags* -P,
210 -p, -r, -i, --inline_index, --disable_rad_check). Resulting FASTA files were trimmed using
211 *PoPoolation* (Kofler *et al.* 2011a) to eliminate low quality regions (--min-length 50 --quality-
212 threshold 20). Reads from all 24 pools were aligned to the threespine stickleback genome
213 (release-084, Ensembl.org) separately using *BWA mem* v. 0.7.13 (Li & Durbin 2009). We then
214 used *SAMtools* (Li *et al.* 2009) v. 1.3.1 to convert the resulting sam files to sorted bam format,
215 keeping only reads with mapping quality above 20 (*samtools view* -q 20). A pileup file was then
216 generated using *SAMtools* v. 1.3.1 (*samtools mpileup* -B), and filtered for completeness by
217 keeping reads with a minimum coverage of five. We then converted the pileup file to a sync file
218 using *PoPoolation2* (Kofler *et al.* 2011b) for further downstream analysis.
219
220 **Statistical analysis**
221 All statistical analyses were performed in *R* 3.2.2 (R Core Team 2015).
222
223 *Variation in environmental variables*
224 To test whether variation in habitat characteristics was greater at the global than regional scale,
225 we compared within-scale variation for each environmental parameter using Bartlett's tests
226 (Bartlett 1937). In addition, we calculated levels of among-lake and among-stream variation for
227 each environmental variable at each scale separately. To calculate among-lake and among-stream
228 variation at each scale, we computed ANOVAs at each scale separately with each environmental
229 variable as dependent variable and watershed as predictor. Among-lake or among-stream

230 variation was calculated by dividing the pair term sum of squares by the total number of pairs in
231 each scale respectively.
232
233 *Univariate and multivariate phenotypic analysis*
234 To reduce the large number of univariate traits, we first performed a principal components
235 analysis (PCA) on the size-standardized trait values for all samples (global and regional)
236 combined. To consider phenotypic differences between lake and stream stickleback across pairs
237 for the global and regional sample sets separately, we then used multivariate analysis of
238 covariance (MANCOVA) models, with Wilks' lambda (λ) as the test statistic. We tested for
239 differences in specific trait sets for the regional and global samples separately using three
240 different MANCOVA models, with dependent variables as: i) all 27 (size-standardized)
241 univariate measurements, ii) the five gill raker traits only, and iii) the PCs derived from the 38
242 Procrustes residuals.
243 To understand the contribution to parallelism of a subset of individual traits likely
244 important to lake-stream divergence (body depth, gill raker number, gill raker spacing, and mean
245 length of the three longest gill rakers), we analyzed each of these traits separately using an
246 analysis of covariance (ANCOVA) at each regional and global scale. The independent variables
247 in every model were the fixed effects of sex, pair, and habitat (lake versus stream), along with all
248 two-way interactions. Centroid size was also included as a covariate (along with its two-way
249 interactions) to control for any remaining size effects.
250
251 *Estimation of genome-wide population differentiation*

252 We evaluated the genetic relationship within pairs by estimating the fixation index (F_{ST}) for each
253 lake-stream pairwise comparison using *PoPoolation2* (Kofler *et al.* 2011b), applying a number
254 of stringent criteria (see below) to define genomic sites for analysis across the entire genome.
255 The accuracy of allele frequency estimation of pooled individuals is highly dependent on
256 sequence coverage (Schloetterer *et al.* 2014). Therefore, to reduce stochastic error and increase
257 the accuracy of F_{ST} estimates, for each pairwise combination we used a minimum minor allele
258 count of two across all pools, a high sequence coverage filter (between five and 500 within each
259 pool), and a large 100-kb non-overlapping sliding window. This window size led to mean of
260 146.74 SNPs per window (± 90.29, median = 123). We visualized population structure by
261 building neighbour-joining trees for both scales separately based on average pairwise F_{ST} values.
262

263 *Evaluation of (non)parallelism at both geographic scales*

264 We used two different approaches to estimate the extent of parallel evolution at the two
265 geographic scales.

266 First, we compared the effect sizes of the habitat and habitat-by-pair interaction terms
267 from the MANCOVA and ANCOVA analyses of phenotype (partial η^2 and η^2 values,
268 respectively) (Oke *et al.* 2016; Stuart *et al.* 2017). A large effect size for the habitat term
269 suggests consistent lake versus stream differences shared across pairs, i.e., parallel evolution. A
270 large effect size for the pair-by-habitat interaction term, on the other hand, suggests idiosyncratic
271 lake-versus-stream differences across pairs, i.e., non-parallel evolution. Thus, if the ratio of the
272 habitat to the interaction effect size is greater in the pairs sampled at a regional scale as
273 compared to the pairs sampled at a global scale, we infer that lake-stream divergence is more
274 parallel at the regional scale.

275 Second, we calculated lake-to-stream divergence vectors for our phenotypic and genetic
276 data. Each vector connects the multivariate lake mean to the multivariate stream mean and
277 quantifies the magnitude and direction of lake-stream divergence for each population pair
278 (Adams & Collyer 2009). Then, to describe the extent to which vectors diverged in parallel
279 across pairs, we calculated: θ, the angle between any two vectors; L, the length of the vector for
280 each pair; and ΔL, the difference in length between any two vectors. Strict parallel divergence
281 between pairs would correspond to $\theta = 0$ and $\Delta L = 0$. Vector analysis for the phenotypic and
282 genetic datasets follows Stuart *et al*. (2017) and is briefly described below:

283 (i) Phenotypic: For each pair, for each trait (Table S2), we ran a t-test comparing the
284 distribution of a trait in a lake to that in its adjoining stream. Concatenating each
285 t-statistic resulted in a 1xN vector of scale-standardized lake-stream differences,
286 thereby constituting our lake-stream divergence vector. We then calculated angles
287 between phenotypic divergence vectors (θ_P) as the arc-cosine of the Pearson
288 correlation for each vector pair. L_P was the multivariate Euclidian length of each
289 vector, and ΔL_P was the difference in length between any two divergence vectors.
290 We tested for differences in θ_P and ΔL_P between global and regional scales using
291 a two-tailed t-test.

292 (ii) Genetic: Using the sync file of allele frequencies extracted with *PoPoolation*, we
293 first calculated the frequency of the minor allele at each SNP. We then used PCA
294 (*R prcomp*) to reduce our large SNP dataset into a smaller number of quantitative
295 axes of genetic differentiation. We saved populations' scores of all the resulting
296 24 axes. We calculated the centroid for each lake and each stream, for these 24
297 axes, to obtain vectors in genotypic space. Thus, each row of the resulting matrix

represents the estimate of genetic divergence between the two habitats of the same pair, thereby constituting the genetic lake-stream divergence vector for each pair. First, we performed this analysis on a set of 'outlier' SNPs whose lake-stream F_{ST} values fell within the top 5% of estimated values for each of the 12 lake-stream pairs, thus representing markers putatively linked to selected loci (175,302 outlier loci). To test for differences in genetic divergence between scales, we performed the genetic vector analysis as described above and across both scales to obtain measures of $\theta_{OUTLIERS}$ and $\Delta L_{OUTLIERS}$. We tested for differences in $\theta_{OUTLIERS}$ and $\Delta L_{OUTLIERS}$ between global and regional scales using a two-sided t-test. Second, we calculated θ, L, ΔL by excluding these outlier SNPs to obtain a set of markers likely to represent neutral population genetic processes (327,291 neutral loci - hereafter θ_G, L_G, and ΔL_G). We tested for differences in θ_G and ΔL_G between global and regional scales using a two-sided t-test. Values of θ_G, L_G, and ΔL_G were used further to investigate potential constraints on divergence (see below). Finally, we considered whether neutral processes could be responsible for phenotypic divergence by testing for a linear relationship between θ_P values and averaged between-watershed neutral F_{ST} at each scale separately using linear models with F_{ST} as predictor and θ_P as response variable.

RESULTS

Variation in environmental variables

Overall, environmental variation is higher at the global scale. For all environmental variables (in both lake and stream habitats), variation among watersheds was significantly greater at the global

321 than regional scale (Table 1, Fig. S3). Similarly, among-lake and among-stream variation was
322 greater at the global than at the regional scale (Fig. S4).
323
324 **Univariate and multivariate phenotypic analysis**
325 Consistent with previous work, we observed variable levels of (non)parallelism in univariate and
326 multivariate traits. The first and second PCs for the 27 univariate measurements explained 24.7%
327 and 17.5% of the total variance, respectively, and every trait except the length of the dorsal fin
328 loaded negatively on the first axis. The within-pair difference between lake and stream fish on
329 PC1 was always in the same direction, indicating that any habitat-by-pair interaction on PC1 was
330 due to differences in the magnitude of divergence, rather than the direction of divergence (Fig.
331 1). The MANCOVA models revealed significant effects of habitat, pair, and their interaction on
332 all sets of traits at both regional and global scales (Table S3).
333 ANCOVAs for individual traits revealed that the effects of habitat, pair, and their
334 interaction were always significant at both the regional and global scales (Table S4).
335 Specifically, stream fish were generally deeper bodied than lake fish for the regional pairs, often
336 with similar magnitudes of difference. However, in the global pairs, stream fish were sometimes
337 not deeper bodied than their lake counterparts (Fig. 2A, B). Lake fish also generally had more
338 and longer gill rakers than stream fish (Fig. 2C-F), although this difference was more
339 pronounced at the regional scale. Gill raker spacing was greater in stream fish at the regional
340 scale but not at the global scale (Fig. 2G, H).
341
342 **Evaluation of (non)parallelism at both geographic scales**
343 *Differences in effect sizes of multivariate models*

344 Significant differences in effect sizes from the MANCOVAs and ANCOVAs revealed that
345 phenotypic parallelism was greater at the regional than at the global scale. Indeed, the ratio of the
346 habitat term to the habitat by pair interaction term was always greater at the regional than at the
347 global scale – across all models. In the MANCOVA models, the averaged ratio was 3.2 at the
348 regional scale and 1.5 at the global scale. In the ANCOVA models, the averaged ratio was 6.7 at
349 the regional scale and 0.3 at the global scale (Table 2, Fig. 3; see Tables S3 and S4 for more
350 detailed results about these models). This finding suggests that lake-stream divergence in
351 phenotypic traits was more parallel at the regional than global scale.
352
353 *Vector analysis*
354 F_{ST} extracted from the sliding window analysis revealed that lake-stream populations in each
355 pair were each other's sister taxon, regardless of the geographic scale of comparison (Table S5,
356 Fig. S5). This pattern is consistent with evolutionarily independent lake-stream divergence in
357 each pair, although ongoing gene flow could also contribute to this pattern.
358 Overall, we found phenotypic divergence to be more parallel at the regional than global
359 scale. Multivariate vector analysis of the phenotypic data revealed a continuum of lake-stream
360 parallelism at both scales (Tables S6 and S7). The mean angle between any two phenotypic
361 vectors (θ_P) was 61.63° ± 12.24sd at the regional scale and 90.17° ± 15.98sd at the global scale
362 (Fig. 4A; $t = 5.49$, df = 26.23, $P = 8.97e^{-06}$). In addition, phenotypic vector lengths, which
363 measure the magnitude (but not direction) of lake-stream divergence, also exhibit greater
364 similarity among regional pairs than among global pairs. Pairwise comparisons of vector lengths
365 (ΔL_P) averaged -2.85 ± 10.57sd locally, and 6.52 ± 7.98sd globally (Fig. 4C; t = 3.16, df =
366 14.00, $P = 0.007$).

367 At the genetic level, divergence was significantly more parallel at the regional than global
368 scale, but only for outlier loci: The mean angle between any two outlier genetic vectors
369 ($\theta_{OUTLIERS}$) was 85.51° ± 5.81sd at the regional scale and at 89.15° ± 3.83sd the global scale (Fig.
370 4B; t = 2.02, df = 24.21, P = 0.04). The mean angle between any two neutral genetic vectors (θ_G)
371 was 89.32° ± 2.72sd at the regional scale and 90.03° ± 1.81sd at the global scale (t = 0.85, df =
372 24.36, P = 0.41). Genetic vector lengths were significantly smaller at the regional than global
373 scale at both outlier and neutral loci. Outlier vector lengths ($\Delta L_{OUTLIERS}$) differed by a mean of
374 1.49 ± 4.12sd and 10.29 ± 13.05sd at the regional and global scales, respectively (Fig. 4D; t =
375 2.49, df = 16.75, P = 0.02). Neutral genetic vector lengths (ΔL_G) differed by a mean of 0.46 ±
376 1.54sd and 6.12 ± 6.77sd at the regional and global scales, respectively (t = 3.16, df = 15.44, P =
377 0.006). Finally, θ_P and averaged between-watershed neutral F_{ST} were not significantly associated
378 at either scale (regional: t = -0.88, df = 13, P = 0.39, R^2 = -0.24; global: t = 1.12, df = 13, P =
379 0.28, R^2 = 0.29).
380 If different watersheds share the same genetic architecture underlying adaptive traits, we
381 would expect outlier loci under selection to overlap across population pairs when lake-stream
382 divergence proceeds in parallel. However, 63% of the outlier loci were unique to a specific
383 watershed across all watersheds, whereas 61% and 77% of outliers were unique to a specific
384 watershed at the regional and global scales, respectively (21% and 4% were shared across a
385 minimum of two watersheds at the regional scale and global scale, respectively). As an overall
386 summary, comparisons of vector pairs showed less parallelism at the global scale than at the
387 regional scale, at both the phenotypic and genetic outlier levels; for neutral genetic loci,
388 (non)parallelism was similar at both scales. Importantly, perfect parallel divergence is non-
389 existent at both phenotypic and genetic levels and at both geographic scales.

390

391 DISCUSSION

392 We assessed the influence of geographic scale on the repeatability of adaptive radiation by
393 quantifying levels of phenotypic and genetic parallelism in lake-stream population pairs of
394 threespine stickleback. In agreement with previous work conducted over restricted spatial scales
395 (Lavin & McPhail 1993; Hendry & Taylor 2004; Berner *et al.* 2008; 2009; Kaeuffer *et al.* 2012;
396 Stuart *et al.* 2017), we found a continuum of phenotypic and genetic parallelism, with lake-
397 stream divergence being more pronounced for some traits, and in some population pairs, than in
398 others. At the phenotypic level, parallelism was especially notable in body depth and gill raker
399 number – also consistent with previous work. When comparing between geographic scales,
400 parallelism in phenotypes and genetic outliers was greater at the regional scale (Vancouver
401 Island) than at the global scale (Europe and both sides of North America). In contrast,
402 parallelism was similar across scales for neutral genetic loci. We suggest that the observed
403 disparities in phenotypic and genetic parallelism between scales are likely the result of
404 differences in environmental variation, selection pressures, and genetic architecture of traits
405 between scales. We develop these ideas further in the text below.

406

407 *Phenotypic (non)parallelism*

408 Univariate, multivariate, and vector analyses all revealed that phenotypic parallelism was greater
409 at the regional than the global scale (Fig. 4A, C). This difference between scales was especially
410 pronounced in specific morphological traits, such as body depth and gill raker length, that are
411 known to have a strong heritable basis and that have been previously implicated in adaptive

412 parallel evolution (Sharpe *et al.* 2008; Berner *et al.* 2008; Hendry *et al.* 2011; Berner *et al.* 2011;
413 Kaeuffer *et al.* 2012; Miller *et al.* 2014; Oke *et al.* 2016; Moser *et al.* 2016).

414 Lake-stream body depth divergence was highly parallel in that stream fish always had
415 deeper bodies than lake fish; yet the level of this parallelism was almost always greater at the
416 regional than the global scale (Figs. 1-4). A similar pattern emerged for gill raker number, with
417 strong parallelism evident in lake-stream divergence at the regional scale, consistent with
418 previous studies (Berner et al. 2008; 2009; Kaeuffer et al. 2012; Stuart et al. 2017). Although
419 lake-stream parallelism in these traits was also present at the global scale, it was less pronounced
420 than at the regional scale (Table 2, Fig. 3). For instance, also in accordance with previous studies
421 (Berner et al. 2008; Kaeuffer et al. 2012), gill raker spacing and length exhibited low levels of
422 parallelism (Fig. 2E-H), yet the parallelism that was present in these traits was greater at the
423 regional than at the global scale (Fig. 3B).

424 With environmental variation across watersheds being greater at global than regional scales
425 (Figs. S3, S4), our data are consistent with the hypothesis that reduced environmental variation
426 among watersheds at smaller spatial scales enhances (or enables) stronger parallel evolution. For
427 instance, divergent selection related to diet, foraging mode, and swimming performance has been
428 implicated in variation in body shape and gill rakers between lake and stream stickleback. In
429 lakes, sticklebacks generally feed on small limnetic prey (Berner *et al.* 2008; 2009; Kaeuffer *et*
430 *al.* 2012), leading to selection for an elongated body shape (Webb 1984; Walker 1997) and
431 longer/more numerous gill rakers (Bentzen & McPhail 1984). In streams, stickleback commonly
432 feed on benthic prey, leading to selection for deeper bodies and shorter/less numerous gill rakers
433 (Bentzen & McPhail 1984; Berner *et al.* 2008; 2009; Hendry *et al.* 2011; Kaeuffer *et al.* 2012).
434 Body depth and gill raker number are both known to be strongly heritable (Hermida *et al.* 2002;

435 Aguirre *et al.* 2004; Oke *et al.* 2016; McPhail), and as such, the stronger parallelism of these
436 traits at regional than at global scales presumably reflects greater determinism of selection owing
437 to more consistent ecological differences between these habitat types across replicate watersheds
438 on smaller spatial scales.
439
440 *Genetic (non)parallelism*
441 Like phenotypic parallelism, genetic parallelism at outlier loci was more pronounced at the
442 regional than the global scale. In contrast, for neutral loci, genetic parallelism was similar at the
443 two geographic scales. Why might we observe greater parallelism at the regional versus global
444 scales for outlier genotypes? One explanation is that these outliers are associated with the
445 underlying genetic basis of adaptive phenotypes, which – as described above – show greater
446 parallelism at the regional than the global scale. However, phenotypic parallelism does not
447 necessarily lead to genetic parallelism if different genetic changes are capable of attaining
448 similar phenotypic outcomes (Kautt *et al.* 2012; Conte *et al.* 2012; Westram *et al.* 2014; Le
449 Moan *et al.* 2016). The greater parallelism of outlier loci at regional than global scales might thus
450 reflect more similar genetic architectures and higher levels of shared standing genetic variation
451 available to selection at smaller geographical scales (Barrett & Schluter, 2008; Agrawal &
452 Stinchcombe, 2009; Flint & Mackay, 2009; Terekhanova *et al.*, 2014; Saltz *et al.*, 2017; Llaurens
453 *et al.*, 2017; Bassham *et al.*, 2018). In support of this idea, we found that Vancouver Island lake-
454 stream pairs shared more outlier loci than did lake-stream pairs at the global scale (21% of
455 outliers were shared across a minimum of two pairs at the regional scale but only 4% of outliers
456 were shared across a minimum of two pairs at the global scale).
457 In contrast to the outlier loci, we detected no differences between geographic scales in the

levels of (non)parallelism at neutral loci – at least when quantified using the *direction* (θ_G) of genetic vectors. However, neutral genetic vectors were somewhat more similar in *magnitude* (ΔL_G) at the regional than the global scale. This last result might suggest a role for gene flow in reducing divergence between watersheds over smaller spatial scales – that is, they are less genetically "independent" not only in their colonization but also in their contemporary genetic connections (Stuart *et al.* 2017; Berner & Roesti 2017; Haenel *et al.* 2018). It is also possible that these patterns could be driven by a difference in the effect of drift in shaping the extent of lake-stream neutral genetic divergence between scales. However, the lack of differences in θ_G between scales, and the lack of a relationship between θ_P and the average between-watershed neutral F_{ST} at either scale, imply that neutral processes are unlikely to be responsible for phenotypic divergence at either scale.

Constraints on parallel evolution at regional versus global scales

Lake-stream pairs at both scales showed relatively low levels of parallel evolution, especially for multivariate traits. Indeed, the mean angles between any two phenotypic vectors (θ_P) were 61.63° ± 12.24 sd and 90.17° ± 15.98 sd at the regional and global scales, respectively. Similarly, the mean angles between any two genetic outlier vectors ($\theta_{OUTLIERS}$) were 85.51° ± 5.81 sd at the regional scale and 89.15° ± 3.83 sd at the global scale. Multiple factors can restrict the level of parallel evolution at both scales (Bolnick *et al.* 2018). For instance, a number of ecological variables that differ between lakes and streams are known to affect the degree of stickleback divergence, including diet (Berner *et al.* 2008; Kaeuffer *et al.* 2012; Ravinet *et al.* 2013), parasite load (Eizaguirre *et al.* 2011), flow regimes (generally higher in streams: Jiang *et al.* 2015), and predators (predatory fish and birds likely more common in lakes: Reimchen *et al.* 1985;

481 Reimchen 1994). However, the direction and magnitude of lake-stream divergence in these
482 factors is not always consistent (Berner *et al.* 2008; Kaeuffer *et al.* 2012; Stuart *et al.* 2017).
483 Parasite communities are not universally more diverse in lakes than streams (Feulner *et al.*
484 2015), flow can be low in many stream reaches (Moore *et al.* 2007), and fish and bird predators
485 vary dramatically among different lakes – and among different streams (Moodie & Reimchen
486 1976). Thus, variation across watersheds in the consistency of the ecological differences between
487 lake and stream habitats could disrupt patterns of parallel evolution. We found this type of
488 among-watershed environmental variation to be highest at the global geographic scale, and
489 suggest that this non-parallel ecological divergence is likely to be a strong contributing factor to
490 the non-parallel phenotypic and genetic divergence. However, it is noteworthy that univariate
491 traits, such as body depth and gill raker number, were highly parallel in comparison to
492 multivariate traits. One possible explanation is that "simple" univariate traits generally
493 experience more parallel selection than "complex" multivariate traits for which multiple
494 evolutionary solutions are possible to the same ecological challenge (Thompson *et al.* 2017).
495 At the genetic level, several factors can constrain the level of parallel evolution. Parallel
496 evolution at loci associated with ecologically-relevant phenotypes is likely shaped by
497 environmental heterogeneity for the same reasons as those mentioned for phenotypes above (Orr
498 2005). Physically proximate populations are also be expected to be more closely related
499 genetically (both because of colonization and gene flow), and therefore could be expected to
500 display greater levels of parallelism due to sharing a more similar molecular basis of phenotypes
501 (Barrett & Schluter 2008; Conte *et al.* 2012). For instance, with partially-shared pools of
502 standing genetic variation, closely related populations might have more similar genetic solutions
503 to similar adaptive problems (Barrett & Schluter 2008). The Vancouver Island populations in our

study likely diverged from a marine ancestor at a similar time ~ 5,000 years ago (Stuart *et al.* 2017). In contrast, the global scale populations are estimated to have diverged at quite different times: as early as 5,000-15,000 years ago in Alaska (Reger & Pinney, 1996; Cresko *et al.*, 2004) to hundreds to thousands of years in Europe and Iceland (Moser *et al.* 2012; Lucek *et al.* 2014b; Roesti *et al.* 2015). This discrepancy between scales in divergence times might also explain the observed differences in parallel evolution; for the reasons outlined above, populations with similar divergence times might be more parallel than populations with diverse divergence times. Gene flow is another factor known to effect adaptive divergence (Slatkin, 1987; Lenormand, 2002; Garant *et al.*, 2007) and therefore also evolutionary (non)parallelism. On the one hand, populations exchanging genes will become more genetically similar, which can promote parallelism. On the other hand, gene flow between populations in distinct environments can impede regional adaptation (Garant *et al.*, 2007). Thus, gene flow within watersheds containing populations inhabiting distinct lake and stream habitat types should generally constrain their adaptive differentiation – as has frequently been inferred for lake-stream stickleback (Hendry *et al.*, 2002; Moore *et al.*, 2007; Berner *et al.*, 2009; Stuart *et al.*, 2017). If levels of gene flow are high within some watersheds and low in others, parallelism could be restricted, in particular as reflected by ΔL (Stuart *et al.* 2017). We detected lower ΔL_P and ΔL_G at regional than global scales, suggesting that the role of within-watershed gene flow in constraining adaptive differentiation could be more consistent at smaller spatial scales. However, $\Delta L_{OUTLIERS}$ was not different between the regional and global scales, which could imply similar levels of within-watershed gene flow across scales for outlier loci. The differences in ΔL_P may then reflect either differing levels of phenotypic plasticity between scales (Oke *et al.* 2016), or that our genetic markers have not included the loci associated with the traits experiencing parallel adaptive

527 divergence between habitats, which might show distinct patterns of admixture to those described
528 here (Nosil *et al.* 2009).
529
530 *Implications for adaptive radiation*
531 Threespine stickleback are a remarkable example of adaptive radiation: they show dramatic
532 phenotypic and evolutionary divergence from very small to very large geographical scales (Bell
533 & Foster, 1994; McKinnon and Rundle 2002). Moreover, most of this divergence has played out
534 over just a few thousand generations; and, in some cases, impressive freshwater divergence from
535 a marine ancestor has evolved in less than fifty years (Kimmel *et al.*, 2012; Lescak *et al.*, 2015;
536 Bassham *et al.*, 2018). At the same time, different stickleback populations in the adaptive
537 radiation can still interbreed in nearly all cases (Hendry, 2009); and they show at most two forms
538 in a single location (but see: Hippel von & Weigner, 2004). Hence, the applicability of the
539 stickleback radiation to inferences about other classic adaptive radiations should be limited to the
540 early stages of those radiations. Yet this potential limitation is also a strength in some respects;
541 by capturing multiple replicates of the very early stages of adaptive radiation, we can more easily
542 draw inferences about the processes that promote and constrain adaptive radiation – because we
543 are removed from concern over confounding effects of non-causal factors that accumulate after
544 the radiation is largely complete.
545 From this perspective, we found that, when replicate adaptive radiations are farther apart
546 in space, and therefore are more likely to be independent, they show greater dissimilarities as
547 reflected in lower phenotypic and genetic parallelism. We suggest that this scale-dependent
548 repeatability is primarily driven by ecological rather than genetic factors. That is, the set of
549 divergent environments in a given location will differ more from the same set of divergent

environments in another location when those locations are situated farther apart in space. Indeed, the largest differences between scales appears to derive from phenotypes and genetic outliers, as opposed to neutral genetic markers. Further effort should be directed toward exploring both the ecological and genetic causes of scale-dependent determinism.

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AUTHOR CONTRIBUTIONS

AP, DH, APH, and RDHB designed the study. YS and DIB provided sequence and environmental data from the regional pairs. FAvH, MK, TK, SS and BKK provided fish samples and collected environmental data from the global pairs. AP and DH extracted sequence data from the global pairs and phenotypic data from both scales. AP and DH analyzed the data. AP and DH wrote the manuscript with input from all coauthors.

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TABLES AND FIGURES

Table 1. Within-scale mean and standard deviation in environmental variables. Results from Bartlett's tests of homogeneity of variances are presented with K-squared (K^2), degrees of freedom (df), and *P*-values (*P*-value). *P*-values < 0.05 are in bold.

	Mean		Standard deviation		Bartlett test		
	Regional	Global	Regional	Global	K^2	df	*P*-value
Lake area (m^2)	8.50^{e+05}	1.13^{e+08}	7.16^{e+05}	2.25^{e+08}	45.98	1	**<0.001**
Lake perimeter (m)	6.19^{e+03}	7.19^{e+04}	5.84^{e+03}	1.00^{e+05}	19.56	1	**<0.001**
Lake-stream elevation (m)	106.33	100.17	72.80	159.75	2.56	1	0.109
Lake depth (cm)	14.02	33.25	12.93	35.96	3.84	1	**0.004**
Stream depth (cm)	73.14	75.37	738.62	4438.49	3.25	1	0.070
Stream width (cm)	505.62	2016.57	122.96	2883.69	22.39	1	**<0.001**
Stream flow (cm/s)	3.72	131.60	5.16	130.22	23.06	1	**<0.001**

Table 2. Results from MANCOVAs on the multivariate traits (A) and ANCOVAs on single traits (B) at both regional and global scales. Effect sizes (Partial η^2) and their lower and upper 95% confidence limits (Lo.CI; Up.CI) of the habitat (Hab.) and habitat by pair interaction (Hab. x Pair) as well as their ratios (Ratio: Hab./Hab. x Pair) are reported.

(A) - MANCOVAs

	REGIONAL							GLOBAL						
	Hab.	Lo. CI	Up. CI	Hab.xPair	Lo. CI	Up. CI	*Ratio*	Hab.	Lo. CI	Up. CI	Hab.xPair	Lo. Ci	Up. CI	*Ratio*
Univariate	0.81	0.79	0.85	0.35	0.34	0.46	2.30	0.41	0.30	0.89	0.23	0.22	0.35	1.75
Gill rakers	0.45	0.41	0.65	0.09	0.06	0.19	5.04	0.03	0.18	0.05	0.03	0.02	0.11	0.95
Body shape	0.85	0.85	0.88	0.38	0.34	0.46	2.25	0.34	0.03	0.41	0.19	0.18	0.25	1.82

(B) - ANCOVAs

	REGIONAL							GLOBAL						
	Hab.	Lo. CI	Up. CI	Hab.xPair	Lo. CI	Up. CI	*Ratio*	Hab.	Lo. CI	Up. CI	Hab.xPair	Lo. Ci	Up. CI	*Ratio*
Body depth	0.48	0.39	0.72	0.02	0.02	0.11	21.20	0.02	0.01	0.21	0.03	0.02	0.18	0.54
Gill raker number	0.29	0.12	0.58	0.09	0.05	0.14	3.39	0.01	0.01	0.11	0.03	0.02	0.10	0.54
Mean gill raker length	0.03	0.02	0.11	0.10	0.10	0.34	0.22	0.00	0.00	0.29	0.04	0.04	0.14	0.03
Gill raker spacing	0.15	0.10	0.27	0.08	0.07	0.20	1.35	0.00	0.00	0.25	0.02	0.02	0.12	0.00

Figure Legends

Figure 1: Mean and standard error of score on PC1 of the 27 univariate measurements for regional (A) and global (B) lake-stream pairs. The direction of phenotypic divergence is consistent but the magnitude of phenotypic divergence varies.

Figure 2: Mean and standard error of body depth (A, B), number of gill rakers (C, D), mean gill raker length (E, F) and gill raker spacing (G, H) for regional (left panels) and global (right panels) lake-stream pairs.

Figure 3: Phenotypic parallel divergence is higher in regional versus global comparisons. (A) Effect size of the habitat-by-pair interaction term versus effect size of the habitat term for regional and global pairs. (B) Ratio of the habitat effect size to the habitat-by-pair interaction effect size for regional pairs (x-axis) versus global pairs (y-axis) for all traits examined. In (A) and (B) effect size measured as η^2 for single traits and partial η^2 for multivariate traits. In (A), traits found below the 1:1 line show more parallelism (large, consistent effect of habitat) than non-parallelism (large, but inconsistent effect of habitat). Traits above the line show the opposite pattern. In (B) traits found below the 1:1 line show greater parallelism in regional than in the global pairs.

Figure 4: Pairwise angles between phenotypic vectors (Panel A, θ_P), outlier genetic vectors (Panel B, $\theta_{OUTLIERS}$), as well as differences in vector length between phenotypic vectors (Panel C, ΔL_P) and outlier genetic vectors (Panel D, $\Delta L_{OUTLIERS}$) at regional (grey circles) and global (black triangles) scales. Overall mean values are represented by a straight line (±SE). Significance in mean differences is represented by two stars ($P < 0.01$) or one star ($P < 0.05$).

Figure 1

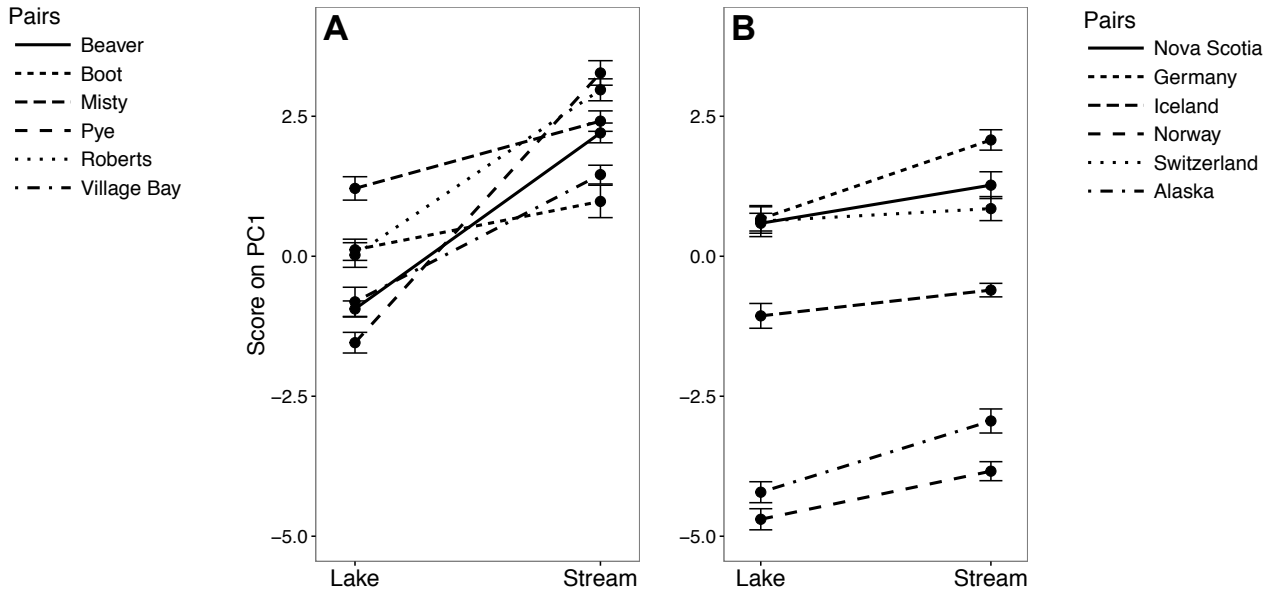
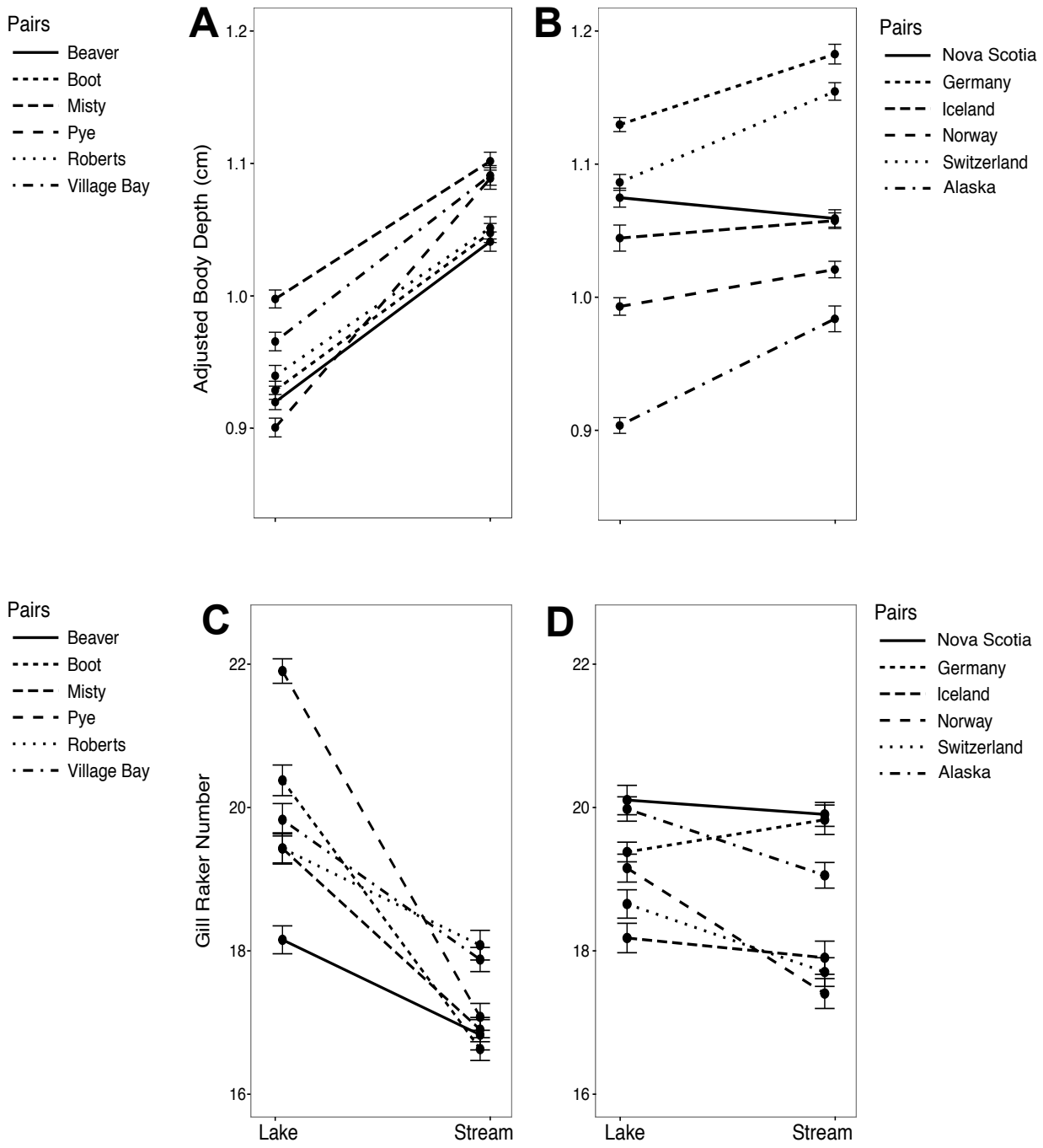

Pairs
Beaver
Boot
Misty
Pye
Roberts
Village Bay
A
B
Score on PC1
2.5
0.0
−2.5
−5.0
Lake
Stream
Pairs
Nova Scotia
Germany
Iceland
Norway
Switzerland
Alaska

Figure 2


A
B
C
D
Pairs
Beaver
Boot
Misty
Pye
Roberts
Village Bay
Nova Scotia
Germany
Iceland
Norway
Switzerland
Alaska
Adjusted Body Depth (cm)
Gill Raker Number
1.2
1.1
1.0
0.9
22
20
18
16
Lake
Stream

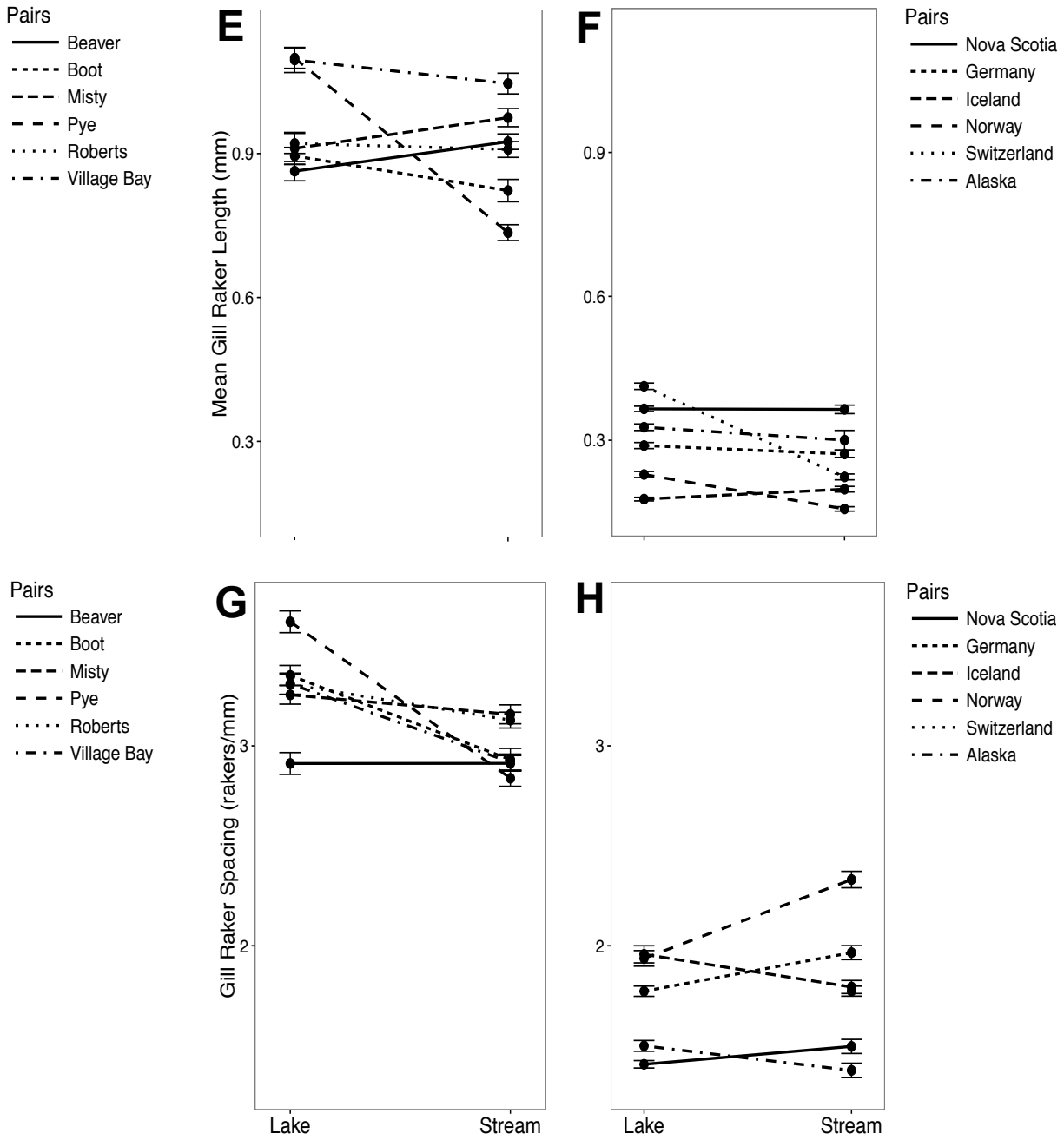

Pairs
Beaver
Boot
Misty
Pye
Roberts
Village Bay
E
Mean Gill Raker Length (mm)
0.9
0.6
0.3
F
Pairs
Nova Scotia
Germany
Iceland
Norway
Switzerland
Alaska
Pairs
Beaver
Boot
Misty
Pye
Roberts
Village Bay
G
Gill Raker Spacing (rakers/mm)
3
2
Lake
Stream
H
Pairs
Nova Scotia
Germany
Iceland
Norway
Switzerland
Alaska

Figure 3

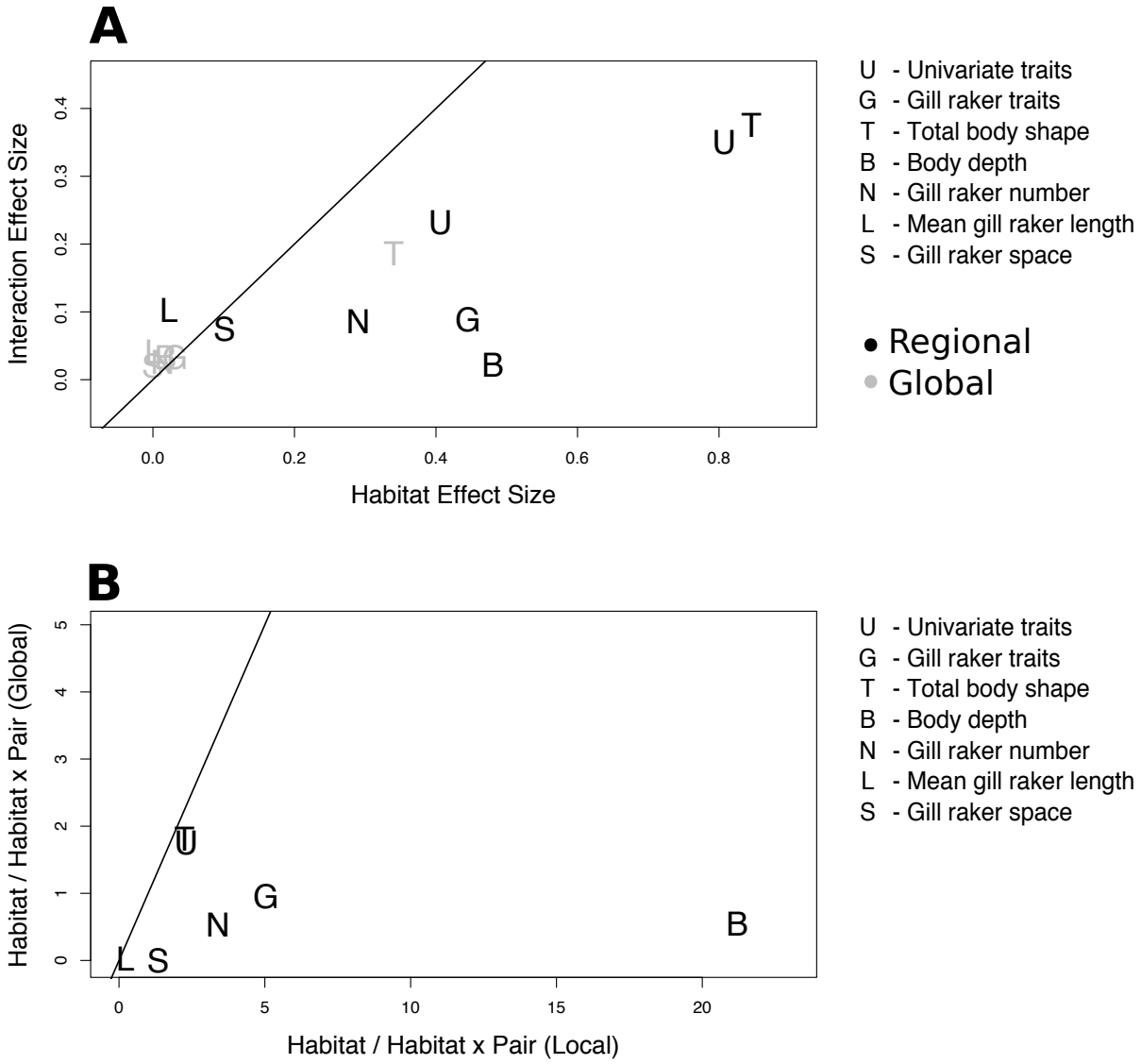
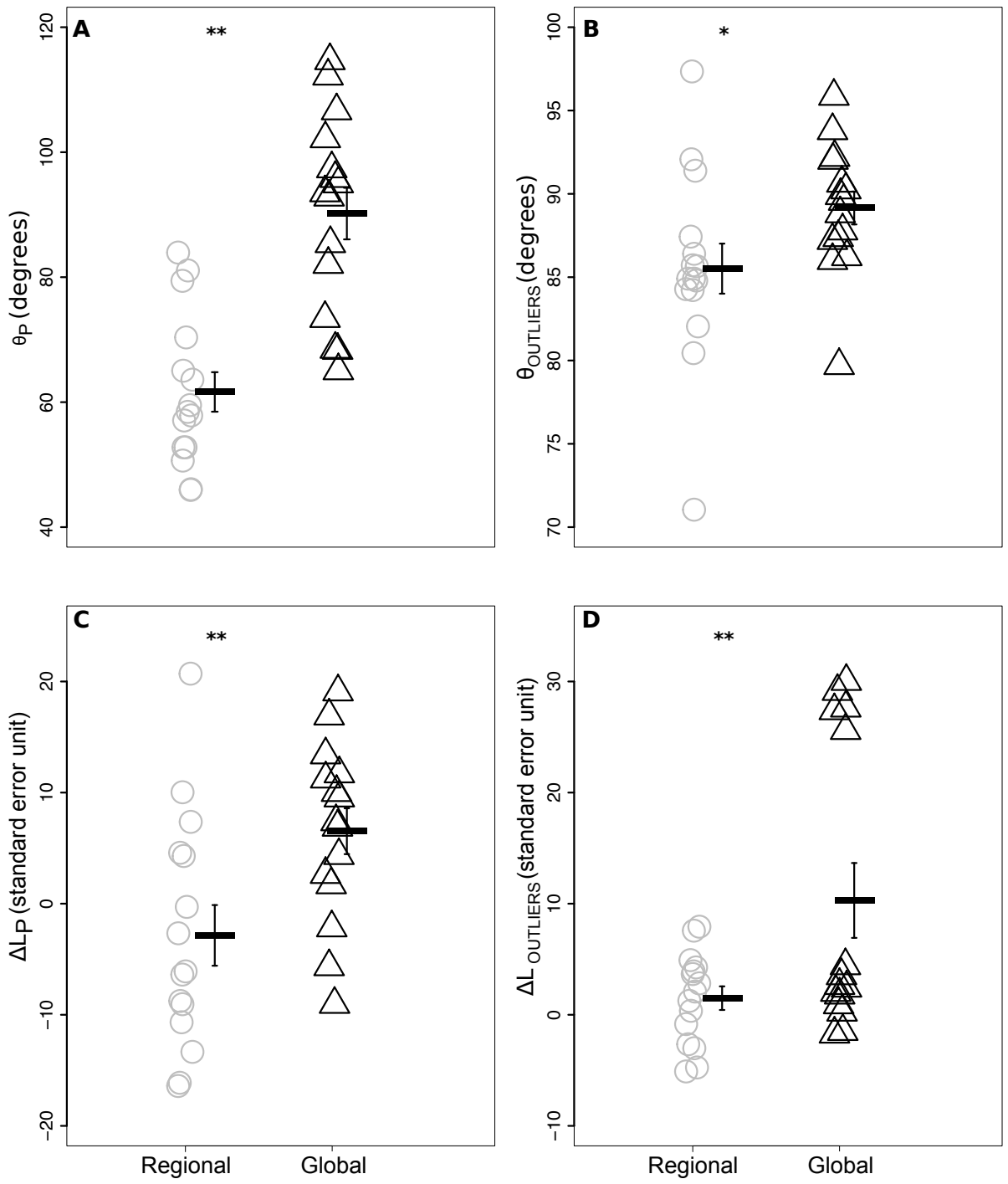

A
Interaction Effect Size
Habitat Effect Size
0.0
0.1
0.2
0.3
0.4
0.0
0.2
0.4
0.6
0.8
U
T
U
T
L
S
N
G
B
U - Univariate traits
G - Gill raker traits
T - Total body shape
B - Body depth
N - Gill raker number
L - Mean gill raker length
S - Gill raker space
Regional
Global
B
Habitat / Habitat x Pair (Global)
Habitat / Habitat x Pair (Local)
0
1
2
3
4
5
0
5
10
15
20
U
G
N
B
L
S
U - Univariate traits
G - Gill raker traits
T - Total body shape
B - Body depth
N - Gill raker number
L - Mean gill raker length
S - Gill raker space

Figure 4


A
B
C
D
**
*
**
**
θP (degrees)
θOUTLIERS (degrees)
ΔLP (standard error unit)
ΔL OUTLIERS (standard error unit)
Regional
Global
Regional
Global