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Weak but parallel divergence between kōaro (*Galaxias brevipinnis*) from adjacent lake and stream habitats

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ABSTRACT

Background: Fish in New Zealand and elsewhere in the temperate Southern Hemisphere rarely show the adaptive divergence in sympatry or parapatry seen elsewhere in the world.

Hypothesis: Galaxiid fish in high-elevation lakes will show parallel morphological shifts across six lake–stream ecotones, possibly accompanied by genetic divergence.

Organism: Kōaro, the climbing galaxias, which is often the sole fish species in New Zealand lakes that lack introduced trout.

Methods: Geometric morphometric analyses of photos taken of live fish collected from lakes and streams to measure the extent and direction of body shape divergence; microsatellite genotyping to measure genetic differentiation.

Results: Kōaro show weak or no genetic differentiation between adjacent lake and stream habitats, but do show generally parallel shifts in body shape between lakes and streams.

Keywords: diadromy, *Galaxias brevipinnis*, parapatric speciation, phenotypic change vector analysis, phenotypic plasticity.

INTRODUCTION

New species frequently originate as the result of populations adapting to occupy distinct ecological niches (Schluter, 2001; Nosil, 2012). Comparisons of populations occurring across sharp habitat transitions provide some of our best opportunities to investigate the early stages of intraspecific divergence, and to identify factors that favour the evolution of new species. For fishes, lakes and streams provide distinct environments with contrasting hydrodynamic properties and prey communities. In numerous fish species, populations that occur in lentic and lotic environments differ in body shape and other morphological characteristics (McGuigan *et al.*, 2003; Collin and Fumagalli, 2011; Samways *et al.*, 2015; Ramler *et al.*, 2016), and this divergence may occur in parallel across replicate lake–stream pairs. In threespine stickleback (Hendry *et al.*, 2002; Berner *et al.*, 2008; Kaeuffer *et al.*, 2012; Stuart *et al.*, 2017) and African cichlids (Theis *et al.*, 2014),

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populations in adjacent lake and stream habitats have diverged genetically as well as morphologically, and occur at various points along the speciation continuum from undifferentiated to reproductively isolated.

To date, documented parapatric divergence between lake and stream fish has been largely a Northern Hemisphere phenomenon (Seehausen and Wagner, 2014), with Australian rainbow fish representing a rare example in southern latitudes (McGuigan *et al.*, 2003). New Zealand has a relatively diverse freshwater fish fauna for its size and latitude (35 described native species, of which 31 are endemic, spend at least part of their life cycle in fresh water). Despite this diversity, freshwater fish in New Zealand show little if any evidence for the recent adaptive diversification seen in fishes in broadly similar environments in North America and Europe. Almost all species are either diadromous or stream-resident, and lakes are generally occupied by larval forms of stream fish and by habitat generalists, with very few lake specialists.

There are several possible explanations for the apparent rarity of trophic polymorphism and adaptive divergence along habitat gradients in New Zealand freshwater fish. It is possible that the species present (most of which belong to the families Galaxiidae and Eleotridae) are constrained by a lack of genetic variation or by life-history characteristics, or that for some reason the environment provides less ecological opportunity for divergence than is present elsewhere. Alternatively, it could be that adaptive diversity has in fact evolved, but that it has either been overlooked or largely eliminated following the introduction of non-native species such as brown trout (*Salmo trutta*). Trout have had widely documented impacts on native galaxiids, frequently excluding them from large parts of their former range (Crowl *et al.*, 1992), which could have obscured previously established diversity.

Kōaro (*Galaxias brevipinnis*) is a medium-sized galaxiid (typically 10–20 cm total length as adults) native to New Zealand and southeast Australia. It is ancestrally amphidromous: adults live and spawn in streams, and pelagic larvae rear in the ocean for 4–5 months before returning to streams as post-larval 'whitebait'. However, kōaro readily establish landlocked populations upstream of lakes, which provide an alternative pelagic rearing environment for larvae (McDowall and Allibone, 1994; Augspurger, 2017). In some lakes, mostly in remote high-elevation areas, adult kōaro occur in the lake itself as well as in inflow streams (Meredyth-Young and Pullan, 1977), although introduced fish species appear to be responsible for largely displacing adult kōaro from other lakes (Chadderton, 2001; Rowe *et al.*, 2008). It is largely unknown whether lake and stream kōaro that occur in adjacent habitats are locally adapted subpopulations or single well-mixed populations. In the Arawera Lakes on the North Island, kōaro caught in the lake differed in life history from fish caught upstream (Young, 2002). Kōaro collected from non-adjacent lentic versus lotic habitats around the South Island differed in morphology, and rearing experiments suggested this was a plastic response to hydrological regime (Dunn, 2012). To date, no study has tested for morphological and genetic differentiation across multiple lake–stream ecotones, or sought to identify environmental factors that influence the degree of divergence.

We investigated whether kōaro in lakes around New Zealand's South Island differ morphologically and genetically from kōaro in adjacent streams. We hypothesized that body shape divergence would occur in parallel across independent lake–stream transitions. We also hypothesized that if populations varied in progress towards adaptive divergence, sites showing greater divergence in body shape would also feature greater divergence at neutral genetic markers. Finally, we hypothesized that body shape divergence would be greater at sites with a steeper stream gradient, representing a sharper contrast between lake and stream habitats.

METHODS

Site selection and sampling

We visited seven lakes on the South Island of New Zealand (Fig. 1, Table 1) over a two-month period during the austral summer (November 2016 to January 2017). Sites were selected on the basis of accessibility and either past information that adult kōaro were present in the lake or similarity to lakes known to contain kōaro. At each site, the elevation and lake surface area were obtained from topographical maps (topomap.co.nz). The stream gradient over the area of sampling was estimated by obtaining the elevation and GPS coordinates (in UTM) with a handheld GPS device (Garmin eTrex 30, Garmin International, Olathe, KS, USA) at the trapping sites in the stream closest to and farthest

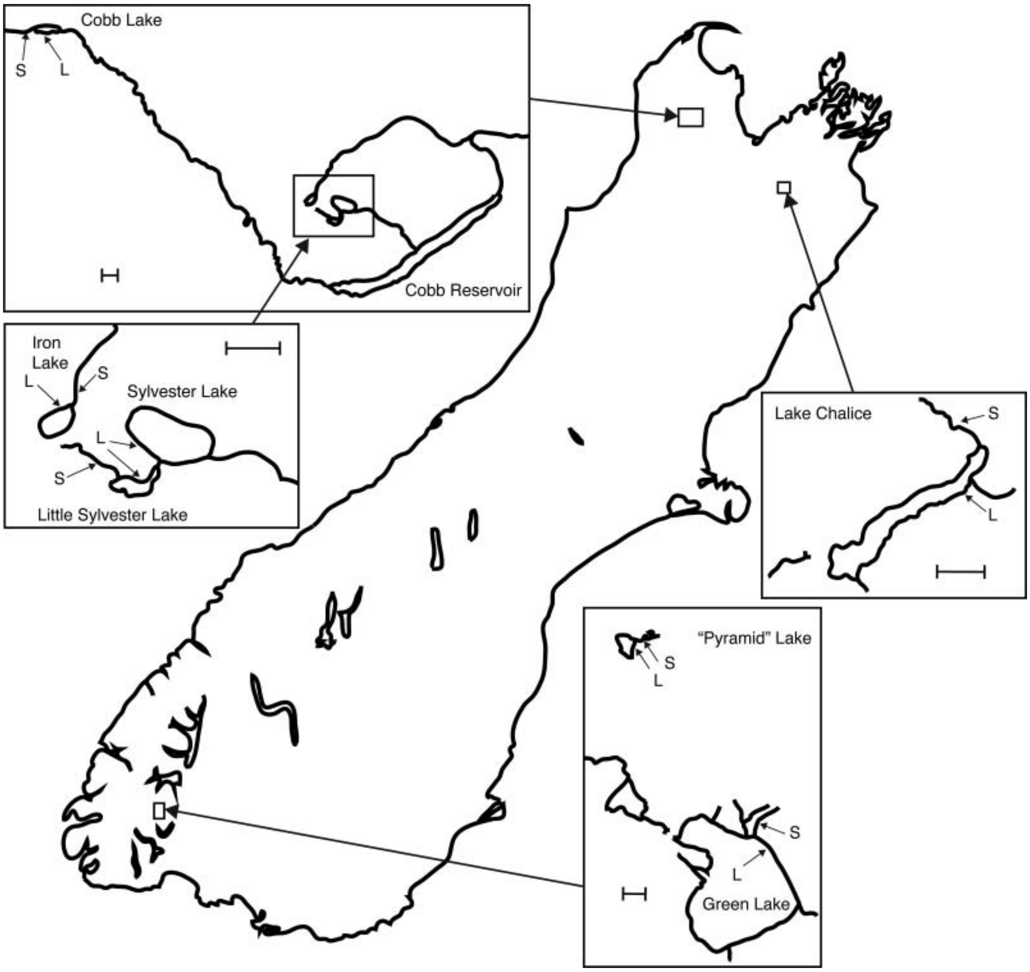

Fig. 1. Map of the sites included in the study. Insets show the approximate location of the stream (S) and lake (L) trapping at each site, and each scale bar corresponds to a distance of 500 m.

Table 1. Sampling site location and characteristics

Site	Latitude (UTM 59)	Longitude (UTM 59)	Elevation (m)	Lake area (ha)	Stream gradient
Lake Chalice	5396415	693153	750	52.5	0.237
Lake Cobb	5453845	627079	1089	18.3	0.009
Green Lake [1]	4926927	686655	822	566.6	0.072
Iron Lake [2]	5448427	635828	1440	7.9	0.193
Pyramid Lake	4931347	684173	744	16.9	0
Sylvester Lakes [3]	5447850	636623	1333	41.2	0.255

[1] Trout likely present. [2] Outlet stream sampled. [3] Two small connected lakes.

from the lake. The approximate average gradient was calculated as the change in elevation divided by the horizontal distance over the area of the stream in which traps were set.

Fish were sampled from both lake and stream habitats using collapsible mesh traps (Kilwell Baitcatcher, 43 × 25 × 25 cm, Kilwell Sports Ltd., Rotorua, New Zealand). Between 4 and 15 traps were set at each lake or stream site that was trapped, depending on the extent of trappable habitat accessible on foot. In lakes, traps were set along a stretch of 80–900 m of shoreline at depths between 0.5 and 3 m. In streams, traps were set starting just above the inlet to the lake, and as far upstream as suitable trapping habitat (areas of >20 cm depth and low to moderate flow) was accessible. One lake (Iron Lake) lacked an appreciable inlet and traps were therefore set in the outlet stream, and in another (Green Lake) we set traps in two small nearby streams. Traps were left overnight (12–20 hours) and retrieved as soon as possible the following day. One site (Pyramid Lake) was sampled on two occasions, several weeks apart.

As kōaro are considered to be in decline across New Zealand (Goodman *et al.*, 2014) and as population sizes are unknown for the populations studied, we elected to sample fish non-lethally for body shape (photographs) and genetics (fin clips). We aimed to sample 30 fish from each lake or stream, although this was not always possible. For photography, fish were placed in custom-built transparent acrylic boxes filled with clean water from the lake or stream. Each fish was placed in the best-fitting of several box sizes, and if necessary an acrylic insert was used to confine the fish to the edge of the box nearest the camera. Several photos were taken of each fish with a Canon EOS 5D digital camera and a Compact-Macro lens EF 50 mm 1:2.5, with a ruler in the photo to provide scale. The camera was set on manual with the focal length fixed at 0.5 m in order to standardize the distance at which each photo was taken in field conditions.

Following photography, a small piece of the caudal fin of each fish was clipped using clean dissecting scissors and placed in a labelled tube with 99% ethanol. The fish were allowed to recover and then released near the site of capture. All animal use was approved by the Otago University Animal Ethics Committee (protocol 59/16) and site access was approved by the Department of Conservation (authorization 51959-RES).

DNA extraction, microsatellite genotyping, and genetic analysis

We extracted genomic DNA using Chelex (Bio-Rad) following a modification of Casquet *et al.* (2012); a small fragment of fin tissue was transferred to 1.5 mL tubes with 400 μL of 5% Chelex solution and 40 μg of proteinase K was added. Following incubation, the samples were heated to 95°C for 10 minutes. We genotyped fish at twelve microsatellite loci previously found to be polymorphic in *G. brevipinnis*; two were originally developed for *G. vulgaris* (Waters *et al.*, 1999) and the remaining ten were developed for *G. brevipinnis* (Augspurger, 2017). PCR was carried out in a multiplex (Schuelke, 2000) with loci labelled with FAM, NED, VIC, and PET dyes and loci with non-overlapping allele sizes sharing the same dye (Townsend *et al.*, 2012). Fragment sizes were resolved on an ABI 3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA), with GeneScan 500 (LIZ)™ size standard, and alleles were scored with GENEMAPPER v.4.1 (Applied Biosystems, Foster City, CA). Any samples with ambiguous alleles were re-run in single-plex PCR reactions.

We first pooled lake and stream fish within each site, and calculated pairwise population differentiation among the seven sites using Hedrick's G'_{ST} (Hedrick, 2005) implemented in the *diveRsity* package (Keenan *et al.*, 2013) in the R environment (R Core Team, 2016). Hedrick's G'_{ST} measures differentiation by scaling G_{ST} by its maximum value given the observed heterozygosity. While differentiation statistics are the subject of considerable debate (Whitlock, 2011; Verity and Nichols, 2014), our results were consistent regardless of which measure we used. This analysis revealed that the only undifferentiated sites were Sylvester and Little Sylvester Lakes ($G'_{\mathrm{ST}} = 0.007$, bootstrap 95% CI –0.024 to 0.050), which are separated by a very short (<50 m) stream. Therefore, for all following analyses, we combined lake fish from both sites, excluded the few fish collected in the stream between lakes, and contrasted fish from the longer stream flowing into Little Sylvester Lake with the combined lake fish. Otherwise, we treated each site as an independent replicate, as any lake–stream divergence was expected to have occurred after the founding of the lake population.

Within each site (combining lake and stream fish), we tested for Hardy-Weinberg equilibrium and linkage disequilibrium, and measured allelic diversity and expected heterozygosities at each locus. We then measured the extent of differentiation between lake and stream fish with G'_{ST}, and used the 95% bootstrap confidence intervals to assess whether there was significant lake–stream differentiation.

Geometric morphometric analysis of body shape

Only fish larger than 5 cm standard length were included in shape analysis, to ensure that any post-larval forms were excluded. For each fish, we selected the photo that combined the best visibility of the relevant features (fins, eyes, operculum, mouth) and the straightest positioning. Photos of fish facing right were flipped horizontally so that all images showed fish facing left. Landmarks were placed using the *geomorph* package in R (Adams and Otárola-Castillo, 2013). We chose 16 landmarks to describe body shape, incorporating functional traits including the shape and size of the head, eyes and mouth, the relative depth of the body, the depth and length of the caudal peduncle, and the positioning of the fins (Fig. 2).

We read the landmark data into R, and carried out a generalized Procrustes analysis with the *geomorph* function 'gpagen' to centre, rotate, and scale each image. Dorsoventral arching of the body is a common issue in fish geometric morphometric studies (Valentin *et al.*, 2008; Ingram, 2015), and our photography of conscious fish in water meant that some specimens

Fig. 2. Position of landmarks used to digitize body shape from photographs of kōaro.

were substantially arched up or down. We carried out a principal component analysis on the landmark data with the *geomorph* function ‘plotTangentSpace’, and found that PC1 was very clearly interpretable as dorsoventral arching. We therefore used Burnaby back-projection to recalculate the landmark positions after removing variation along the vector defined by the coefficients of PC1 (Burnaby, 1966; Valentin *et al.*, 2008). This process effectively ‘straightened’ the specimens so they could be compared without the influence of dorsoventral arching.

We next tested for an influence of size on shape, by carrying out an allometric regression of the adjusted coordinates against log-transformed centroid size using the *geomorph* function ‘procD.allometry’. As we detected significant allometry, we computed residuals at each landmark coordinate for each specimen, and added these to the consensus shape from the whole data set to obtain size-adjusted coordinates for all fish. We also used an analysis of variance (ANOVA) to test for variation in log-transformed centroid size associated with lake versus stream habitat, site, and a habitat × site interaction.

We tested for significant differentiation between lake and stream fish within each site by carrying out a Procrustes ANOVA (Goodall, 1991) using the *geomorph* function ‘procD.lm’. This analysis used the distances between specimens in the size-adjusted coordinate space as the response variable; results were qualitatively identical if we instead analysed the data that had been corrected for arching but not for size, and included centroid size as a covariate.

We then tested whether the extent and direction of divergence between lake and stream fish occurred in parallel across multiple sites using phenotypic change vector analysis (PCVA). Using the *geomorph* function ‘trajectory.analysis’, we computed the phenotypic change vector in multivariate body shape phenotype between each pair of lake and stream populations, encompassing the magnitude (Euclidean distance, D_{E}) and direction of divergence (Collyer and Adams, 2007; Adams and Collyer, 2009). Then, we used a permutation test to ask whether pairs of sites differed significantly in the magnitude or direction of divergence. Non-parallel shape change would be indicated by the difference in magnitudes $D_{\mathrm{E(1)}} - D_{\mathrm{E(2)}}$ or the angle between divergence vectors θ being larger than expected by chance.

We used Pearson’s correlation to test whether sites with greater genetic divergence between lake and stream fish (G'_{ST}) also showed greater body shape divergence (D_{E}). We also tested whether D_{E} was predicted by the steepness of the stream gradient, which we used as a proxy for the extent to which the stream represented a hydrologically distinct habitat from the lake.

RESULTS

Genetic analyses

We obtained scorable alleles for 313 individuals (range 19–82 per site), with 96.5% of fish having alleles scored at all 12 loci (Table 2). After the Sylvester and Little Sylvester populations were combined, there was moderate to strong genetic differentiation among all pairs of sites, ranging from $G'_{ST} = 0.32$ (Sylvester Lakes vs. Cobb Lake) to $G'_{ST} = 0.76$ (Iron Lake vs. Pyramid Lake). All measures of genetic differentiation between sites were bounded well away from zero based on bootstrap 95% confidence intervals.

Two sites (Lake Chalice and Cobb Lake) showed deviations from Hardy-Weinberg equilibrium, as well as higher expected heterozygosity and allelic richness than other sites. There was little indication of linkage disequilibrium, with few or no pairs of alleles at any given site deviating significantly from equilibrium. Comparisons of lake and stream fish revealed weakly positive or absent genetic structuring, with G'_{ST} ranging from −0.003 to 0.045 and the confidence interval including zero for each of the six sites.

Geometric morphometric analysis of body shape

We digitized body shape data from a total of 291 *Galaxias brevipinnis* across the six sites (Table 3). Prior to the 'unbending' procedure, the first principal component explained 45.8% of variation in the overall shape data, and clearly corresponded to dorsoventral arching. There was significant allometry in the shape data (Procrustes ANOVA: $F_{1,289} = 42.0$, $P < 0.001$, $R^2 = 0.13$), corresponding primarily to an enlarged head and a shorter caudal peduncle in larger specimens. Log-transformed centroid size differed between sites (ANOVA: $F_{5,279} = 14.7$, $P < 0.0001$) and between lake and stream fish ($F_{1,279} = 8.6$, $P = 0.003$) but there was no significant interaction ($F_{5,279} = 0.79$, $P = 0.56$). The lake–stream difference corresponded to mean standard lengths of 82 mm in lake fish and 91 mm in stream fish.

Four of the six sites showed significant body shape differences between lake and stream fish (Procrustes ANOVA; $P < 0.05$), the exceptions being Pyramid Lake and Lake Chalice. The percentage of variation explained by habitat was generally low, ranging from 2% to 12% (Table 3).

Phenotypic change vector analysis indicated that none of the sites differed from one another in terms of the length or direction of divergence between mean lake and stream

Table 2. Summary of site-level genetic results from 12 microsatellite loci

Site	N_L	N_S	HW-p	H_E	AR	G'_{ST} (95% CI)
Lake Chalice	30	22	0.001	0.77	7.6	0.027 (−0.028 to 0.095)
Lake Cobb	30	28	0.000	0.57	4.6	0.027 (−0.012 to 0.073)
Green Lake	7	12	0.216	0.52	3.8	0.045 (−0.073 to 0.193)
Iron Lake	16	7	0.851	0.48	3.5	−0.003 (−0.080 to 0.113)
Pyramid Lake	40	39	0.135	0.52	3.3	0.006 (−0.019 to 0.041)
Sylvester Lakes	56	26	0.316	0.45	3.8	0.017 (−0.012 to 0.055)

Note: N_L and N_S are the number of lake and stream fish genotyped; HW-p is the significance of a multi-locus exact test for deviation from Hardy-Weinberg equilibrium, H_E is the expected heterozygosity, and AR is the average allelic richness across loci. G'_{ST} values measure divergence between lake and stream fish within the site.

Table 3. Site-level geometric morphometric comparisons of lake and stream fish

Site	N_L	N_S	F	P	R^2	D_E
Lake Chalice	24	17	1.30	0.195	0.03	0.0095
Lake Cobb	30	29	2.19	0.028	0.04	0.0099
Green Lake	6	11	2.11	0.027	0.12	0.0189
Iron Lake	15	5	2.31	0.033	0.11	0.0198
Pyramid Lake	37	36	1.45	0.127	0.02	0.0076
Sylvester Lakes	55	26	5.55	0.001	0.07	0.0142

Note: N_L and N_S are the number of lake and stream fish used for morphometric analyses; F, P, and R^2 are from Procrustes ANOVAs on the residual shape data at each site; D_E is the Euclidean distance between lake and stream means from the phenotypic change vector analysis.

body shapes, with the exception of Pyramid Lake, which showed negligible lake–stream divergence (Fig. 3). Pyramid Lake had a significantly shorter D_E than Sylvester Lakes ($P = 0.026$) and a marginally shorter D_E than Green Lake ($P = 0.078$) or Iron Lake ($P = 0.061$). Pyramid Lake also had a significantly different angle of divergence than Lake Chalice, Cobb Lake, and Sylvester Lakes ($P < 0.01$). All other pairwise comparisons of D_E and divergence angle were non-significant ($P > 0.1$).

Neither genetic divergence (G'_{ST}; $r = -0.12$, $P = 0.81$) nor stream gradient ($r = 0.32$, $P = 0.54$) was correlated with the extent of morphological divergence (D_E) between lake and stream fish (Fig. 4).

DISCUSSION

We found modest and roughly parallel divergence in body shape between lake and stream kōaro, with little or no evidence of genetic differentiation.

Lake kōaro were generally deeper bodied with larger heads and shorter, thicker caudal peduncles than fish collected from adjacent streams. These patterns are broadly similar to those previously reported for kōaro and related galaxiids collected from lentic and lotic habitats (Dunn, 2012). Shape divergence was not an artefact of fish positioning or size, as it remained when shape data were corrected for arching and allometry. In fact, the lake body shape was more like that of larger fish given the observed allometric relationship, despite the fact that lake fish were slightly smaller than stream fish on average (albeit with broad overlap in the size distributions). The trajectory of shape divergence contrasted with patterns seen in some other lake–stream populations of fish; for example, stream stickleback (*Gasterosteus aculeatus*) and minnows (*Phoxinus* sp.) are typically deeper-bodied than fish in lakes (Kaeuffer *et al.*, 2012; Ramler *et al.*, 2016; Stuart *et al.*, 2017). The difference may be attributable to habitat preference: stream kōaro typically occur in fast-flowing water and can climb and swim against considerable flow, while other species occur primarily in backwaters and pools. In contrast, lake kōaro are benthic and negatively buoyant, not pelagic like lake-dwelling stickleback and minnows. The more streamlined form of stream kōaro may therefore be an adaptation or plastic response to improve performance in higher flow.

Microsatellite data indicate that the six sites we sampled are all substantially genetically isolated, even when they are in the same catchment (e.g. Cobb Lake, Iron Lake, and Sylvester Lakes). We expect that they are largely independent populations and that any

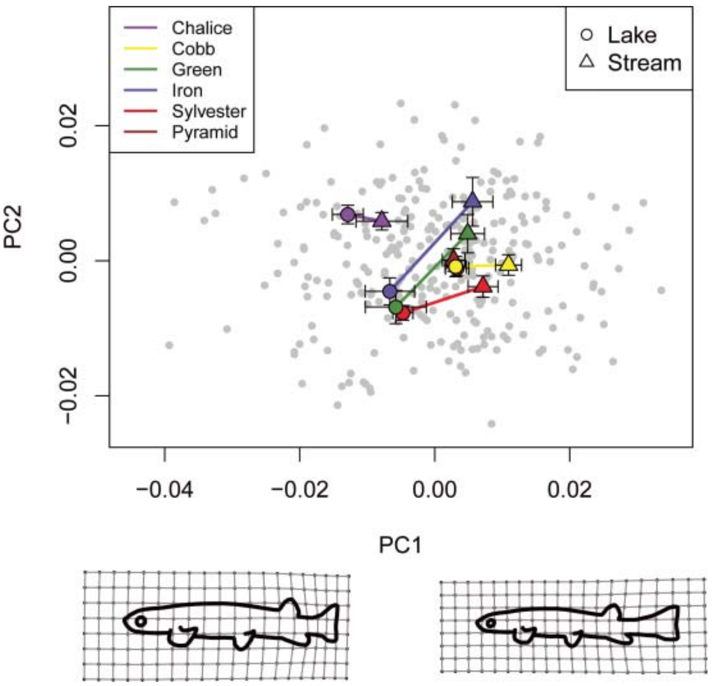

Fig. 3. Phenotypic change vector analysis of body shape in multiple adjacent lake–stream habitats. Lines connect the mean PC1 and PC2 scores for lake fish and stream fish at a given site, and error bars indicate ± 1 standard error. Deformation grids show the shape variation captured by PC1, with the fish outline transformed to the shape representing the minimum and maximum observed values of PC1 (with PC2 fixed at zero).

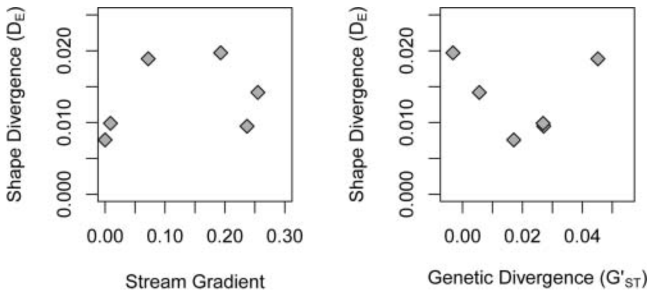

Fig. 4. Relationship between the magnitude of morphological and genetic divergence, and between the magnitude of morphological divergence and the stream gradient at each site. Neither genetic divergence ($r = 0.12$, $P = 0.81$) nor stream gradient ($r = 0.32$, $P = 0.54$) was significantly correlated with the extent of morphological divergence.

exchange of migrants is very rare. Despite the probable isolation of these populations, genetic diversity as measured by expected heterozygosity is reasonably high. H_E ranged from values comparable to those in the mid-sized Lake Okareka (0.43–0.46) to values comparable to the large and well-mixed kōaro populations in Lakes Wanaka and Wakatipu (0.65–0.74), in a previous study using a subset of the loci used here (King *et al.*, 2003).

Despite there being a reasonable level of genetic variation for natural selection to potentially act upon, there has been little if any divergence between habitats at putatively neutral microsatellite loci. The ages of the lakes imply sufficient time for divergence, as Lake Chalice dates to a landslide ca. 2160 years BP (Adams, 1981), Green and Pyramid Lakes date to a landslide ca. 12,000 years BP (Hancox and Perrin, 2009), and Sylvester Lakes, Iron Lake, and Lake Cobb date to glacial retreat ca. 13,000 years BP (Shulmeister *et al.*, 2001). While it is unknown when kōaro colonization occurred and whether the lakes have been continuously habitable, the timescale is comparable to cases of lake–stream or within-lake population differentiation or speciation in many temperate fishes (Seehausen and Wagner, 2014). Therefore, neither genetic variation nor time appear likely to be the cause of the lack of population differentiation between lake and stream kōaro.

Life-history constraints may favour a plastic response to local conditions rather than local adaptation to lake and stream habitats. Kōaro larvae are small and rear in pelagic environments, either the ocean or a lake. A number of galaxias species in New Zealand have larvae that can rear in the stream, but these stream resident species appear to be the descendants of a single landlocking event by a kōaro-like ancestor, so the evolution of stream residence appears to be rare. Spawning of lacustrine kōaro populations is believed to occur in summer in the inlet streams, as spawning within the lake itself has not been reported (Rowe *et al.*, 2002). It is therefore likely that lake and stream adults use similar spawning and larval rearing habitats. Temporal segregation of spawning could still allow for some divergence (Young, 2002), but given the protracted spawning and larval pelagic phase of kōaro, significant isolation is unlikely.

The modest but significant and generally parallel shifts in body shape likely result from phenotypic plasticity, as has been found in other cases of lake–stream divergence. Plasticity can either facilitate or hinder local adaptation, and has been shown to enhance the degree of parallelism in lake and stream stickleback body shape (Oke *et al.*, 2016). An alternative strategy to plasticity is 'matching habitat choice' in which individuals sample environments and settle wherever their phenotype confers high fitness (Bolnick *et al.*, 2009; Edelaar *et al.*, 2017). For example, if more streamlined kōaro are better able to climb or swim against steep gradients, they may settle in stream habitats and potentially facilitate early stages of lake–stream divergence. However, there is direct evidence that *G. brevipinnis* body shape responds to changes in flow regime (Dunn, 2012), and theory predicts that in most conditions, plasticity will be a more likely solution to matching habitat choice when genetic divergence does not occur (Edelaar *et al.*, 2017).

We did not detect a correlation between the extent of shape divergence and stream gradient as a proxy for the dissimilarity between lake and stream habitats. However, our study was clearly limited by the small number of lakes that were accessible and that had both lake and stream adult kōaro present. There appeared to be some variation among sites in the magnitude or trajectory of lake–stream divergence (Lake Chalice and Cobb Lake appear somewhat different from Sylvester Lakes, Iron Lake or Green Lake), but aside from Pyramid Lake none were significantly different, potentially reflecting limited statistical power. Furthermore, some sites are more appropriate to testing our hypotheses than others: Iron Lake could only be sampled in an outlet stream, while Green Lake is believed to have trout present (though it has not been formally surveyed). We did find that the site with the lowest gradient (Pyramid Lake) had the smallest lake–stream body shape divergence, but considerably more sites would be necessary to test for environmental drivers of the magnitude of divergence and the extent of parallelism (Stuart *et al.*, 2017).

We did not detect any population differentiation or incipient speciation in what appears to be a conducive scenario: a sharp habitat transition and a lack of competing species. The flexible adult habitat requirements of widespread native fish species likely allow the rapid formation of distinctive lake-resident forms where conditions permit.

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