

Article

Phenotypically Induced Intraspecific Variation in the Morphological Development of Wetland and Stream *Galaxias gollumoides* McDowall and Chadderton

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Abstract: The hypothesis that contrasting hydrology induces divergent intraspecific phenotypic plastic responses in non-migratory freshwater fish was investigated. Morphologies of wetland and stream *Galaxias gollumoides* from South Island, New Zealand, at different stages of ontogeny, were examined. Phenotypic responses were tested for in a 2 × 2 factorial laboratory based controlled reciprocal transplant experiment with flow (current or no current) and source habitat (wetland or stream), as treatments. There was a shift in the overall head morphology of wetland current treatment *G. gollumoides* away from the wetland no current treatment, and toward the stream current treatment, demonstrating convergence in head morphology in the presence of flow of wetland and stream sourced captive *G. gollumoides*. Morphologies of captive reared *G. gollumoides* were also compared to developmental trajectories of morphological characters during the ontogeny of field reared first year, and adult conspecifics. In combination, experimental and field results support the hypothesis, finding habitat hydrology to be the potential mechanism inducing and maintaining intraspecific morphological divergence in *G. gollumoides*. Recognition of this mechanism inducing morphological divergence between populations also aids the taxonomic description of long genetically recognised lineages of co-members of the *Galaxias vulgaris* species complex.

Keywords: *Galaxias gollumoides*; morphology; hydrological environment; phenotypic plasticity

1. Introduction

Habitat hydrology is a strong selective pressure on fish [1–3]. Intraspecific morphological and biological variation may result from the influence of the proximate hydrological environment of a habitat inducing a phenotypic plastic response from early in an individual's ontogeny [3–8]. Variation may also arise from genotypic differences between populations through the process of local adaptation. Additionally, these two processes may interact with environmental factors, superimposing their effects on the ranges of morphological characters a genotype can produce [9–15].

During the embryonic period, meristic characters such as vertebrae number are considered to be the most labile, being directly influenced by the proximate environment, before becoming fixed [16,17]. Vertebrae number influences body flexibility and swimming mode, meaning their early determination may influence an individual's morphology [17–20]. Morphologies, unlike meristic characters however, continue to develop and change as an individual develops, to maintain a functional form, being induced by the hydrodynamics of the habitat [17,21–23]. Evidence of such hydrologically induced plasticity of

phenotypes comes from both field observations and experimental investigations, typically between stream (flowing) and lake (non-flowing) dwelling conspecifics [24–26]. Evidence also comes from within flowing habitats, for example, Kelley et al. [27] experimentally demonstrated that *Melanotaenia australis* (Castelnau) reared in high velocities had a more robust body compared to slow velocity treatment reared conspecifics. Imre et al. [7] experimentally showed that differences in the form of the caudal fin could be induced, and maintained in *Salvelinus fontinalis* (Mitchill) reared in high and low water velocities, similar to the field observations of McLaughlin and Grant [28], between first year *S. fontinalis*, occupying faster and slower velocity sections of streams. Moreover, both Shuai et al. [29] and Reyes Corral and Aguirre [19] found water turbulence, correlated with different altitudes, temperatures and, channel gradients to be important, with those individuals raised in higher turbulence treatments having more streamlined forms compared to individuals reared in lower turbulence treatments.

Species within Galaxiidae similarly display substantial variation in body form across a range of habitats from fast flowing intermittent and perennial streams, to wetlands, and lakes [30–42]. Intraspecific variation occurs between life history strategies, but is particularly pronounced in non-migratory species, such as those in the *Galaxias vulgaris* species complex (sensu Allibone and Wallis [42–47] on South and Stewart islands in New Zealand. This has led to a situation of morphologically similar, but genetically distinct species and undescribed lineages due to occurrence in similar habitat types, and vice versa, which has hindered formal descriptions [44,45]. Dunn et al. [48] hypothesised that the contrasting hydrological environments of lentic and lotic habitats occupied by *Galaxias vulgaris* species complex taxa is the mechanism inducing phenotypic plastic responses in intraspecific character divergence in contrasting habitats, and interspecific morphological convergence in similar habitats. Dunn et al. [48] found support for this hypothesis by comparatively assessing the morphologies of three species representing the two main morphotypes (flathead—*Galaxias vulgaris* Stokell *sensu stricto*, and roundhead—*Galaxias gollumoides* McDowall and Chadderton), recognised within the *G. vulgaris* species complex [49,50], and the inferred extant, facultative diadromous ancestor (*Galaxias brevipinnis* Günther), within the framework of contrasting habitat types. Dunn et al. [48] found lentic *Galaxias* to have longer bodies; and shorter, stouter caudal peduncles; and longer, wider heads with larger mouths; compared to lotic *Galaxias*, which typically have overall shorter bodies, slenderer caudal peduncles, and shorter flatter heads with smaller mouths [48]. A major contrast in morphology between habitat types is the shape of the pectoral fins, shifting from a longer than wider ‘oar’ shape in lentic *Galaxias*, to a much broader, more trapezium-like ‘paddle’ form in lotic *Galaxias* [48]. These shifts in *Galaxias* morphologies were interpreted as functional responses to the hydrodynamic requirements of habitats, because they afford better sustained swimming, turning, and braking abilities in faster flowing water; and quick starts and forward propulsion in more stationary water columns [48]. Of significance was the divergence of morphological characters, in the same direction between lentic and lotic habitats, from a similar starting body configuration in the three closely related species, from multiple habitats across the ranges of *G. gollumoides* and *G. vulgaris*, and in eastern South Island landlocked populations of *G. brevipinnis*. Further investigations of *G. gollumoides* populations and their breeding biology by Dunn [51] suggest habitat mediated responses are occurring at or affecting all life stages.

Here, we test the hypothesis that the broader scale intraspecific morphological differences identified by Dunn et al. [48] are phenotypic responses to the contrasting hydrological environments of wetland and stream habitats, being induced early, and shifting during ontogeny to maintain a functional form. The phenotypic response aspect was tested at a finer scale in a laboratory based controlled reciprocal transplant experiment with captive reared *G. gollumoides* morphologies being compared with the morphologies of field reared conspecifics in wetland and stream habitats in uncontrolled conditions. Field reared juvenile wetland and stream *G. gollumoides* were collected temporally to also examine the ontogenetic shift aspect of intraspecific morphological divergence.

2. Materials and Methods

2.1. Study Design

This study utilised three component datasets to comparatively assess the effect of hydrological environments on *G. gollumoides* morphology. All *G. gollumoides* examined were sourced either from a no-slow flow wetland or a fast flowing stream habitat, located on Four Mile Creek in the Matura River system, being part of a longer term study on *G. gollumoides* undertaken by Dunn [51]. Morphological datasets were generated from *G. gollumoides* collected as larvae and raised in captivity under experimental conditions; *G. gollumoides* juveniles were collected temporally from the field, and adult *G. gollumoides* were collected once from the field.

2.2. Captive *Galaxias gollumoides*

Larval *G. gollumoides* sourced from wetland and stream habitats were reared in a laboratory-based, controlled, reciprocal transplant experiment (sensu Robinson and Parsons [12]), with water flow treatments of either current or no current.

Larvae were collected from wetland and stream habitats on 27–28 October 2006. All larvae either possessed yolk sacs or appeared to have only recently started exogenous feeding; finfolds were also present, indicating that they had likely hatched within the previous week [52–54].

Galaxias gollumoides were maintained in an aerated, trickle through flow facility specifically designed and constructed for the experiment. The facility comprised twelve, 7 L polypropylene plastic containers (35.5 cm (L) × 23.5 cm (W) × 12 cm (D)), arranged in four blocks of three. This design allowed for a 2 × 2 (source habitat × flow) factorial design with four experimental treatments: wetland no current, wetland current, stream no current, and stream current. Three replicate control (same flow as source habitat) and three replicate treatment (opposite flow to source habitat) containers for both source (wetland and stream) habitats were then randomly assigned within each block to control for position effects (Figure 1A). Water levels were maintained at a depth of approximately 8 cm. Mean (±1 SE) water velocity was 0.013 ± 0.002 m·s⁻¹, and 0 m·s⁻¹, in the current and no current treatment containers, respectively.

Fifteen larvae (due to availability at study habitats) were randomly introduced into each replicate container, giving a total of 45 larvae for each treatment and raised in this facility for one year. *Galaxias gollumoides* were then transferred to four (one per treatment; Figure 1B) aerated 120 L tanks within an outdoor facility based on Dunn and O'Brien [51,55]. Mean (±1 SE) water velocity in outdoor tanks created using power heads was 0.16 ± 0.01 m·s⁻¹ and 0 m·s⁻¹, in current and no current flow treatments, respectively. Cover was provided in the form of a layer of cobbles on the tank floor. Minimum and maximum temperatures over the period October 2006–October 2007 were recorded as −2.3–33.2 °C and −0.3–27.3 °C for air and water, respectively. All *G. gollumoides* were fed the same diet, initially consisting of ground dried fish flakes and live *Artemia* nauplii, before being transitioned to frozen bloodworms at 8 months [55]. After twenty-seven months in captivity, *G. gollumoides* were taken as specimens, being anaesthetised using 0.6 mL·L⁻¹ 2-phenoxyethanol, then euthanized with a clove oil overdose (2 mL·L⁻¹), and fixed in individual vials containing 10% formalin with subsequent transfer to 70% ethanol for storage after seven days, based on McDowall [31].

During the experiment, mortalities of captive *G. gollumoides* occurred. Mortalities were greatest in the first eight months, particularly during the larval stage before metamorphosis, being greatest in both current treatments, and least in the wetland no current treatment. Once placed into the larger outdoor tanks, mortality rates decreased in all treatments. After twenty-seven months in captivity, overall survivorship was 35%, and within treatments being 56%—wetland no current, 34%—wetland current, 21%—stream no current, and 28%—stream current.

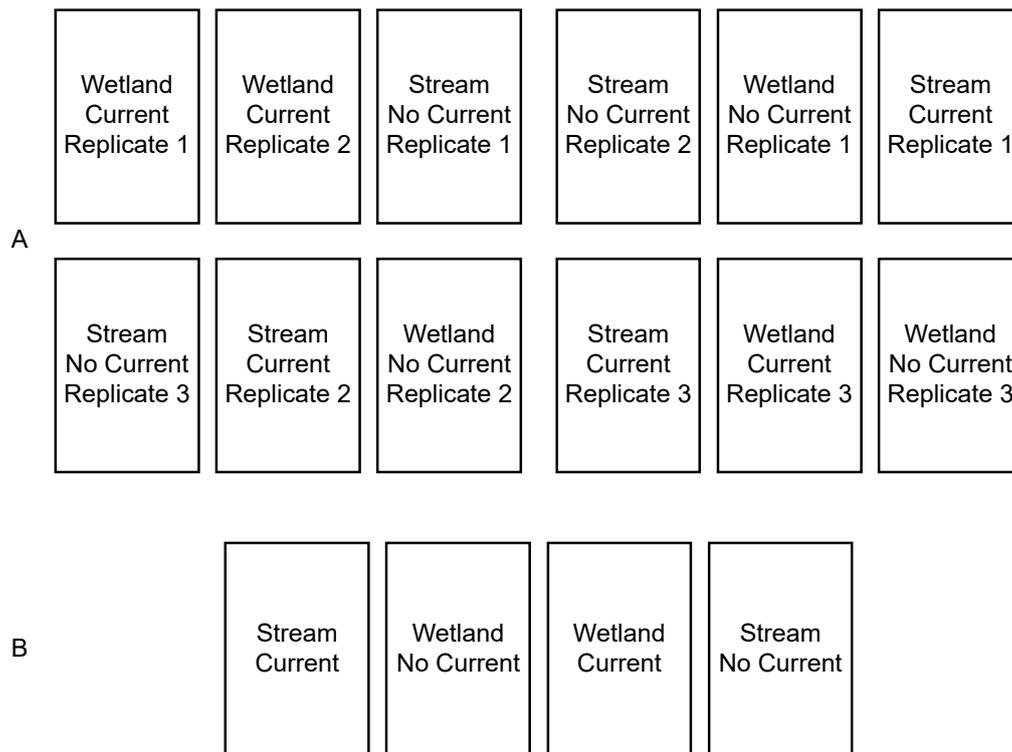


Figure 1. Schematic of experimental treatment allocation within: (A) the initial larval facility with 3 replicates for each treatment, and (B) the outdoor facility, with a single tank per treatment.

2.3. Field *Galaxias gollumoides*

The influence of contrasting hydrological environments on intraspecific differences in ontogenetic morphological development was also assessed in field reared juvenile *G. gollumoides*. The 2006 year class was followed, with populations being sampled seasonally (3 month intervals) from spring 2006–2007. *Galaxias gollumoides* were captured using either passive (overnight Gee’s minnow trapping; (1/8 inch mesh, Cuba Speciality Manufacturing Company, United States of America) or active methods (electrofishing; Kainga EFM300; NIWA Instrument Systems, New Zealand), as appropriate to the wetland and stream habitats, respectively. Five juvenile *G. gollumoides* (due to availability) from each habitat were taken as specimens (as above) each season, being termed field *G. gollumoides*.

2.4. Adult *Galaxias gollumoides*

Five adult ($\geq 1+$ year class) *G. gollumoides* were collected from each study habitat in Four Mile Creek and taken as specimens using the above methods on 8–9 April 2006, as part of a wider scale study of non-migratory *Galaxias* morphology [48].

2.5. *Galaxias gollumoides* Morphometrics

Formalin preserved *G. gollumoides* were measured using needle point digital Vernier callipers, following the techniques described by McDowall [31,40,49,56–58] and several additional measures described below (Table 2 and Table A1), to generate morphological character sets for each specimen. Measures and ratios for each *G. gollumoides* were standardised by that individual’s own standard length (SL), to reduce the effects of allometric growth, to standardise for differences in the size between populations, and to create ratios for further analyses following McDowall [31,40,49,56–58], Crow et al. [46], and Raadik [59].

Additional measures and their interpretations between treatments or habitats used or developed included the following. Body girth, calculated as the distance to the mid lateral line from the ventral

surface at the vent divided by the body depth at the vent, was interpreted as being either greater or lesser, compared to other groupings. Fin shape was calculated as:

$$\text{Fin shape} = \text{absolute} \left(\frac{\text{maximum width of fin} - \text{maximum width of fin base}}{\text{maximum length of fin}} \right) \quad (1)$$

For pectoral and pelvic fins, a smaller value for fin shape was interpreted as describing a shorter narrower, more ‘oar’ like shape, compared to a larger value describing a longer, broader, more ‘paddle’ shaped fin. For anal and dorsal fins, a smaller fin shape value represented more equal length and width, and was hence interpreted as being more square, compared to a more oblong form in a longer than wider fin. For the caudal fin, aided by the calculation of fork depth, a smaller value was interpreted as representing a more truncate form, compared to a larger value representing a more emarginate form. Caudal flange length, interpreted as longer or shorter compared to other groupings, was measured from the anterior edge of the caudal flange to a projected line passing vertically through the hypural crease. Three additional measures further described head shape, interpreted as being wider or narrower when compared between groupings. Inter-nostril width was the horizontal distance between anterior nares; maximum orbit width was the widest width between the outer edges of the two orbits; while the distance from the base of the orbit to the mid ventral line of the head, divided by head width, described the anterior profile of the head.

Character sets were generated for a total of 24—wetland no current, 14—wetland current, 9—stream no current, and 13—stream current, captive *G. gollumoides* specimens, with differences in numbers arising from differential mortality, as discussed above.

Field collected larvae were too small in spring 2006 to measure using the morphological techniques described below, while measurement of a limited number of characters, such as those described by Jones and Closs [54] and Jarvis et al. [60] for larval *Galaxias* and *Gobiomorphus* respectively, would have resulted in character sets incompatible with older specimens. But by summer 2007, *G. gollumoides* had metamorphosed and grown sufficiently, attaining juvenile forms, allowing morphological methods to be used. Thus, character sets were generated for each of the five specimens collected in summer, autumn, winter, and spring 2007 at each habitat, resulting in a dataset comprised of 20 wetland and 20 stream field *G. gollumoides*.

Character sets were also generated for five wetland and five stream adult *G. gollumoides*.

2.6. Statistical Procedures

To investigate possible differences in captive *G. gollumoides* morphologies, length standardised morphological data for each individual specimen (owing to low numbers surviving in some treatments), relativized by the mean for a morphological character, were initially summarised using non-metric multidimensional scaling (MDS) ordinations in PRIMER 6.1.12 [61]. The strength and direction of relationships (Spearman ρ correlations) between individual mean relativised morphological ratios and MDS dimension scores were then calculated in Statistica 6.0 [62] and used to interpret the two-dimensional MDS plot (Figure 2). To better identify possible morphological differences between experimental treatments, analyses were conducted on overall *G. gollumoides* morphologies (morphological MDS), and variables split into groupings describing the three primary regions following Matthews [25]; these being the body (body MDS), fins (fin MDS), and head (head MDS) of *G. gollumoides* (morphological variables listed in Table 2 and Table A1). To examine differences in morphologies between the four treatments (differences significant at $p < 0.05$), non-parametric one-way ANOSIM (analysis of similarity) analyses in PRIMER were conducted on the Euclidean distance dissimilarity matrix underlying each individual MDS ordination. ANOSIM uses the statistic R ; a test based on the rank similarities within and between *a priori* groupings in the dissimilarity matrix, ranging from -1 to $+1$, and typically between 0 and 1 [63]. The interpretation of MDS and ANOSIM results were assisted by calculation of the centroid (mean dimension 1 and 2 scores), for each treatment in each analysis.

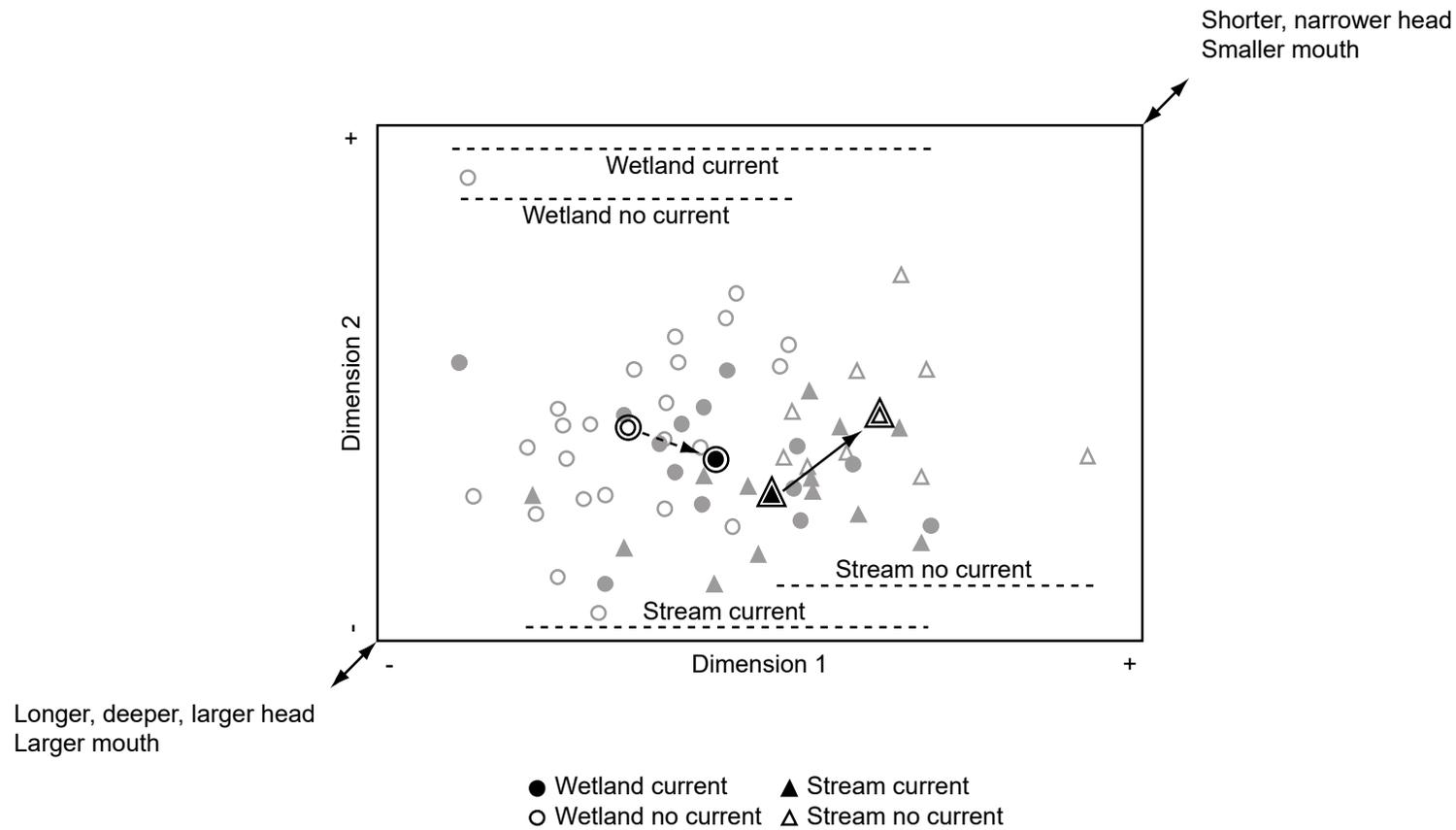


Figure 2. Captive head Multi Dimensional Scaling (MDS) ordination of nineteen mean relativised standard length standardised morphological variables for *G. gollumoides* individuals within the four experimental treatments. Descriptors are interpreted from Spearman ρ correlations of morphological variables and dimension scores significant at $p < 0.05$ (Table 2). Arrows indicate negative and positive directions across dimensions. Diagonal arrows indicate significant correlations of variables with both dimensions. Spread across dimension 1 for each treatment is given by dashed lines. Black outlined symbols are centroids for each treatment with arrows indicating direction of shifts between source treatment and the reciprocal. Grey symbols following the same convention as the centroids represent each individual *G. gollumoides* for each treatment.

To examine the hypothesis that field *G. gollumoides* morphology was diverging between the hydrological environments of wetland and stream habitats during ontogeny, developmental trajectories of individual morphological variables were investigated temporally and compared with adults collected from the same habitats. Given that morphological characters were examined individually as reaction norms (based on Imre et al. [7]), this required a different statistical approach compared to the captive *G. gollumoides*, where morphology was assessed only at the end of the experiment. Thus, for each morphological variable individually, the mean of length standardised morphological ratios at each seasonal sampling period, at each habitat for wetland and stream field *G. gollumoides*, were individually plotted against the number of days since the summer sampling period as a continuous, independent *x* axis variable.

Possible differences in developmental trajectories between wetland and stream field *G. gollumoides*, for each individual morphological variable, were tested individually using homogeneity of slopes and *y*-intercepts tests in GraphPad Prism 3.0 [64]. The interpretation of wetland and stream field *G. gollumoides* developmental trajectory slope direction for each individual morphological variable, was based on visual assessment of linear trend lines in Statistica 6.0 [62]. Furthermore, three patterns of developmental trajectories trend line slopes between wetland and stream field *G. gollumoides* were recognised, based on their configuration at the spring 2007 sampling period. Divergent—departure of trend lines indicating divergence in the ontogenetic development of a character (Figure 3A,B); convergent—trend lines approach one another due to disproportionately faster or slower development relative to standard length (Figure 3C); and parallel—similar intervals separated developmental rates and trend lines at all sampling periods (Figure 3D). To further examine the hypothesis that first year field *G. gollumoides* morphology was converging to the hydrological environment of their habitat, the direction of developmental trajectories for each individual morphological variable was assessed as to whether it approached or contradicted the mean adult *G. gollumoides* state for that morphological variable from the same habitat.

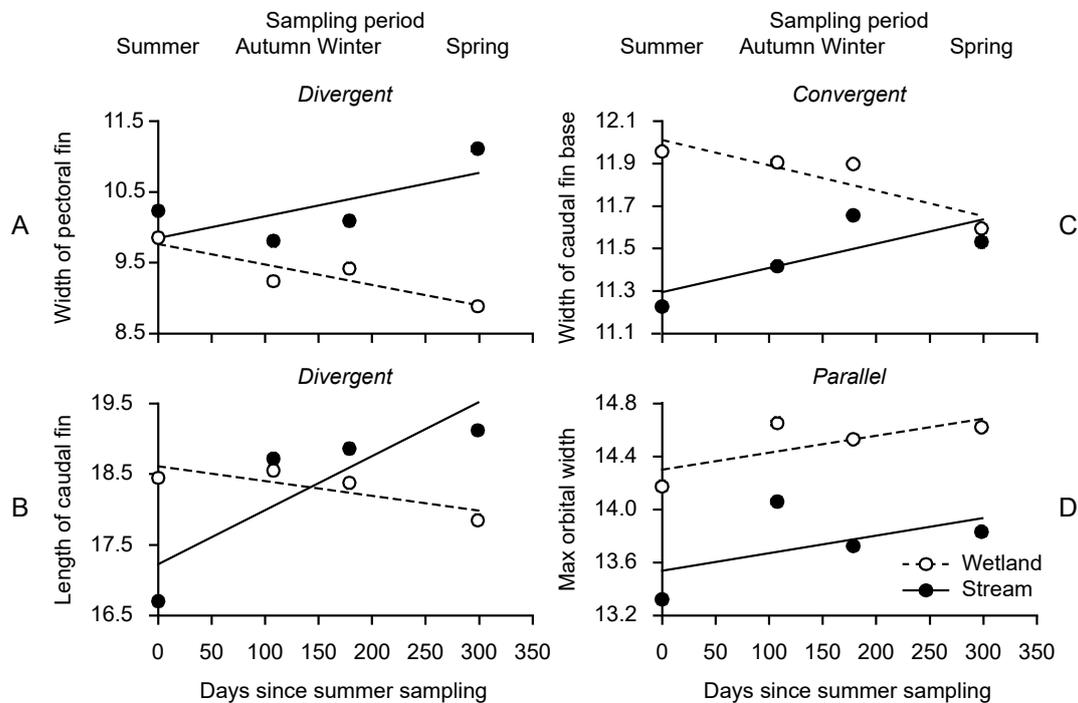


Figure 3. Developmental trajectories of wetland and stream field *G. gollumoides*. Plots show the three general patterns of developmental trajectories: (A,B)—divergent; (C)—convergent; and (D)—parallel; based on seasonally measured mean standard length standardised morphological variables and linear trend lines.

3. Results

3.1. Experimentally Induced Morphological Convergence

There was significant separation of captive *G. gollumoides* morphologies between the four treatments in the reciprocal transplant experiment for overall morphology ($R = 0.111$, $p = 0.006$), body ($R = 0.250$, $p = 0.001$), and head ($R = 0.195$, $p = 0.001$) ordinations, but not for the fin MDS ($R = 0.065$, $p = 0.07$). Low R values (0–1 scale) indicate that while differences in morphologies are present, they are comparatively small, meaning that in addition to the differential mortality between treatments, results must be considered with some caution.

The examination of pairwise comparisons of head variables (the most informative grouping) demonstrate that differences between treatments exist (Table 1). The non-significant ANOSIM pairwise test result between the two current treatments, and the shift in the wetland current treatment centroid away from that of the wetland no current, and toward the stream current treatment centroid, demonstrates convergence in head morphology in the presence of flow of wetland and stream sourced captive *G. gollumoides* (Figure 2). Moreover, this shift occurred despite all captive *G. gollumoides* being provided the same diet and resulted in almost identical mouth forms of wetland and stream sourced captive *G. gollumoides* (Table 2). Unexpectedly, in the absence of flow, there was a significant divergence of *G. gollumoides* head morphologies between the two no current treatments.

To further interpret patterns represented in the head MDS (Figure 2), Spearman ρ correlations between ranked, mean relativised, length standardised, morphological variables and MDS dimension 1 and 2 scores were calculated (Table 2). Overall, the head morphologies of captive reared *G. gollumoides* were separated across dimension 1 and 2 from those with longer, deeper, larger heads, and larger mouths, toward the bottom left of Figure 2, to those with shorter, narrower heads and smaller mouths in the upper right.

Table 1. Results of pairwise two-way ANOSIM examining similarities and dissimilarities in head morphologies of captive *G. gollumoides* within the four experimental treatments, based on the mean relativised standard length standardised morphological variables used to construct the Euclidean distance matrix underlying the head MDS. **Significant** and **Not significant**—treatments significantly and not significantly different at $p < 0.05$, respectively. ANOSIM R values for each pairwise test are given in parentheses.

		Significant (0.154)	
		Stream current	Stream no current
Not significant (0.076)	Wetland current	Not significant (0.026)	Significant (0.181)
	Wetland no current	Significant (0.279)	Significant (0.451)

Table 2. Spearman ρ correlations of mean relativised standard length standardised morphological variables with the captive *G. gollumoides* head MDS dimension 1 and 2 scores, and untransformed minimum—mean—maximum percentages of standard length standardised variables within each experimental treatment. Bold text—significant at $p < 0.05$.

Variable	Dimension 1		Dimension 2		Wetland		Stream	
	Spearman ρ	p Value	Spearman ρ	p Value	No Current	Current	No Current	Current
Head length	−0.517	<0.001	−0.402	0.001	21.7–23.2–25.2	21.5–23.1–24.8	19.5–22.3–23.2	21.5–22.9–23.8
Snout length	−0.276	0.033	−0.506	<0.001	5.6–6.6–7.3	6.0–6.5–6.9	5.5–6.2–6.9	5.8–6.7–7.2
Snout length/Orbit diameter	−0.548	<0.001	−0.286	0.027	2.4–2.7–3.0	2.3–2.7–3.3	2.1–2.4–2.6	2.3–2.7–2.9
Post-orbital head length	−0.215	0.098	−0.224	0.085	10.1–11.5–12.9	10.3–11.2–12.0	9.7–11.1–12.5	10.1–11.1–12.0
Cheek Length	−0.655	<0.001	−0.292	0.023	16.7–18.1–19.2	16.5–17.8–19.2	16.2–16.9–17.6	16.6–17.6–18.5
Head width	−0.103	0.432	−0.263	0.042	12.8–13.6–14.4	13.1–13.8–15.0	12.1–13.6–14.5	13.1–14.0–14.9
Inter nostril width	−0.211	0.106	−0.358	0.005	6.0–6.5–7.2	5.4–6.3–6.8	5.9–6.3–6.7	6.0–6.5–6.8
Head depth	−0.626	<0.001	−0.127	0.335	11.5–12.5–13.6	11.8–12.5–13.8	10.9–11.9–12.8	11.0–12.0–13.1
Head width/Head depth	−0.686	<0.001	0.123	0.351	2.2–2.6–2.9	1.9–2.5–2.9	2.1–2.2–2.6	1.9–2.4–2.7
Anterior Head width/Head depth	−0.846	<0.001	0.081	0.541	1.0–1.2–1.7	1.0–1.1–1.4	0.9–1.0–1.1	0.9–1.1–1.2
Diameter of orbit	−0.787	<0.001	0.126	0.338	5.1–5.7–6.5	4.9–5.5–6.1	4.2–5.1–5.6	4.8–5.1–5.6
Sub inter-orbital width	−0.515	<0.001	−0.181	0.167	8.1–9.1–10.0	8.4–9.0–9.7	8.0–8.5–9.1	8.2–8.8–10.2
Maximum inter-orbital width	−0.772	<0.001	−0.085	0.516	15.0–16.5–17.7	15.0–16.2–17.5	14.4–15.2–16.1	14.3–15.8–17.3
Length of upper jaw	−0.307	0.017	−0.394	0.002	6.9–8.8–9.7	7.8–8.8–9.6	8.0–8.5–9.0	7.8–9.0–10.4
Length of lower jaw	−0.177	0.175	−0.505	<0.001	6.2–7.7–9.0	6.5–7.4–8.3	6.7–7.5–8.0	6.7–7.6–8.9
Width of gape	0.243	0.061	−0.319	0.013	7.0–8.3–9.4	7.6–8.5–9.3	7.2–8.5–9.5	7.8–8.8–10.0
Depth of gape	−0.330	0.010	−0.340	0.008	7.3–8.8–10.0	7.7–8.5–9.5	7.2–8.2–9.1	7.7–8.9–11.0
Depth of gape/Width of gape	−0.909	<0.001	0.148	0.258	2.1–2.5–2.8	1.7–2.2–2.7	1.3–1.9–2.1	1.7–2.1–2.6
Width of upper lip	−0.151	0.249	−0.809	<0.001	0.7–1.3–1.8	1.2–1.4–1.7	1.0–1.2–1.4	1.2–1.4–1.7

3.2. Morphology of *Galaxias gollumoides* in Contrasting Hydrological Environments

All morphological variables displayed differences in ontogenetic developmental trajectories between wetland and stream sourced field *G. gollumoides* (Table A1). All individual morphological variable trend lines had either negative or positive slopes indicating allometric growth, with no variables displaying isometric growth. The majority of morphological variables showed divergent (55%) developmental trajectory slope patterns, 36% were described as convergent, and 10% as parallel. Developmental trajectories for the majority (56.5%) of field *G. gollumoides* morphological variables also approached the mean values obtained for adult *G. gollumoides* specimens collected from the same source habitats (Table A1).

Interestingly, 38% of first year field *G. gollumoides* morphological variable developmental trajectories showed a contradiction in one habitat, but an approach in the other habitat to the adult state in the same source habitats, with only 5.5% of cases showing a contradiction in both habitats (Table A1). However, only a small number of morphological variables had either significantly different developmental trajectory slopes or *y*-intercepts (Table A1; Figure 3); yet, reaction norm results have relevance as they support functional form predictions, especially if interpreted in a more qualitative manner [65].

Head shape in stream field *G. gollumoides* was typically smaller, flatter (dorso-ventrally compressed) and narrower, being more oval shaped when viewed anteriorly. During ontogeny, stream field *G. gollumoides* heads lengthened, deepened and widened, but were always flatter, especially the internostril width, approaching the condition of stream adults. In comparison, wetland field *G. gollumoides* head length was initially greater than stream conspecifics, but decreased during ontogeny toward the adult condition. Meanwhile, wetland field *G. gollumoides* head width and depth were always greater, but more equal than stream field conspecifics, resulting in a more circular form from earlier in ontogeny (Table A1). Diameter of the orbit (a distinctive character of *G. gollumoides*), and maximum inter-orbital width increased in a parallel manner (Figure 3D), with wetland field *G. gollumoides* having larger orbit diameters, and a greater width between the outside edges of the lenses of both orbits, compared to stream field conspecifics, consistent with the narrower head of stream *G. gollumoides* (Table A1).

The caudal region and fin displayed contrasting developmental trajectories. Body width at the vent of field *G. gollumoides* increased and the caudal peduncle of stream field *G. gollumoides* shifted from being relatively stocky, to the slenderer form of stream *G. gollumoides* adults (Table A1). Conversely, the wetland field *G. gollumoides* caudal peduncle shortened, approaching the wetland *G. gollumoides* adult condition, but its depth decreased slightly, opposite to the stockier adult form.

Concomitant ontogenetic shifts in individual characters describing first year field *G. gollumoides* caudal fin form typically contradicted the adult form in one habitat (Table A1; Figure 3B,C). Caudal fin length decreased and increased in wetland and stream field *G. gollumoides* respectively, but were always longer than adults in both habitats, with the adult stream caudal fin length both predicted and observed to be longer than wetland conspecifics. The caudal fork form also changes during ontogeny. The initially deep fork in field wetland *G. gollumoides* diminished, but fork depth deepened in stream conspecifics, resulting in significantly different slopes of developmental trajectories (Table A1). While the caudal fork depth trajectory of wetland field *G. gollumoides* approached the truncate—rounded fin of adults, it appears that as stream *G. gollumoides* pass their first year, relative fork depth also diminishes, resulting in stream adults having only a slightly forked fin.

Marked ontogenetic changes were also observed in the pectoral, anal, and dorsal fins, of field *G. gollumoides*, typically displaying divergent slope patterns and contradicting the condition of adults. The length of the pectoral fin developed faster in stream field *G. gollumoides*, resulting in 'oversized' fins relative to the body size, but during ontogeny the pectoral fin widened (Table A1; Figure 3A), with overall fin shape approaching the paddle form of adults. In wetland field *G. gollumoides*, the pectoral fin was initially longer than in stream conspecifics, but more similar in width, however, these characters decreased during ontogeny, with the overall shape approaching the more elongate oar form of adults. Length of the anal fin of wetland field *G. gollumoides* remained almost constant during ontogeny,

whereas in stream field *G. gollumoides*, anal fin length increased rapidly from summer–autumn becoming longer, but narrower than in wetland conspecifics, then declined, resulting in shorter fins than the adult condition for both habitats. Similarly, in both wetland and stream field *G. gollumoides*, dorsal fin shape reached its maximum size relative to standard length during the juvenile period.

4. Discussion

In combination, our laboratory-based controlled reciprocal transplant experimental and uncontrolled field results demonstrate, albeit with some caution, that the presence or absence of water current moulds *G. gollumoides* morphology from early in ontogeny. In captive *G. gollumoides*, when fed the same diet and held in experimental conditions that only differed by the presence or absence of current, intraspecific morphological shifts were induced. The head of wetland current *G. gollumoides* became flatter and more streamlined, converging with that of stream current *G. gollumoides*, and diverging from wetland no current conspecifics from the same source habitat. In first year field *G. gollumoides* at different stages of ontogeny and adults, the induction and maintenance of intraspecific morphological divergence in response to the continuous influences of the hydrological environment on functional form were observed. These results support the hypothesis that the hydrological environment of a habitat induces an individual's morphology in a particular habitat type, leading to intraspecific morphological divergence through mechanisms such as phenotypic plasticity. Furthermore, the hypothesis that *G. gollumoides* morphology is induced early, and shifts during ontogeny, to maintain a functional form in the contrasting hydrological environments of wetland and stream habitats, is also supported.

That phenotypic divergence is induced early in the ontogeny of *G. gollumoides* when morphological characters are labile is consistent with the findings of similar experiments on salmonids [7,23,28,66]. However, differential accidental and random mortality occurred in the laboratory based reciprocal transplant experiment and was high in treatments involving water current, despite the low velocities involved, meaning that results must be interpreted with some caution. In that, if a phenotype is related to mortality within a particular treatment, this can result in mean morphological differentiation between treatments due to selective mortality rather than plastic morphological shifts [12]. The results, especially the significant divergence of *G. gollumoides* head morphologies between the two no current treatments, could also be interpreted as having arisen from the process of local adaptation. That is, most survivors in current treatments were, by necessity, morphologically similar, as a particular functional form improved chances of survival [67]. However, accidental and random mortality was high and may reduce the strength of such processes and the inferences able to be drawn, to an unknown extent.

Divergent shifts in both wetland and stream field *G. gollumoides* morphology during ontogeny, and in adults, likely represent simultaneous individual- and population-level phenotypic responses in all body regions, to the sustained influence of the hydrological environment, maintaining a functional form that optimises swimming and foraging [7,17,21,68]. Morphological plasticity of head form during ontogeny in contrasting flow regimes is not unexpected [69,70]. The head is the leading edge of the fish that encounters drag in a stream, thus, a dorso-ventrally flattened head as seen in stream *G. gollumoides* and induced in wetland current conspecifics, is more streamlined, thus reducing energy expenditure. A flattened head also has the effect of pushing the body down, allowing a fish to maintain station in flowing water [25,68,69]. In lentic habitats, movements of the body and caudal region generate propulsion [25]. However, this produces lateral movement of the head, increasing drag and energy expenditure [71]. Adult lentic *Galaxias* typically had a larger head and body, and a stocky caudal peduncle [48]. Such a body form, appearing as a deep lateral silhouette, maximises forward thrust, while a larger, heavier head reduces lateral oscillation and energy expenditure during swimming [71].

That intraspecific ontogenetic shifts occurred in the caudal region of field *G. gollumoides* supports similar findings using geometric morphometrics by Crow and McDowall [47], between flathead members of the *G. vulgaris* species complex. As the caudal peduncle provides thrust for locomotion, morphological characters in this region could be expected to shift during ontogeny, due to differences

in morphologies and swimming modes of larvae compared to juveniles and adults [25,70]. Similarly, fins, comprised of rays and membranes, develop early in ontogeny when they are malleable to environmental factors [17]. However, when comparing captive reared *G. gollumoides*, and first year field *G. gollumoides*, relating fin size and shape to hydrology is complex, with conflicting results at different stages of development suggesting high natural variability, or that the role of these fins change during ontogeny as body size and swimming requirements change. However, the similar rate of increase in the width of the pectoral fin base in field *G. gollumoides* from both habitats during ontogeny indicates that differences in pectoral fin length and width, resulting in oversized fins in stream *G. gollumoides*, are phenotypic responses to hydrology, as these two populations are not considered to be genetically distinct [48].

5. Conclusions

Recognition that the hydrological environment is the mechanism inducing intraspecific phenotypic responses resulting in morphological variation between populations and during ontogeny aids the taxonomic description of long recognised lineages within the *G. vulgaris* species complex [44,45]. Identification of the factors influencing variable morphological traits in contrasting hydrological environments conversely allows identification of stable traits, which can be used to distinguish species [47]. The increased understanding of intraspecific variation and the role of the hydrological environment also has implications for the development of more targeted conservation management initiatives [8,72,73], facilitating the maintenance of the diversity of New Zealand's threatened [74], endemic freshwater fish fauna [75].

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Standard length (mm) and untransformed seasonal (summer–autumn–winter–spring) mean percentages of standard-length standardised variables for wetland and stream field, and adult *G. gollumoides*. Slope pattern and directions describe growth trajectories and whether these approach or contradict the morphological condition observed in adults. Results of homogeneity of slope and *y*-intercepts tests: Bold text—significant at $p < 0.05$.

Variable	Wetland	Stream	Slope Pattern	Slope Direction		Adults	Slope		<i>y</i> -Intercept	
				Wetland	Stream		<i>F</i> _(1,4)	<i>p</i> Value	<i>F</i> _(1,5)	<i>p</i> Value
Standard length (mm)	32.7–42.4–44.4–43.8–78.6	26.9–42.7–44.2–43.3–70.2								
Body lengths and depths										
Prepelvic length	52.2–52.7–53.2–52.5–53.0	52.2–51.8–51.6–52.4–51.5	Divergent	Increase	Increase	Approach	0.040	0.851	4.322	0.092
Predorsal length	69.3–70.8–70.1–70.8–69.7	69.2–70.2–69.3–69.5–70.3	Divergent	Increase	Increase	Wetland–Contradict Stream–Approach	1.057	0.362	2.749	0.158
Preanal length	71.8–73.2–73.3–73.6–74.7	72.5–72.0–72.0–73.0–74.0	Divergent	Increase	Increase	Approach	1.679	0.265	2.944	0.147
Pectoral–pelvic length	28.9–31.3–32.1–30.1–30.8	30.8–28.0–29.9–31.0–30.0	Divergent	Increase	Increase	Approach	0.028	0.875	0.435	0.539
Pelvic–anal length	19.6–20.5–20.1–21.1–21.7	20.3–20.1–20.4–20.5–22.5	Divergent	Increase	Increase	Approach	3.827	0.122	0.002	0.965
Predorsal/Preanal length	3.0–2.3–2.2–2.2–1.2	3.6–2.3–2.2–2.2–1.4	Convergent	Decrease	Decrease	Approach	0.552	0.499	0.364	0.573
Length of caudal peduncle	16.0–15.3–14.5–15.3–14.2	14.4–14.9–15.0–15.8–15.1	Divergent	Decrease	Increase	Approach	6.195	0.068	0.320	0.596
Depth of caudal peduncle	8.0–7.9–8.0–7.9–8.9	7.1–8.0–7.6–7.8–9.0	Convergent	Decrease	Increase	Wetland–Contradict Stream–Approach	1.507	0.287	1.963	0.220
Depth/Length of caudal peduncle	1.5–1.2–1.2–1.2–0.8	1.9–1.3–1.1–1.2–0.9	Convergent	Decrease	Decrease	Approach	1.209	0.333	0.310	0.602
Body depth at vent	11.3–11.2–11.3–10.8–12.5	11.0–11.4–11.1–11.5–12.9	Divergent	Decrease	Increase	Wetland–Contradict Stream–Approach	5.204	0.085	0.374	0.568
Body width at vent	5.9–5.8–6.1–6.0–7.2	5.2–6.2–5.9–7.0–7.5	Divergent	Increase	Increase	Approach	7.943	0.048		
Body girth	3.4–2.8–2.6–2.6–1.4	4.5–2.6–2.7–2.4–1.6	Convergent	Decrease	Decrease	Approach	1.545	0.282	0.236	0.647
Pectoral fin										
Length of pectoral fin	14.5–13.7–13.9–14.3–14.1	13.9–15–15.1–15.2–13.8	Divergent	Decrease	Increase	Wetland–Approach Stream–Contradict	2.983	0.159	3.786	0.109
Width of pectoral fin	9.8–9.2–9.4–8.9–10.0	10.2–9.8–10.1–11.1–11.1	Divergent	Decrease	Increase	Wetland–Contradict Stream–Approach	5.889	0.072	6.459	0.052
Width of pectoral fin base	4.4–4.9–4.9–4.8–4.7	4.7–5.2–4.9–5.2–4.9	Parallel	Increase	Increase	Approach	3.12 × 10 ^{−5}	0.996	2.702	0.161
Shape of pectoral fin	1.2–0.7–0.7–0.7–0.5	1.5–0.7–0.8–0.9–0.7	Parallel	Decrease	Decrease	Approach	0.018	0.899	0.860	0.396
Pelvic fin										
Length of pelvic fin	12.7–12.5–13–12.8–12.9	8.5–13.1–13.6–13.6–13.8	Divergent	Increase	Increase	Approach	3.858	0.121	0.242	0.644
Width of pelvic fin	6.7–7.6–8.3–7.0–7.0	5.3–7.9–8.4–8.7–9.4	Divergent	Increase	Increase	Approach	2.834	0.168	0.057	0.821
Width of pelvic fin base	2.8–3.4–3.4–3.1–2.9	2.3–2.9–3.2–3.5–3.4	Divergent	Increase	Increase	Wetland–Contradict Stream–Approach	4.417	0.104	0.488	0.516
Shape of pelvic fin	0.9–0.8–0.8–0.7–0.4	1.3–0.9–0.9–0.9–0.6	Convergent	Decrease	Decrease	Approach	0.804	0.421	4.298	0.093

Table A1. Cont.

Variable	Wetland	Stream	Slope Pattern	Slope Direction		Adults	Slope		y-Intercept	
				Wetland	Stream		F _(1,4)	p Value	F _(1,5)	p Value
Anal fin										
Length of anal fin	17.9–18.1–17.7–17.9–18.7	16.4–18.9–18.7–17.7–18.6	Divergent	Decrease	Increase	Wetland–Contradict Stream–Approach	0.452	0.538	0.005	0.948
Width of anal fin	8.3–8.6–9.1–7.4–8.5	8.4–7.9–8.4–8.1–8.5	Divergent	Decrease	Decrease	Wetland–Approach Stream–Contradict	0.249	0.644	0.168	0.699
Width of anal fin base	13.3–12.5–12.8–12.1–12.7	12.3–12.9–12.9–12.4–11.7	Divergent	Decrease	Increase	Wetland–Approach Stream–Contradict	2.977	0.160	0.104	0.760
Shape of anal fin	0.9–0.5–0.5–0.6–0.3	0.9–0.6–0.5–0.6–0.2	Convergent	Decrease	Decrease	Approach	0.084	0.787	0.170	0.698
Caudal fin										
Length of caudal fin	18.4–18.6–18.4–17.8–16.9	16.7–18.7–18.9–19.1–18.1	Divergent	Decrease	Increase	Wetland–Approach Stream–Contradict	8.567	0.043		
Width of caudal fin	17.5–17.4–20.6–17.6–19.4	18.7–16.5–17.8–16.5–18.5	Divergent	Increase	Decrease	Approach	0.803	0.421	0.814	0.408
Width of caudal fin base	12–11.9–11.9–11.6–12.4	11.2–11.4–11.7–11.5–12.6	Convergent	Decrease	Increase	Wetland–Contradict Stream–Approach	8.783	0.041		
Shape of caudal fin	0.9–0.7–1.1–0.8–0.5	1.7–0.6–0.7–0.6–0.5	Divergent	Decrease	Decrease	Approach	2.456	0.192	0.036	0.858
Length of caudal flange	10.2–10.8–10.6–11.9–10.6	9.0–11.1–10.0–11.1–12.1	Convergent	Increase	Increase	Wetland–Contradict Stream–Approach	0.013	0.916	1.998	0.217
Depth of caudal fork	2.4–2.4–1.6–1.5–1.1	1.8–2.1–2.2–2.3–1.6	Divergent	Decrease	Increase	Wetland–Approach Stream–Contradict	14.870	0.018		
Dorsal fin										
Length of dorsal fin	16.7–17.3–16.4–16.3–19.2	16.3–17.7–17.0–16.7–20.0	Divergent	Decrease	Increase	Contradict	0.480	0.527	0.360	0.575
Width of dorsal fin	7.4–7.7–8.3–7.4–8.3	7.5–6.9–7.4–7.6–8.7	Convergent	Increase	Increase	Approach	0.020	0.893	1.564	0.266
Width of dorsal fin base	11.7–11.5–10.5–9.9–12.0	12.7–10.6–10.6–10.9–12.7	Divergent	Decrease	Decrease	Contradict	0.055	0.826	0.405	0.552
Shape of dorsal fin	0.8–0.5–0.3–0.3–0.2	1.2–0.5–0.4–0.5–0.3	Convergent	Decrease	Decrease	Approach	0.312	0.606	1.131	0.336
Head lengths and depths										
Head length	23.3–23.3–22.6–22.3–22.3	21.9–23.5–22.7–22.8–22.9	Divergent	Decrease	Increase	Approach	2.595	0.183	0.113	0.751
Snout length	6.4–6.5–6.2–6.3–7.1	5.8–6.7–6.3–6.8–7.4	Divergent	Decrease	Increase	Wetland–Contradict Stream–Approach	3.599	0.131	0.100	0.765
Snout length/Orbit diameter	3.8–3.1–2.9–2.8–2.3	4.3–3.3–3.0–3.1–2.5	Convergent	Decrease	Decrease	Approach	0.092	0.777	1.570	0.266
Post-orbital head length	11.9–12.2–11.9–11.7–12.3	10.5–12.2–11.7–11.6–13.0	Convergent	Decrease	Increase	Wetland–Contradict Stream–Approach	1.112	0.351	0.997	0.364
Cheek Length	17.5–17.2–17.3–16.7–16.3	16.5–17.9–17.3–17.0–17.0	Divergent	Decrease	Increase	Wetland–Approach Stream–Contradict	1.110	0.352	0.001	0.974
Head width	14.0–14.4–14.5–14.4–15.0	12.4–13.8–13.7–14.4–15.5	Convergent	Increase	Increase	Approach	5.355	0.082	5.735	0.062
Inter nostril width	6.4–6.2–6.3–5.9–6.2	5.8–6.4–6.1–6.6–6.4	Divergent	Decrease	Increase	Approach	8.792	0.041		

Table A1. Cont.

Variable	Wetland	Stream	Slope Pattern	Slope Direction		Adults	Slope		y-Intercept	
				Wetland	Stream		F _(1,4)	p Value	F _(1,5)	p Value
Head depth	12.2–12.4–12.1–12.2–13.1	11.4–11.9–12.0–11.9–13.4	Convergent	Decrease	Increase	Wetland–Contradict Stream–Approach	2.747	0.173	7.701	0.039
Head width/Head depth	3.5–2.7–2.7–2.7–1.5	4.1–2.7–2.6–2.8–1.7	Convergent	Decrease	Decrease	Approach	0.285	0.622	0.220	0.659
Anterior Head width/Head depth	1.4–1.2–1.2–1.1–0.7	1.7–1.2–1.2–1.1–0.8	Convergent	Decrease	Decrease	Approach	1.190	0.337	0.450	0.532
Diameter of orbit	5.1–5.0–4.9–5.2–3.9	5.1–4.9–4.7–5.2–4.3	Parallel	Increase	Increase	Contradict	0.002	0.968	0.436	0.538
Sub inter-orbital width	8.2–8.5–8.8–9.2–9.4	8.3–7.6–8.6–9.0–10.1	Parallel	Increase	Increase	Approach	0.008	0.934	1.540	0.270
Maximum orbital width	14.2–14.7–14.5–14.6–13.1	13.3–14.1–13.7–13.8–13.6	Parallel	Increase	Increase	Wetland–Contradict Stream–Approach	4.35 × 10 ⁻⁴	0.984	21.341	0.006
Length of upper jaw	8.0–8.5–8.1–8.2–8.9	7.2–8.8–8.2–8.8–9.2	Divergent	Increase	Increase	Approach	1.859	0.244	0.001	0.976
Length of lower jaw	7.6–7.6–7.4–7.3–8.0	6.7–7.8–7.4–7.9–8.0	Divergent	Decrease	Increase	Wetland–Contradict Stream–Approach	6.390	0.065	0.006	0.941
Width of gape	8.4–8.4–8.5–8.8–10.1	7.0–8.2–7.9–8.6–9.8	Convergent	Increase	Increase	Approach	3.452	0.137	4.914	0.077
Depth of gape	7.3–8.1–7.6–7.8–8.3	7.3–8.7–7.6–7.4–8.6	Divergent	Increase	Decrease	Wetland–Approach Stream–Contradict	0.248	0.645	0.016	0.904
Depth of gape/Width of gape	2.7–2.3–2.0–2.1–1.0	4.0–2.5–2.2–2.0–1.3	Convergent	Decrease	Decrease	Approach	3.193	0.149	1.544	0.269
Width of upper lip	1.5–1.4–1.4–1.6–1.6	1.4–1.4–1.4–1.6–1.6	Convergent	Increase	Increase	Approach	0.420	0.552	0.344	0.583

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