

Article

Morphological Convergence and Divergence in *Galaxias* Fishes in Lentic and Lotic Habitats

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Abstract: The influence of contrasting lentic and lotic hydrological environments on the morphology of members of the *Galaxias vulgaris* species complex was examined. Morphological variation between habitat types was investigated by comparison of populations of *Galaxias brevipinnis* (inferred ancestor), *Galaxias gollumoides* (roundhead morphotype) and *Galaxias vulgaris* (flathead morphotype). Interspecific convergence and intraspecific divergence of morphological characters were demonstrated, representing general shifts in morphology towards a common functional form in particular hydrological environments. In all species, more lentic *Galaxias* had longer bodies; shorter, more stout caudal peduncles; longer, narrower pectoral fins; and longer, wider heads with larger mouths. In comparison, lotic *Galaxias* had relatively shorter bodies; more slender caudal peduncles; broader pectoral fins; and shorter flatter heads, with smaller mouths. This study suggests that the hydrological environment of a habitat is an important factor moulding and maintaining an individual fish's morphology to a particular habitat type, most likely representing a phenotypic plastic response.

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

1. Introduction

Fishes display considerable variation in their body forms [1,2]. Equally diverse are the habitats in which freshwater fish occur, which can range across a hydrological continuum from lakes through slow-flowing wetlands, to fast-flowing, steep streams [2,3]. Populations of freshwater fish distributed across this hydrological continuum may display divergent morphologies, such as North American darters [4] and brook char (*Salvelinus fontinalis* (Mitchill); [5,6]). In contrast, different species occurring in the same habitat type may display convergence in morphologies [7–9]. From an ecomorphological perspective, such variation in morphology between habitats can be considered as maintaining a predictable functional form [10–12].

Non-migratory freshwater fish typically show greater intraspecific divergence in morphology than migratory species. This is due, in part, to all life stages occurring in the same habitat type, rather than encountering various hydrological environments at different life stages [5,13–16]. Non-migratory fishes are also often represented by geographically isolated populations across their range, occurring in habitats subject to varying hydrology. Further, at higher latitudes and altitudes, fish assemblages tend to become depauperate, meaning non-migratory species and populations may exploit a wider range

of resources and habitats, and hence, be exposed to a range of selective pressures [3,7,17–19]. Within these habitats, intraspecific morphological variation may arise from the influence of the proximate hydrological environment of a habitat on the phenotype, or through habitat-influenced genotypic differences between populations, or a complex interaction of the two, with environmental factors superimposing their effects on the ranges of phenotypic responses a genotype can produce [20–24].

Species within Galaxiidae display substantial variation in body form across a range of habitats, from intermittent and perennial streams, to wetlands and lakes, across their cool temperate, Southern Hemisphere distribution [19,25–31]. Intraspecific variation in morphology within this group has been examined in the context of landlocked and diadromous riverine populations of *Galaxias truttaceus* Valenciennes [32] and *Galaxias maculatus* (Jenyns) [13,33–35], morphological character displacement in sympatric stream-dwelling *Galaxias* [36] and eco-morphological character displacement in sympatric *Paragalaxias* in Tasmanian lakes [37]. Diet and behaviour may also contribute to morphological variation [38], for example, in *Aplochiton zebra* Jenyns within lakes, resource polymorphism [39,40], and turbidity [41], and for *Galaxias platei* Steindachner, predation risk and trophic status [42] have been reported, while maternal investment into oocytes across an altitudinal and habitat productivity gradient in non-migratory *Galaxias* [43] has also been identified as a factor resulting in morphological variation.

In New Zealand, the *Galaxias vulgaris* species complex (*sensu* Allibone and Wallis [44]), occurring across eastern South and Stewart islands occupy wetland and stream habitats which impose differing hydrodynamic challenges. It is now recognised that there is convergence and divergence of body form within the *Galaxias vulgaris* species complex, which has led to a situation of morphologically similar, but genetically distinct species and undescribed lineages due to the occurrence in similar habitat types, and vice versa, which has hindered formal descriptions [45,46]. This disparity between *Galaxias* morphology and genetics led us to hypothesise that the contrasting hydrological environments of lentic and lotic habitats occupied by these taxa is the mechanism inducing intraspecific character divergence in contrasting habitats and morphological convergence in similar habitats. We further hypothesise that these shifts in morphology are phenotypic plastic responses to maintain an overall functional form. To examine these hypotheses, 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 [47,48] and the inferred extant, facultative diadromous ancestor (*Galaxias brevipinnis* Günther), were assessed within the framework of contrasting habitat types as defined by quantitative hydrological characteristics.

2. Materials and Methods

2.1. Study Design

For each of *G. brevipinnis*, *G. gollumoides* and *G. vulgaris*, specimens, environmental data were collected concurrently from two differing habitat types, in close proximity, within the same sub-catchment, and where possible on the same watercourse, replicated across at least three major catchments during the austral summer–autumn of 2006–2009 (Table 1). Within sub-catchment pairing was designed to account for small-scale variations between habitats, possibly due to geographic, climatic [3,11] and genetic factors [9,35]. Sampled habitats ranged across a hydrological continuum from lakes, through no- to slow-flowing wetlands, to fast flowing steep streams, across eleven major catchments (Table 1). These habitats were defined as either lentic—lakes and wetlands, or lotic—streams and rivers, based on their physical attributes. *Galaxias gollumoides* and *G. vulgaris* were sampled from wetland and stream habitats across their distributional range as they do not occur in lakes [29,49]. For *G. brevipinnis*, landlocked stocks from east coast South Island sub-montane lakes and streams were sampled. *Galaxias brevipinnis* and *G. vulgaris* from several unpaired habitats were included in analyses, as suitable within-catchment pairs could not be found. Similarly, after initial consideration of morphological data, coastal Big Creek *G. brevipinnis* and Island Hill *G. gollumoides* (type locality) were removed from the final dataset due to marked differences in the morphologies of these populations

compared to congeneric populations. For *G. brevipinnis*, this removed the effects of variation imposed by a diadromous life history, and for *G. gollumoides*, interspecific competition results in this population existing in marginal wetland habitat not comparable to South Island habitats, and the impracticality of collecting a full set of hydrological environment data for this habitat.

2.2. *Galaxias* Sampling

At each habitat, fish were captured using either active (electrofishing; Kainga EFM300; NIWA Instrument Systems, New Zealand) or passive methods (overnight Gee's minnow trapping; 1/8 inch mesh, Cuba Specialty Manufacturing Company, United States of America) as appropriate to the habitat type. Captured fish were anaesthetised using 0.6 mL·L⁻¹ 2-phenoxyethanol, length measured (Total Length ± 0.5 mm), weighed to the nearest 0.1 g, and identified to species using the keys of McDowall [29,50]. Ten adult *Galaxias* at each habitat were then randomly selected and euthanised with a clove oil overdose (2 mL·L⁻¹). Five *Galaxias* were fixed in individual vials containing 70% ethanol, and five in 10% formalin with subsequent transfer to 70% ethanol for storage after seven days.

2.3. *Galaxias* Genetics

Galaxias genetic sequences, using material from pectoral fin clips, were examined to confirm species identity of field-collected ethanol preserved specimens, and to investigate within and between catchment haplotype variation. Protocols employed for total DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of *Galaxias* mitochondrial DNA (mtDNA) cytochrome *b* gene (cyt *b*) followed BurrIDGE et al. [51]. Complete *Galaxias* cyt *b* was amplified with primers cytb-Glu and cytb-Thr [52] and sequenced with cytb-Glu, yielding an alignment of 764 base pairs (bp). DNA sequences were deposited in GenBank (Accessions MT409195-210). Phylogenetic relationships among mtDNA sequences were reconstructed via maximum parsimony analysis [53], using PAUP*4.0b10 [54], with the heuristic search algorithm and random sequence addition. Ten heuristic searches were employed to recover up to 5000 equally-most-parsimonious topologies. Parsimony bootstrap analysis [55], also employed 10 heuristic searches for each of 500 bootstrap pseudo-replicates, with up to 500 equally-most-parsimonious topologies retained per heuristic search. The hierarchical partitioning of genetic variation between habitat types within each species was investigated by analysis of molecular variance (AMOVA) using Arlequin 3.1 [56,57] incorporating simple pairwise difference distance among haplotypes. Partitioning of variation between *a priori* habitat types (lentic, lotic; 'populations') was assessed within catchments (Table 1) and relative to the variation between catchments ('groups').

2.4. Aquatic Invertebrates

To assist the understanding of possible morphological differences of *Galaxias* mouths and heads, aquatic invertebrates were sampled from representative substrates within each habitat. A 0.5 mm mesh, triangular kick net, was employed with sampling methods based on the C1—hard-bottomed, semi-quantitative; and C2—soft-bottomed, semi-quantitative protocols of Stark et al. [58]. Composition of dominant taxa was assessed in the field on live invertebrates, identified at appropriate taxonomic levels [58], using the keys of Chapman and Lewis [59] and Winterbourn et al. [60] and assigned coded abundances, based on protocol P1 of Stark et al. [58].

Table 1. Location of study habitats within river catchments (in italics), habitat type (lentic or lotic), *Galaxias* species collected and all other fish species present.¹ habitat name not gazetted by the New Zealand Geographic Board, but locally recognised.² unpaired habitat. – no other fish species present.

Catchment and Habitat	Habitat Type	Species	Latitude	Longitude	Altitude (m)	All Other Fish Species Present in Study Habitat
<i>Waimakariri River</i>						
Lake Marymere	Lentic	<i>G. brevipinnis</i>	43°06'56.91"	171°51'27.98"	616	<i>Gobiomorphus breviceps</i>
Slovens Stream Tributary	Lotic	<i>G. brevipinnis</i>	43°05'40.18"	171°50'55.65"	579	<i>Anguilla dieffenbachii</i> , <i>G. breviceps</i> , <i>Salmo trutta</i>
Waimakariri Spring ¹	Lentic	<i>G. vulgaris</i>	43°00'55.00"	171°48'39.44"	503	<i>A. dieffenbachii</i> , <i>S. trutta</i>
Kowai River	Lotic	<i>G. vulgaris</i>	43°19'53.69"	171°51'55.45"	438	<i>G. breviceps</i>
<i>Rakaia River</i>						
Lake Ida	Lentic	<i>G. brevipinnis</i>	43°14'08.93"	171°32'33.11"	679	<i>G. breviceps</i> , <i>Oncorhynchus mykiss</i> , <i>S. trutta</i>
Ryton River	Lotic	<i>G. brevipinnis</i>	43°16'41.83"	171°32'29.81"	537	<i>O. mykiss</i> , <i>S. trutta</i>
Moss Burn	Lentic	<i>G. vulgaris</i>	43°13'38.19"	171°29'22.38"	561	<i>G. breviceps</i>
Harper River	Lotic	<i>G. brevipinnis</i> ² , <i>G. vulgaris</i>	43°13'04.44"	171°28'18.15"	539	<i>Galaxias paucispondylus</i> , <i>G. breviceps</i> , <i>O. mykiss</i>
<i>Ashburton River</i>						
Lake Camp	Lentic	<i>G. brevipinnis</i> ²	43°36'49.65"	171°03'26.86"	680	<i>Gobiomorphus cotidianus</i> , <i>G. breviceps</i>
Ashburton River	Lotic	<i>G. vulgaris</i> ²	43°35'08.37"	171°09'53.74"	629	<i>G. paucispondylus</i> , <i>G. breviceps</i> , <i>S. trutta</i>
<i>Hinds River</i>						
Hinds River Tributary	Lentic	<i>G. vulgaris</i>	43°42'12.35"	171°20'12.85"	413	<i>G. breviceps</i>
Hinds River	Lotic	<i>G. vulgaris</i>	43°43'16.16"	171°21'25.50"	382	<i>A. dieffenbachii</i> , <i>G. breviceps</i>
<i>Waitaki River</i>						
Blue Lake 1 ¹	Lentic	<i>G. brevipinnis</i>	43°41'35.61"	170°09'51.20"	760	–
Blue Lake 2 ¹	Lentic	<i>G. brevipinnis</i> ²	43°41'36.77"	170°09'53.47"	754	–
Blue Lake 4 ¹	Lentic	<i>G. brevipinnis</i> ²	43°41'41.36"	170°09'55.71"	758	–
Blue Lake 5 ¹	Lentic	<i>G. brevipinnis</i> ²	43°41'43.95"	170°09'56.89"	756	–
Lagoon Stream ¹	Lotic	<i>G. brevipinnis</i>	43°47'26.29"	170°06'45.50"	617	<i>S. trutta</i>
Lake Poaka Tributary	Lentic	<i>G. vulgaris</i>	44°12'13.48"	170°05'24.82"	495	<i>Galaxias macronasus</i> , <i>G. breviceps</i> , <i>O. mykiss</i>
Fraser Stream	Lotic	<i>G. vulgaris</i>	44°12'51.47"	170°02'03.81"	527	<i>G. paucispondylus</i> , <i>G. breviceps</i> , <i>S. trutta</i>
Blue Stream	Lotic	<i>G. vulgaris</i> ²	43°41'53.99"	170°09'43.55"	717	–
Big Creek	Lotic	<i>G. brevipinnis</i> ²	46°09'31.68"	170°09'06.33"	2	<i>Gobiomorphus huttoni</i>
<i>Mokoreta River</i>						
Mokoreta River Tributary	Lentic	<i>G. gollumoides</i>	46°19'36.85"	169°15'21.86"	211	–
Mokoreta River	Lotic	<i>G. gollumoides</i>	46°20'12.74"	169°17'47.75"	321	<i>A. dieffenbachii</i>
<i>Mataura River</i>						
Four Mile Creek Wetland ¹	Lentic	<i>G. gollumoides</i>	45°22'55.12"	168°40'56.16"	347	<i>G. breviceps</i>
Four Mile Creek ¹	Lotic	<i>G. gollumoides</i>	45°21'38.64"	168°42'33.19"	373	<i>G. breviceps</i>
<i>Aparima River</i>						
Hamilton Burn Tributary	Lentic	<i>G. gollumoides</i>	45°38'15.59"	168°05'38.04"	382	–
Hamilton Burn	Lotic	<i>G. gollumoides</i>	45°36'45.49"	168°04'57.89"	433	–
<i>Waiau River</i>						
Fred Burn	Lentic	<i>G. gollumoides</i>	45°20'13.72"	167°55'35.26"	414	–
Whitestone River Tributary	Lotic	<i>G. gollumoides</i>	45°21'30.10"	167°54'05.73"	434	<i>A. dieffenbachii</i> , <i>G. breviceps</i>
<i>Freshwater River</i>						
Island Hill Wetland ¹	Lentic	<i>G. gollumoides</i> ²	46°54'26.55"	167°50'37.17"	32	<i>G. breviceps</i>

2.5. Hydrological Environment Data

For each habitat, physical characteristics were described by measured or calculated quantitative flow, channel/basin form, flow pattern, substratum and physical disturbance variables, hereafter collectively termed as hydrological environment data (as given in Table 2 and Table S1). For each wetland and stream habitat, measurements of current velocity, water depth and channel widths in run habitats were taken; and discharge, hydraulic radius, Froude number, Reynolds number and tractive force calculated. Channel slope in wetlands and streams was measured, but in lakes was considered to be zero. At all habitats, 50 substratum particles were randomly selected and measured to calculate substratum parameters.

Table 2. Spearman ρ correlations of mean relativised hydrological environment variables with hydrological MDS dimension 1 and 2 scores. Bold text—significant at $p < 0.05$.

Hydrological Environment Variable	Dimension 1		Dimension 2	
	Spearman ρ	p -Value	Spearman ρ	p -Value
<i>Flow</i>				
Discharge ($\text{m}^3 \cdot \text{s}^{-1}$)	−0.961	<0.0001	0.257	0.179
Maximum velocity ($\text{m} \cdot \text{s}^{-1}$)	−0.905	<0.0001	0.372	0.047
Hydraulic radius (m)	−0.619	<0.0001	−0.525	0.003
Flow depth (m)	−0.600	0.001	−0.532	0.003
<i>Channel form</i>				
Habitat slope ($\text{m} \cdot \text{m}^{-1}$)	−0.736	<0.0001	0.171	0.374
Habitat depth (m)	0.396	0.033	−0.059	0.762
<i>Flow pattern</i>				
Froude number	−0.902	<0.0001	0.402	0.030
Critical velocity	−0.599	0.001	−0.517	0.004
Reynolds number	−0.971	<0.0001	0.254	0.183
<i>Substratum</i>				
Substratum d_{25} (mm)	−0.059	0.761	0.773	<0.0001
Substratum d_{50} (mm)	0.287	0.131	0.611	<0.0001
Substratum d_{84} (mm)	0.234	0.222	0.270	0.157
Substratum d_{mean} (mm)	0.066	0.734	0.286	0.133
<i>Physical disturbance</i>				
Tractive force ($\text{N} \cdot \text{m}^{-2}$)	−0.896	<0.0001	−0.026	0.892

Mean relativised hydrological environment data for each habitat were initially summarised using a non-metric multidimensional scaling (MDS) ordination in PRIMER 6.1.12 [61], hereafter termed the ‘hydrological environment MDS’. The strength and direction of relationships (Spearman ρ correlations) between individual relativised hydrological environment variables and MDS dimension scores were then calculated in Statistica 6.0 [62] and used to interpret the two-dimensional MDS plot. One-way analysis of similarity (ANOSIM) in PRIMER was performed to test if *a priori* habitat (lentic and lotic) groupings were statistically distinct [63]. SIMPER (Similarity percentages) analysis was also conducted in PRIMER to explore which hydrological environment variables explained within and between *a priori* habitat type similarity and dissimilarity.

2.6. Galaxias Morphometrics

Formalin preserved *Galaxias* from each habitat were measured using needlepoint digital Vernier calipers following the techniques described by McDowall [26,31,37,47,64,65] and several additional measures are described below (as given in Table 3, Figure 1), to generate morphological character sets for each specimen. Measures and ratios for each *Galaxias* were standardised by that individual’s 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 [26,31,37,47,64,65], Crow et al. [36] and Raadik [66].

Table 3. Spearman ρ correlations of mean relativised standard length (SL) standardised morphological variables with morphological multidimensional scaling (MDS) dimension 1 and 2 scores, and interpretation of uni-variate ANOVAs on untransformed SL standardised variables within lentic and lotic habitats for each *Galaxias* species. Bold text—significant at $p < 0.05$.

Variable	Dimension 1		Dimension 2		<i>G. brevipinnis</i>		<i>G. gollumoides</i>		<i>G. vulgaris</i>		Convergence
	Spearman ρ	<i>p</i> -Value	Spearman ρ	<i>p</i> -Value	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	
Standard length					Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
<i>Body lengths and depths</i>											
Prepelvic length	−0.374	0.045	0.174	0.366	Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
Predorsal length	−0.376	0.044	−0.093	0.631	Shorter	Longer	Longer	Shorter	Longer	Shorter	
Preanal length	−0.691	<0.001	−0.075	0.699	Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
Pectoral–pelvic length	0.030	0.877	0.053	0.786	Greater	Lesser	Greater	Lesser	Greater	Lesser	Convergence
Pelvic–anal length	−0.429	0.020	−0.101	0.602	Lesser	Greater	Lesser	Greater	Greater	Lesser	
Predorsal/Preanal length	0.955	<0.001	−0.108	0.578	Lesser	Greater	Lesser	Greater	Lesser	Greater	Convergence
Length of caudal peduncle	0.515	0.004	0.038	0.843	Shorter	Longer	Shorter	Longer	Shorter	Longer	Convergence
Depth of caudal peduncle	−0.133	0.493	0.083	0.668	Deeper	Shallower	Deeper	Shallower	Deeper	Shallower	Convergence
Depth/Length of caudal peduncle	0.524	0.004	−0.029	0.883	Stouter	Slenderer	Stouter	Slenderer	Stouter	Slenderer	Convergence
Body depth at vent	0.004	0.982	0.240	0.209	Shallower	Deeper	Deeper	Shallower	Deeper	Shallower	
Body width at vent	−0.186	0.335	−0.221	0.250	Narrower	Wider	Wider	Narrower	Wider	Narrower	
Position of the lateral line	0.935	<0.001	−0.119	0.538	More dorsal	More ventral	More dorsal	More ventral	More dorsal	More ventral	Convergence
<i>Pectoral fin</i>											
Length of pectoral fin	−0.047	0.810	−0.115	0.552	Shorter	Longer	Shorter	Longer	Shorter	Longer	Convergence
Width of pectoral fin	0.419	0.024	−0.184	0.340	Narrower	Wider	Narrower	Wider	Narrower	Wider	Convergence
Width of pectoral fin base	−0.255	0.182	−0.246	0.198	Narrower	Wider	Narrower	Wider	Narrower	Wider	Convergence
Shape of pectoral fin	0.879	<0.001	−0.030	0.879	More oar like	More paddle like	More oar like	More paddle like	More oar like	More paddle like	Convergence
<i>Pelvic fin</i>											
Length of pelvic fin	−0.225	0.240	0.010	0.960	Longer	Shorter	Shorter	Longer	Shorter	Longer	
Width of pelvic fin	0.625	<0.001	−0.023	0.907	Narrower	Wider	Narrower	Wider	Narrower	Wider	Convergence
Width of pelvic fin base	−0.049	0.800	−0.183	0.341	Narrower	Wider	Narrower	Wider	Wider	Narrower	
Shape of pelvic fin	0.935	<0.001	−0.079	0.684	More oar like	More paddle like	More oar like	More paddle like	More oar like	More paddle like	Convergence
<i>Anal fin</i>											
Length of anal fin	0.183	0.343	0.283	0.137	Shorter	Longer	Shorter	Longer	Shorter	Longer	Convergence
Width of anal fin	0.556	0.002	0.460	0.012	Narrower	Wider	Narrower	Wider	Wider	Narrower	
Width of anal fin base	0.499	0.006	−0.058	0.766	Shorter	Longer	Shorter	Longer	Shorter	Longer	Convergence
Shape of anal fin	0.321	0.089	−0.728	<0.001	More square	More oblong	More square	More oblong	More square	More oblong	Convergence
<i>Caudal fin</i>											
Length of caudal fin	0.540	0.003	0.211	0.271	Longer	Shorter	Longer	Shorter	Shorter	Longer	
Width of caudal fin	0.023	0.905	0.664	<0.001	Wider	Narrower	Narrower	Wider	Narrower	Wider	
Width of caudal fin base	0.052	0.790	0.555	0.002	Wider	Narrower	Wider	Narrower	Wider	Narrower	Convergence
Shape of caudal fin	0.503	0.005	0.407	0.028	More emarginate	More truncate	More truncate	More emarginate	More truncate	More emarginate	
Length of caudal flange	0.162	0.402	0.379	0.042	Longer	Shorter	Shorter	Longer	Longer	Shorter	
Depth of caudal fork	0.442	0.016	−0.236	0.217	Shallower	Deeper	Deeper	Shallower	Shallower	Deeper	
<i>Dorsal fin</i>											
Length of dorsal fin	−0.055	0.778	0.268	0.159	Longer	Shorter	Longer	Shorter	Shorter	Longer	
Width of dorsal fin	0.498	0.006	0.416	0.025	Narrower	Wider	Narrower	Wider	Wider	Narrower	
Width of dorsal fin base	0.028	0.885	−0.015	0.937	Wider	Narrower	Narrower	Wider	Narrower	Wider	
Shape of dorsal fin	0.189	0.326	−0.681	<0.001	More square	More oblong	More oblong	More square	More square	More oblong	

Table 3. Cont.

Variable	Dimension 1		Dimension 2		<i>G. brevipinnis</i>		<i>G. gollumoides</i>		<i>G. vulgaris</i>		Convergence
	Spearman ρ	<i>p</i> -Value	Spearman ρ	<i>p</i> -Value	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	
<i>Head lengths and depths</i>											
Head length	−0.527	0.003	0.183	0.343	Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
Snout length	−0.548	0.002	0.041	0.831	Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
Snout length/Orbit diameter	0.746	<0.001	−0.413	0.026	Lesser	Greater	Lesser	Greater	Lesser	Greater	Convergence
Post-orbital head length	−0.563	0.001	0.304	0.109	Longer	Shorter	Shorter	Longer	Longer	Shorter	
Cheek Length	−0.497	0.006	0.158	0.414	Longer	Shorter	Shorter	Longer	Longer	Shorter	
Head width	−0.564	0.001	0.103	0.593	Wider	Narrower	Wider	Narrower	Wider	Narrower	Convergence
Inter nostril width	−0.332	0.079	−0.103	0.595	Wider	Narrower	Wider	Narrower	Wider	Narrower	Convergence
Head depth	−0.159	0.410	0.656	<0.001	Deeper	Shallower	Deeper	Shallower	Deeper	Shallower	Convergence
Head width/Head depth	0.860	<0.001	−0.281	0.140	Robuster	Flatter	Robuster	Flatter	Robuster	Flatter	Convergence
Diameter of orbit	0.237	0.216	0.453	0.014	Larger	Smaller	Smaller	Larger	Smaller	Larger	
Sub interorbital width	−0.011	0.953	0.399	0.032	Wider	Narrower	Narrower	Wider	Wider	Narrower	
Max orbital width	−0.224	0.244	0.534	0.003	Wider	Narrower	Narrower	Wider	Wider	Narrower	
Position of the eye	0.857	<0.001	0.049	0.800	Wider	Narrower	Wider	Narrower	Wider	Narrower	Convergence
Length of upper jaw	−0.678	<0.001	0.001	0.994	Longer	Shorter	Longer	Shorter	Shorter	Longer	
Length of lower jaw	−0.633	<0.001	0.072	0.711	Longer	Shorter	Longer	Shorter	Longer	Shorter	Convergence
Width of gape	−0.521	0.004	0.233	0.225	Wider	Narrower	Wider	Narrower	Wider	Narrower	Convergence
Depth of gape	−0.604	0.001	0.078	0.686	Deeper	Shallower	Deeper	Shallower	Deeper	Shallower	Convergence
Depth of gape/Width of gape	0.839	<0.001	−0.222	0.247	Smaller	Larger	Smaller	Larger	Smaller	Larger	Convergence
Width of upper lip	−0.388	0.038	0.084	0.664	Wider	Narrower	Wider	Narrower	Narrower	Wider	

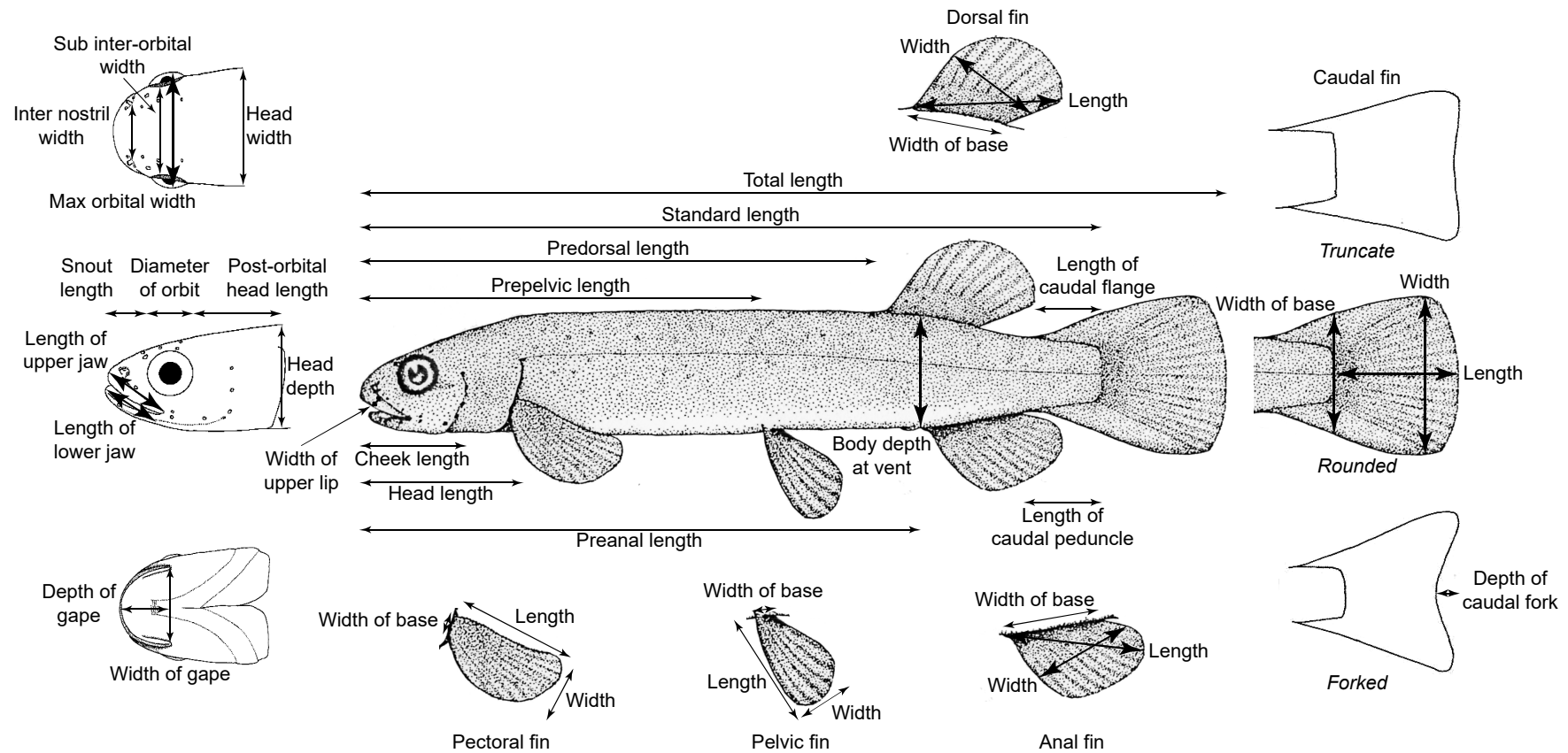


Figure 1. Morphological measures used to create ratios for further analyses. See text for further descriptions. Drawings modified from the originals by R. M. McDowall.

Additional measures and their interpretations between *a priori* groupings (species: *G. brevipinnis*, *G. gollumoides* and *G. vulgaris*, or habitat type: lentic and lotic), 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, 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 calculation of fork depth, a smaller value was interpreted as representing a more truncated form, compared to a larger value representing a more emarginate form (Figure 1). 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.

To investigate possible differences in *Galaxias* morphologies, a mean for each morphological variable ratio was calculated from the character sets for each species, for each sampled habitat, and initially summarised by MDS, using the methods described above, termed the ‘morphological MDS’. *A priori* groupings were used for the interpretation of initial analyses. Spearman ρ rank correlations were performed to assess the strength of relationships between individual mean relativised morphological ratios and MDS dimension scores in Statistica. Non-parametric one-way ANOSIM analyses with pairwise tests were conducted separately to examine differences between the *a priori* groupings.

2.7. Hydrological Environment–*Galaxias* Morphology Relationships

The hypothesis that the hydrological environment of a habitat was influencing variation in *Galaxias* morphology was initially assessed via principal components analysis (PCA) in PRIMER, using the same data sets utilised in the MDS ordinations. Spearman ρ correlations assessing the relationships between principal components (PC) scores and individual variables included in the analyses were conducted in Statistica. As PCA is an eigenvalue-based method, this allowed the extraction of axes with meaningful, absolute distance scales, as opposed to those of MDS, which only preserve the rank order of the dissimilarity matrix [61]. To examine the relationships between *Galaxias* morphology and source hydrological environment of habitats, linear regression in Statistica was conducted on the loadings of the first two principal components of the hydrological environment and morphological PCA’s.

To investigate the hypothesis of *Galaxias* morphologies diverging between habitat types, one-way analysis of variance (ANOVA) in Statistica was used to test for differences between the means of each morphological variable in lentic and lotic habitats for each species individually. Standard length relativised data from each specimen were used, with data for each variable and for each species individually, being checked for normality using Shapiro–Wilks’s *W*-tests in Statistica.

3. Results

3.1. Hydrological Environment of Lentic and Lotic Habitats

MDS analysis of hydrological environment data found a significant separation of lentic and lotic habitats (ANOSIM $R = 0.282$, $p < 0.001$; Figure 2), although, within both habitat groups, there

was a large spread of habitats. SIMPER analyses indicated that the majority of similarity within the lentic habitat group was explained by variables describing depth (habitat depth: 49.6%; flow depth (mean depth of wetland channel): 10.4%). Whereas in lotic habitats, discharge (33.0%) and the flow descriptors Reynolds and Froude numbers (22.9% and 13.6% respectively) accounted for the majority of similarity across these habitats. It was these same variables combined, that accounted for over 60% of the dissimilarity between lentic and lotic habitat groups.

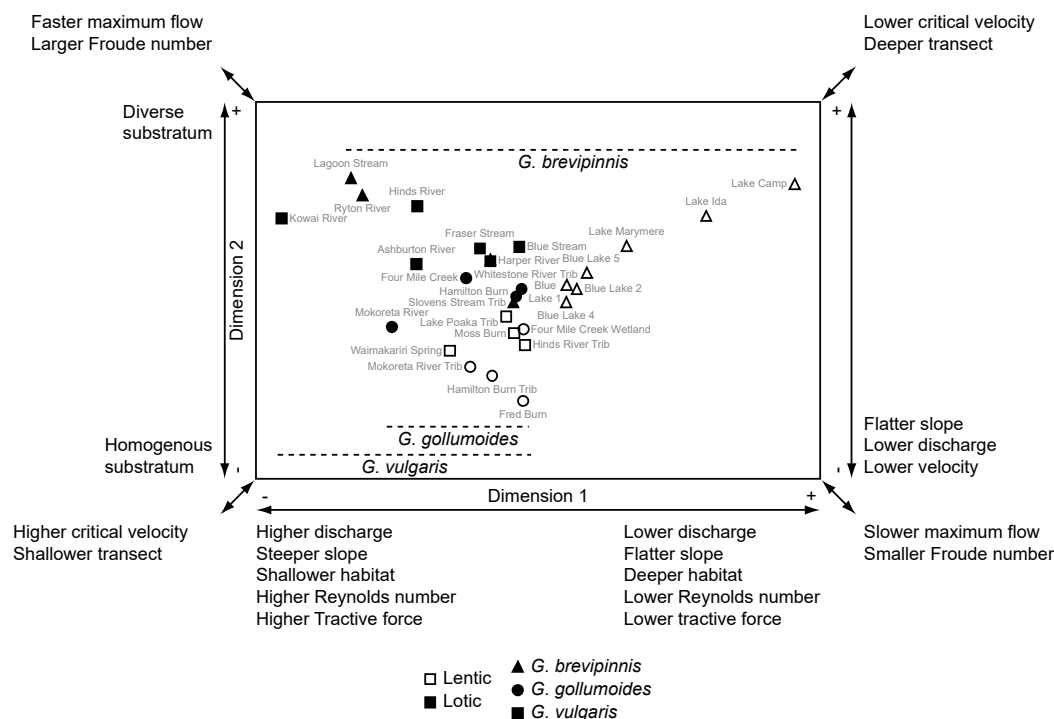


Figure 2. Hydrological environment multidimensional scaling (MDS) ordination of fourteen mean relativised hydrological variables for twenty-nine sampled *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* habitats. Descriptors are interpreted from Spearman ρ correlations of hydrological environment 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 species is given by dashed lines.

Significant Spearman ρ correlations (Table 2) between mean relativised hydrological environment variables and hydrological environment MDS dimension scores were used to interpret Figure 2. Reynolds number, a measure of flow turbulence, was negatively correlated with dimension 1, while flow depth and Substratum d_{25} were negatively and positively correlated, respectively, with dimension 2 (Figure 2).

3.2. Aquatic Invertebrates

Lentic and lotic habitats had distinctly different aquatic invertebrate community compositions (Figure 3). Lotic habitats were dominated by Ephemeroptera, Plecoptera and Trichoptera, whereas lentic habitats were dominated by the micro-crustaceans, Copepoda, Cladocera and Ostracoda.

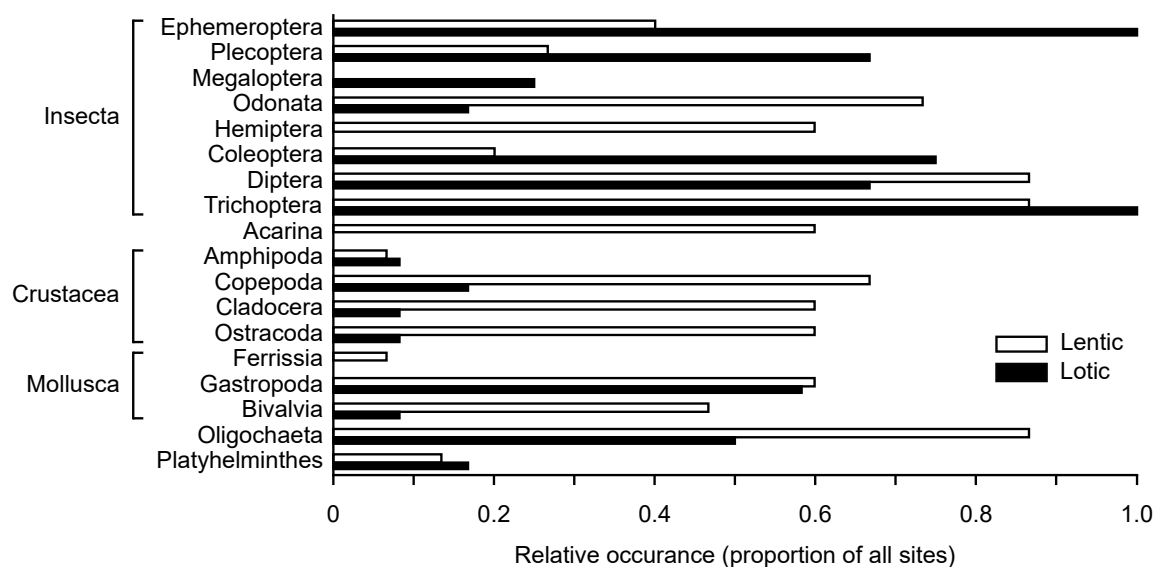


Figure 3. Composition of aquatic invertebrate fauna in lentic and lotic *Galaxias* habitats. Bars indicate the proportion of habitats sampled in which a particular taxonomic grouping occurred. Taxonomic groups follow those of Stark et al. [58].

3.3. *Galaxias* Morphology in Lentic and Lotic Habitats

Morphological data were initially summarised by MDS (Figure 4), with dimension 1 being negatively correlated with pre-anal length (Spearman $\rho = -0.691$; Table 3) and positively correlated with the ratio pre-dorsal/pre-anal length (Spearman $\rho = 0.955$), a measure describing the relative position of these two fins on the trunk. The shape of the anal fin was negatively correlated (Spearman $\rho = -0.728$), and the width of the caudal fin (Spearman $\rho = 0.664$) positively correlated with dimension 2. Thus, across dimension 1, the morphologies of *Galaxias* were distributed from those with longer bodies with more stout caudal peduncles, longer, wider heads with larger mouths to those with shorter body lengths, more slender caudal peduncles, shorter more flatter heads with smaller mouths, but with longer, broader fins. In terms of species, this separation was influenced by the different morphologies of lentic *G. brevipinnis* and *G. vulgaris* through to lotic populations of all species. Across dimension 2, there was a separation of *Galaxias* morphologies from those with slimmer anal and dorsal fins and shorter heads to those possessing longer anal, caudal and dorsal fins; and wider, deeper, longer heads.

One-way ANOSIM analyses revealed significant differences in *Galaxias* morphologies between lentic and lotic habitat types ($R = 0.155$, $p = 0.006$), despite the observed overlap when displayed in two dimensions (Figure 4), and significant differences between the three species in terms of overall morphologies ($R = 0.347$, $p < 0.001$). Pairwise tests indicated that the greatest morphological dissimilarities (larger R -value) occurred between *G. brevipinnis* and *G. gollumoides* ($R = 0.508$, $p < 0.001$), then *G. gollumoides* and *G. vulgaris* ($R = 0.404$, $p < 0.001$) and least between *G. brevipinnis* and *G. vulgaris* ($R = 0.219$, $p = 0.011$).

Galaxias brevipinnis displayed the greatest amount of variation in morphology across dimension 1 (Figure 4, Table S2) and occurred across the greatest range of habitats (Figure 2). *Galaxias gollumoides* morphology showed greater variability across both dimensions than *G. vulgaris* (Figure 4), yet the range of habitats occupied by *G. gollumoides* was less than the range of *G. vulgaris* (Figure 2).

Paired habitats within sub-catchments (Table 1) were separated across dimension 1 with the lentic habitat within a pair to the left, and the lotic habitat to the right in all cases except for *G. vulgaris* in the Rakaia River catchment. This separation across morphological dimension 1 also resulted in no within sub-catchment groupings being evident for any species.

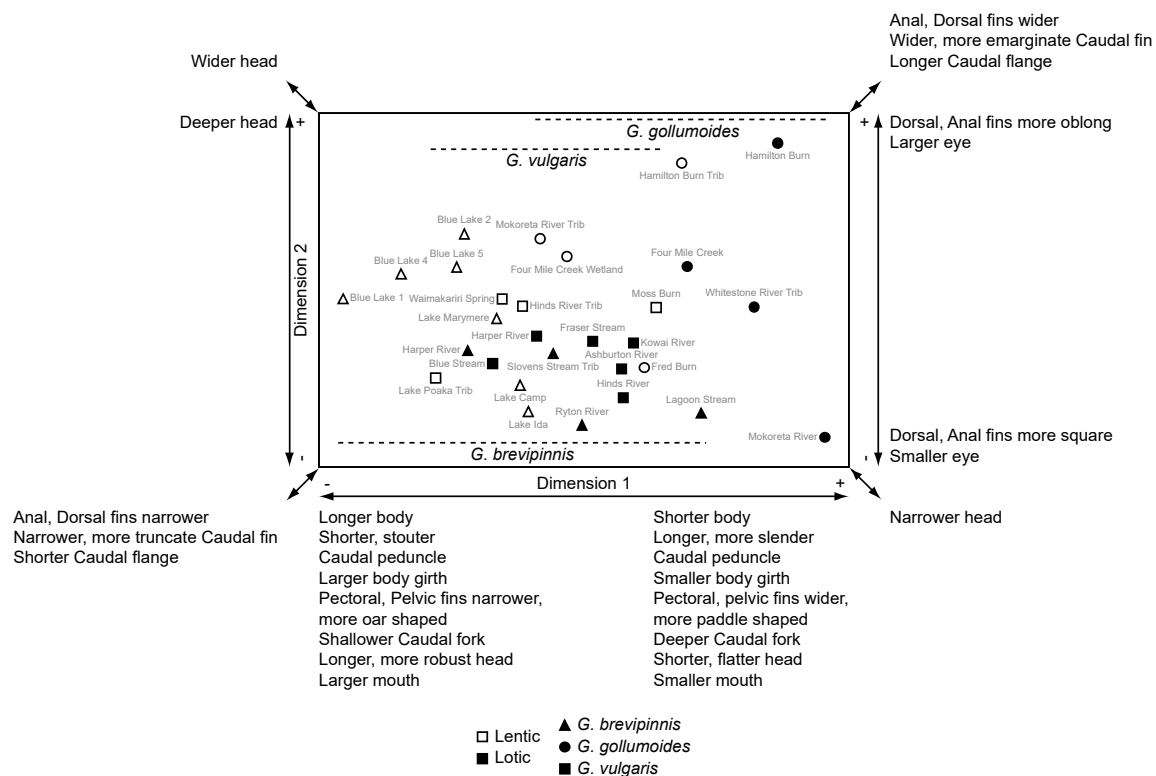


Figure 4. Morphological multidimensional scaling (MDS) ordination of fifty-three mean relativised standard length standardised *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* morphological variables from twenty-nine habitats. Descriptors are interpreted from Spearman ρ correlations of morphological variables and dimension scores significant at $p < 0.05$ (Table 3). Arrows indicate negative and positive directions across dimensions. Diagonal arrows indicate significant correlations of variables with both dimensions. Spread across dimension 1 for each species is given by dashed lines.

3.4. Galaxias Genetic Considerations

Phylogenetic relationships of *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* from habitats sampled in this study, and representative samples of other members of the *G. vulgaris* species complex, and diadromous *Galaxias fasciatus* Gray and *Galaxias postvectis* Clarke as outgroups, were initially summarised using a strict consensus topology (Figure 5).

This indicated strong support (>99% parsimony bootstrap proportions (BP)) for the partitioning of *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* as distinct species. There was also support (>70% BP) for the roundhead (*Galaxias anomalus* Stokell, *Galaxias eldoni* McDowall, *G. gollumoides*, *Galaxias pullus* McDowall) and flathead (*Galaxias depressiceps* McDowall and Wallis, *Galaxias* “northern”, *Galaxias* “southern”, *Galaxias* “species D”, *Galaxias* “Teviot”, *G. vulgaris*) clades within the *G. vulgaris* species complex.

Groupings within *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* were less obvious, and neither were there clear distinctions between lentic and lotic pairs within sub-catchments for each species. However, there appears to be greater support (>70% BP) for groupings within *G. gollumoides* than either *G. brevipinnis* or *G. vulgaris*.

Hierarchical analysis of genetic variation by AMOVA indicated that for those paired *Galaxias* populations included in the analysis, there was significant partitioning of genetic variation between major catchments, explaining 65.8% of the overall genetic variation (Table 4). Conversely, genetic variation was not significantly partitioned between lentic and lotic habitats within major catchments. Further, the significant partitioning of genetic variation within lentic and lotic habitats is likely due to differences between habitats of the same type in different major catchments.

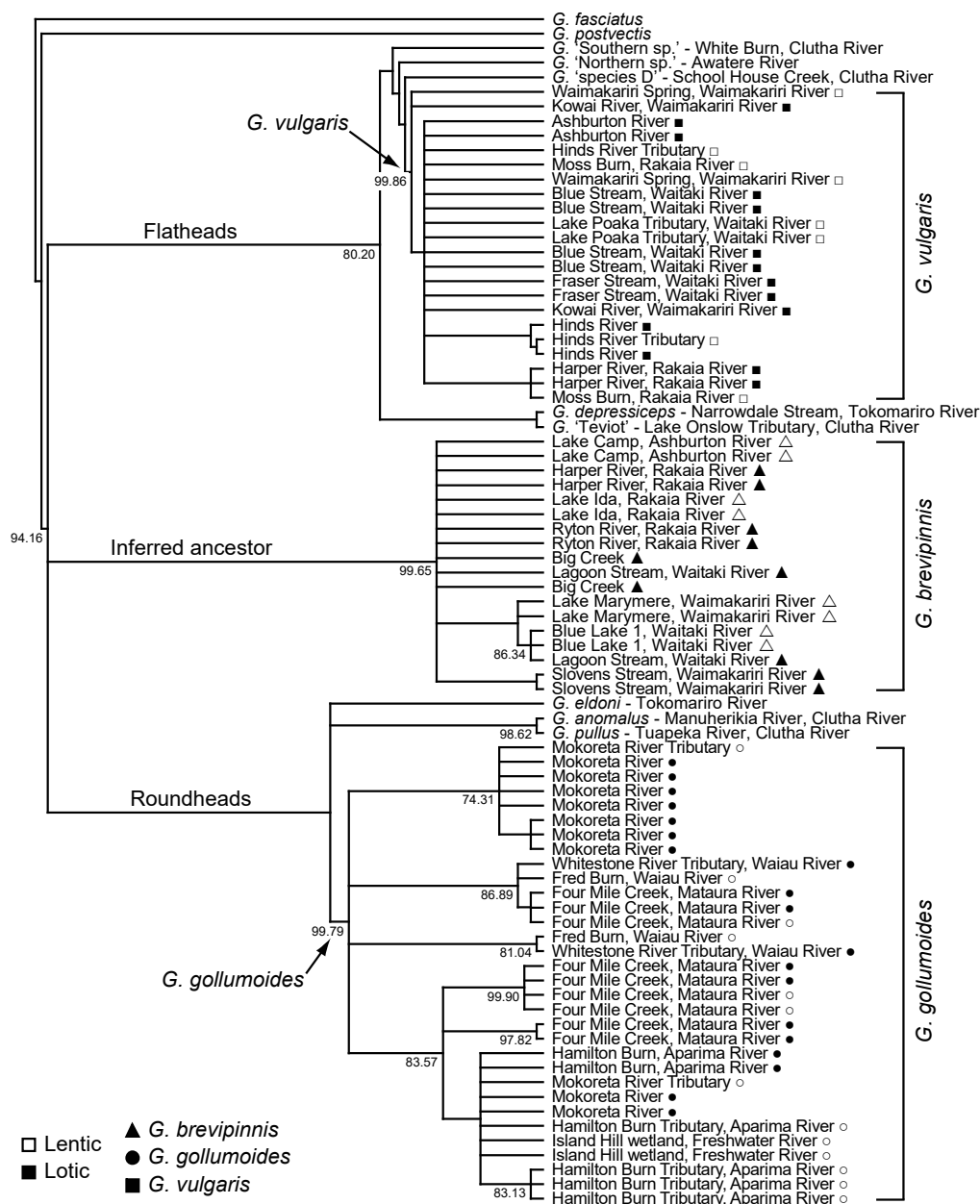


Figure 5. Phylogenetic relationships (strict consensus topology) of *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* from sampled habitats in this study, and representatives of other lineages within the two recognised morphotypes of the *G. vulgaris* species complex. A minimum of two specimens were included for each habitat. Values at nodes represent parsimony bootstrap percentages (when exceeding 70%).

Table 4. Results of analysis of molecular variance (AMOVA) analysis examining partitioning of *Galaxias* haplotype variation between and within river catchments and lentic and lotic habitat types. Bold text—significant at $p < 0.05$.

Variance Component	Observed Partition		<i>p</i> -Value	Φ Statistic
	Variance	% of total		
Between catchments	11.53	65.79	<0.0001	$\Phi_{CT} = 0.658$
Between habitats within catchments	−2.06	−11.73	0.929	$\Phi_{SC} = −0.343$
Within habitats	8.05	45.94	<0.0001	$\Phi_{ST} = 0.541$

3.5. Relationships between the Hydrological Environment and *Galaxias* Body Form

Linear regression of hydrological environment PC and morphological PC loadings were employed to test that the patterns observed above are consistent with the hypothesis that the hydrological environment was influencing *Galaxias* morphology, rather than being the product of genetic variation in localised populations. Linear regression revealed significant relationships between overall *Galaxias* morphology and the hydrological environment of habitats (Figure 6). There was a negative relationship between both hydrological environment PC 1 and morphological PC 1 ($r^2 = 0.199$, $p = 0.018$; Figure 6A) and hydrological environment PC 2 and morphological PC 2 ($r^2 = 0.247$, $p = 0.0061$; Figure 6B). Examination of initial hydrological environment and morphological PC analyses indicated congruence with the MDS analyses. The first two hydrological environment PCs accounted for 45.2% and 24.2% of the overall variation, with eigenvalues of 9.20 and 4.91, respectively. The first two morphological PCs explained 42.2% and 17.7% of the overall variation in *Galaxias* morphology, with eigenvalues of 0.300 and 0.126, respectively. Thus, the linear regressions of PC loadings confirmed the hypothesis that the morphology of the three *Galaxias* species examined were convergent with the hydrological conditions of habitats. That is, the more streamlined *Galaxias* occurred in steeper, faster flowing lotic habitats compared with stockier *Galaxias*, which tended to occur in lentic habitat types.

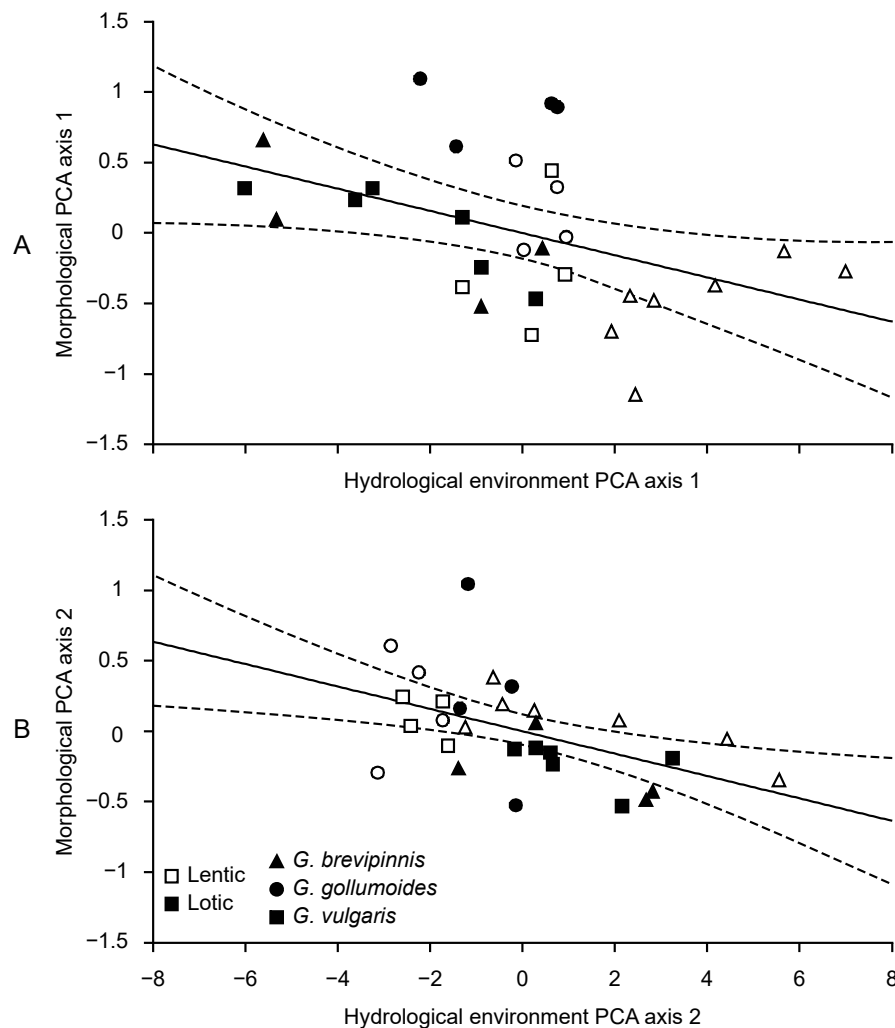


Figure 6. Spearman ρ rank correlations between hydrological environment principle component analysis (PCA) and morphological PCA: A—axis 1; and B—axis 2 scores; suggesting morphological convergence of *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* morphologies in similar hydrological environments. Both correlations were significant at $p < 0.05$.

The hypothesis that *Galaxias* morphologies were diverging between habitat types was further examined. Significant ANOVA results ($p < 0.05$) revealed that 57% of morphological variables individually displayed convergence to habitat type (Table 3 and Table S3). Where convergence to habitat type of a morphological variable was interpreted when the direction of divergence between lentic and lotic means was the same across all three *Galaxias* species.

Of these variables, almost half displayed significant divergence in two or more *Galaxias* species, suggesting gradients of character divergence under similar habitat conditions. These results were congruent with the interpretation of the morphological MDS (Table 3, Figure 4). Importantly, the pectoral fin was significantly wider, and the ratio of head width/head depth indicated a flatter head, in lotic habitats for all three *Galaxias* species. Non-convergent morphological variables were signified by a divergence between habitat types in one species in a direction opposite to that shown by the other two species (Table 3), suggesting that non-convergent variables may not be influenced to the same extent by hydrological conditions as convergent variables. Non-convergent variables occurred in all three groups of body- and head-dimensions and fins.

4. Discussion

This study, using a comparative approach, supports the hypothesis that *G. brevipinnis* (eastern South Island landlocked populations), *G. vulgaris* and *G. gollumoides* (from multiple habitats across their ranges) display both interspecific convergence and intraspecific divergence of morphological characters. Further, the hypothesis that these shifts in morphology are likely phenotypic plastic responses to maintain an overall functional form within the framework of contrasting lentic and lotic habitats is also supported, given the lack of genetic structuring by habitat type.

4.1. Convergence and Divergence of Form

When considered from an ecomorphological perspective, intraspecific morphological divergence is not unexpected, as fish form is a trade-off, balancing the functional requirements of a fish at different life stages and the influence of the hydrological environment of the habitat occupied [11,18]. However, the extent and similarity of morphological divergence displayed by the three *Galaxias* species across multiple characters and regions was unexpected.

In terms of body form, more lentic *Galaxias*, were typified by deeper bodies for their length and larger girths, compared to lotic congeners, consistent with the finding of longer, deeper bodied species, such as *Galaxias argenteus* (Gmelin) in pools or slow flowing habitats [26]. Lentic *Galaxias* are also likely to be ambush predators, presumably using their powerful, stout (deeper and shorter) caudal peduncles for fast start acceleration and swimming to propel them through a relatively stationary water column [67]. A similar shift in caudal peduncle form is seen in the increasingly wetland specialised *Neochanna* [64,68,69]. Habitat form may have further influenced landlocked *G. brevipinnis*. *Galaxias brevipinnis* from deeper lakes with inlet streams, were shorter, more slender, with more pointed snouts and forked tails, characteristic of pelagic swimming lentic fishes [11,12,70], such as *Galaxias maculatus* [71], compared with larger, deeper bodied conspecifics from the shallower, tributary-less, Blue lakes complex. In comparison, more lotic *Galaxias* typically assumed a shorter, slender (shallower and longer), tubular body and caudal peduncle form, more suited to reducing turbulence and maintaining position in the current for drift feeding and life in a cobble substratum [4,11,18,26,29,38,72].

Fins are an integral part of the swimming mechanisms of fishes, and their form and position needs to match the demands of the hydrological environment in which a fish lives [5,6,12,18,73,74]. Thus, it is expected that the fins of lentic and lotic *Galaxias* will differ in size and shape in hydrologically contrasting habitat types. As such, pectoral and pelvic fin shape shifted from a longer than wider 'oar' shape in lentic *Galaxias*, to a much broader, more trapezium like 'paddle' form *sensu* McKenzie [75] in lotic *Galaxias* (Table 3; Figure 4). Enlargement and increased role of lotic pelvic fins is predictable, with the ventral position of the pectorals in *G. brevipinnis*, *G. gollumoides* and *G. vulgaris* creating

more lift and less efficient braking, but better turning and climbing abilities in steep, turbulent rocky streams [10,18,76].

Of the stabilizing median fins, located behind the centre of gravity [26], only the anal fin displayed convergence to habitat type with lentic *Galaxias* having more square (widths and lengths more equal) compared to the longer, more oblong form in lotic *Galaxias*. The absence of dorsal fin convergence to habitat type is paradoxical, suggesting species-specific characteristics and differences in swimming demands. In particular, *G. gollumoides* has very distinctive, rounded and relatively large anal and dorsal fins of similar length and width (see McDowall and Chadderton [65]). However, the position of insertion of these fins did converge to habitat, with the distance between the dorsal and anal fin origins of lentic *Galaxias* increasing, indicating the dorsal was positioned more anterior to the anal, compared to lotic *Galaxias*. This shift in fin insertion position is likely due to an anterior shift in the centre of gravity due to a larger, heavier, head [26,77]. This distance increased the most in *G. brevipinnis* and least in *G. gollumoides*, consistent with McDowall [50], but was always greater in lentic conspecifics. Interestingly, an anterior fin position contrasts to Humphries' [32] finding that in landlocked, lake dwelling *Galaxias truttaceus* Valenciennes, dorsal and anal fins shifted posteriorly, relative to those of riverine, diadromous conspecifics.

The caudal fin of fishes has importance in locomotion, producing forward thrust [11,12,18,67] and varies in Galaxiidae from forked–emarginate–truncate–rounded [26,29]. Despite predictions based on interspecific differences between groupings of *Galaxias*, *Neochanna* and *Galaxiella* occurring across hydrological gradients [26,31,64,78–81], there was little caudal fin convergence to habitat type, except for the width of the base being narrower in lotic *Galaxias*, which also had more slender caudal peduncles. However, significant correlations between caudal fin variables and morphological MDS dimensions were observed. In this analysis, caudal fins of lotic *Galaxias* were longer, wider and more emarginate—forked in shape, whereas more lentic *Galaxias* had shorter, narrower, truncate—rounded, caudal fins. Depth of the caudal fork generally increased in more lotic *Galaxias*. However, some lacustrine, possibly pelagic, *G. brevipinnis* populations also had moderately deeply forked fins as did the lacustrine, pelagic *Paragalaxias dissimilis* (Regan) [37]. Caudal fork depth was greater in lentic compared to lotic *G. gollumoides*, with caudal lobes becoming quite rounded. Interestingly, the development of caudal flanges appeared to be variable between individuals, across all species and habitats, showing no overall trend, contrasting with McDowall and Burridge's [79] assertion that flanges are strong in species, such as in *Galaxiella* and *Neochanna*, occupying wetlands [78,81,82].

In flowing water, a flatter head has the effect of pushing the more streamlined body down and is considered to shift and dampen the effects of pressure and friction drag toward the caudal peduncle region, allowing the fish to maintain station in flowing water [11,12]. In comparison, in lentic habitats, a more robust head, body and caudal peduncle, appearing as a deep lateral silhouette, produces maximum thrust in a quick start, with a larger, heavier, head reducing lateral oscillation and energy expenditure during swimming [77]. Such habitat convergent morphologies were observed in this study, with lentic *Galaxias* having larger heads and deeper longer bodies than lotic *Galaxias* which had shorter, flatter heads (Table 3; Figure 4). This morphology is characteristic of *G. brevipinnis* and flathead morphotypes, including *G. vulgaris* [45–47], but that even lotic *G. gollumoides* displayed this same convergence in form to habitat type indicates that habitat hydrology is probably inducing intraspecific morphological divergence.

Mouth shape can be influenced by diet, but is also an integral component of the head, its form being influenced by the habitat type. In this study, *Galaxias* occurred in lentic and lotic habitats with contrasting invertebrate communities, thus if diet was influencing *Galaxias* mouth shape, as opposed to hydrodynamic constraints in lentic and lotic habitats, it could be expected that lentic *Galaxias* specialising on micro-crustacea and smaller, lentic Trichoptera and Diptera, would have smaller mouths compared to the larger mouths of lotic *Galaxias* consuming larger Ephemeroptera, Plecoptera and Trichoptera. However, lentic *Galaxias* had larger mouths for their length, compared with lotic *Galaxias* as inferred from the ratio of depth/width of gape and also deeper and wider gapes. The larger

mouth within a larger, more robust, wider and deeper head of lentic *Galaxias*, compared to the smaller mouths, with lower jaw underbites, within the flattened, more streamlined heads of lotic *Galaxias* suggests mouth shape appears more influenced by overall head shape, itself influenced by the hydrological environment of habitats.

4.2. Phenotypic Basis for Morphological Shifts

Evidence presented here supports the hypothesis that observed differences in *Galaxias* morphology are likely phenotypic responses to the hydrological environments of contrasting lentic and lotic habitats. This finding is important as previous studies have found discordance between the morphology and genetics amongst members of the *G. vulgaris* species complex, which has hindered formal taxonomic description of lineages [45,46,83].

Crow et al. [36] found morphological and genetic variation between catchments within *G. gollumoides* and *G. "southern"*, similar to the findings of the current study. Yet this is not unexpected for non-migratory *Galaxias* from geographically isolated populations [19,44], which have been further fragmented by anthropogenic activities and introduced salmonids [30,84]. Using fine scale amplified fragment length polymorphism (AFLP) analysis of genomic DNA, Crow et al. [36] also found genetic variation within both species between habitats within streams, contrasting with the AMOVA results based on mtDNA *cyt b* in this study. Moreover, the use of paired habitats in this study, in close proximity and hydrologically connected, allowed for possible gene flow between habitats within sub-catchments. Further, Crow et al. [36] found morphological variation between habitats within streams for *G. gollumoides*, but not *G. "southern"*. This led them to speculate that these differences in *G. gollumoides* morphology may be due to random genetic drift and/or local adaptation. However, our results suggest that differences in the hydrological environment of habitats may induce modification of the phenotype and also maintain these divergent morphologies. Our results support our ecomorphological predictions of functional form in different habitat types, likely due to different hydrodynamics imposing different swimming requirements [67,74,85,86]. Moreover, that these patterns were observed at multiple habitats across the species' ranges, further suggests that divergence in morphology likely arises from phenotypic plastic responses to habitat hydrology [6,23], thus providing the context in which to directly test this experimentally, as examined by Dunn et al [87].

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/5/183/s1>, Table S1. Untransformed minimum–mean–maximum hydrological environment variable values within lentic and lotic habitats for each *Galaxias* species, Table S2. Summary standard length (mm) and untransformed minimum–mean–maximum percentages of standard length standardised morphological variables within lentic and lotic habitats for each *Galaxias* species; and the Big Creek *G. brevipinnis* and Island Hill *G. gollumoides* populations, which were excluded from analyses, Table S3. Results of one-way ANOVA examining differences in standard length (mm) and untransformed standard length standardised morphological variables (mean percentage \pm 1 standard error) within lentic and lotic habitats for each *Galaxias* species. Bold text—significant at $p < 0.05$.

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91-05, approved in 2006 by the University of Otago Animal Ethics Committee operating in accordance with the New Zealand Animal Welfare Act 1999. Work was undertaken in accordance with the 2002 New Zealand National Animal Ethics Advisory Committee, Ministry for Agriculture and Forestry *Good practice guide for the use of animals in research, testing and teaching*, itself based on the 1995 New Zealand Animal Welfare Advisory Committee, Ministry for Agriculture and Forestry *Code of recommendations and minimum standards for the care and use of animals for scientific purposes*. Specimens were collected on Department of Conservation managed public conservation land under High Impact, Research and Collection permits: CA-17921-FAU (Canterbury Conservancy), OT-17597-RES (Otago Conservancy); and SO-17877-RES and NHS-12-06-SL-1 (Southland Conservancy).

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

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