

Fine-scale habitat preferences influence within-river population connectivity: a case-study using two sympatric New Zealand *Galaxias* fish species

JONATHAN M. WATERS* AND CHRISTOPHER P. BURRIDGE†

*Department of Zoology, University of Otago, Dunedin, New Zealand

†School of Biological Sciences, University of Tasmania, Hobart, TAS Australia

SUMMARY

1. Understanding the ecological factors governing population connectivity in freshwater systems represents an ongoing challenge for aquatic biologists.
2. We used genetic analysis to test the hypothesis that fine-scale habitat preferences can influence within-river connectivity patterns in freshwater-limited fishes.
3. Genetic variation among sampling sites within systems was compared for co-distributed fish taxa *Galaxias gollumoides* (low-velocity pool/swamp habitat) versus an undescribed species, *Galaxias* 'southern' (high-velocity riffle habitat), using ϕ statistics. These analyses incorporated 1624 bp of mtDNA from 429 galaxiid specimens, encompassing 95 localities across four river systems in Southland, New Zealand.
4. Although similar numbers of haplotypes per river were detected in both taxa, spatial partitioning of this genetic variation within rivers was significantly stronger for *G. gollumoides* (mean $\phi_{ST} = 0.539$) than for *G.* 'southern' (mean $\phi_{ST} = 0.142$). Within-catchment genetic divergence values were also higher for the former species.
5. These findings suggest that a combination of main-channel habitat continuity and high water velocity facilitates relatively high connectivity in *G.* 'southern', whereas *G. gollumoides* populations are genetically isolated in fragmented low-velocity habitats. We conclude that population connectivity can be strongly influenced by habitat preference, leading to profound differences in the phylogeography, diversity and conservation status of freshwater fish lineages.

Keywords: connectivity, ecology, genetics, habitat preference, phylogeography

Introduction

Biological dispersal ability is thought to be a key factor governing spatial biodiversity patterns (Mayr & Diamond, 2001; Renner, 2004; Lomolino, Riddle & Brown, 2005; Gillespie *et al.*, 2012; Strona *et al.*, 2012). Ecological features that influence dispersal potential are hypothesised to strongly affect population connectivity (Nikula, Spencer & Waters, 2011), particularly in freshwater systems (McDowall, 1990; Ward, Woodward & Skibinski, 1994). While numerous studies have shown that major ecological shifts in dispersal potential (e.g. migratory versus non-migratory life history) can influence freshwa-

ter biogeography (Bănărescu, 1990; Leathwick *et al.*, 2008), diversity and population connectivity (Allibone & Wallis, 1993; DeWoody & Avise, 2000; Burridge *et al.*, 2008; McCulloch, Wallis & Waters, 2009), our understanding of the biodiversity impacts of more subtle ecological differentiation (e.g. fine-scale habitat preference) remains limited.

New Zealand's South Island presents an informative system for assessing the influence of fish ecology on population-genetic connectivity. Previous population-genetic and phylogeographic analyses of freshwater-limited galaxiids have revealed substantial within-river genetic differentiation (Allibone *et al.*, 1996; King & Wal-

Correspondence: Jonathan M. Waters, Allan Wilson Centre, Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand. E-mail: jon.waters@otago.ac.nz

lis, 1998; Waters *et al.*, 2001a; Burridge, Crow & Waters, 2007). Regional (among-river) contrasts in genetic diversity have also been noted (Wallis *et al.*, 2001). However, a detailed consideration of the ecological factors shaping within-catchment genetic differentiation in these freshwater-limited taxa is lacking. Here, we analyse phylogeographic data from co-distributed representatives of South Island's *Galaxias vulgaris* species complex (Waters & Wallis, 2001) (Fig. 1) to test the hypothesis that patterns of within-river population connectivity are linked to contrasting habitat preferences.

Our study focuses on two phylogenetically similar (Waters *et al.*, 2010), broadly sympatric freshwater-limited galaxiid taxa (*Galaxias gollumoides* and *G.* 'southern') in rivers of Southland, New Zealand (Fig. 1; Waters *et al.*, 2001b; Burridge *et al.*, 2007, 2008; Crow *et al.*, 2009, 2010). Despite their sympatry and biological similarity (they were considered conspecific until recent decades;

McDowall, 1990), these two taxa exhibit distinct fine-scale habitat preferences, with both field and experimental observations confirming that *G.* 'southern' prefers faster water velocities than does *G. gollumoides*. Although these taxa often occur in direct sympatry or close proximity (Waters *et al.*, 2001a,b; Crow *et al.*, 2010), *G. gollumoides* typically inhabits low-velocity (e.g. swamps, pools, backwaters) habitats, whereas *G.* 'southern' is a strictly high-velocity, riffle-dwelling species (Fig. 2; Burridge *et al.*, 2008; Crow *et al.*, 2010).

We hypothesise that *G.* 'southern' populations are likely to experience relatively high genetic connectivity via main-channel habitat continuity, whereas *G. gollumoides* populations are likely to be relatively genetically fragmented due to their presence in unconnected low-velocity habitats, rather than major river channels (Fig. 2). We test this hypothesis by conducting comparative genetic analyses of these co-distributed taxa across four replicate Southland river drainages.

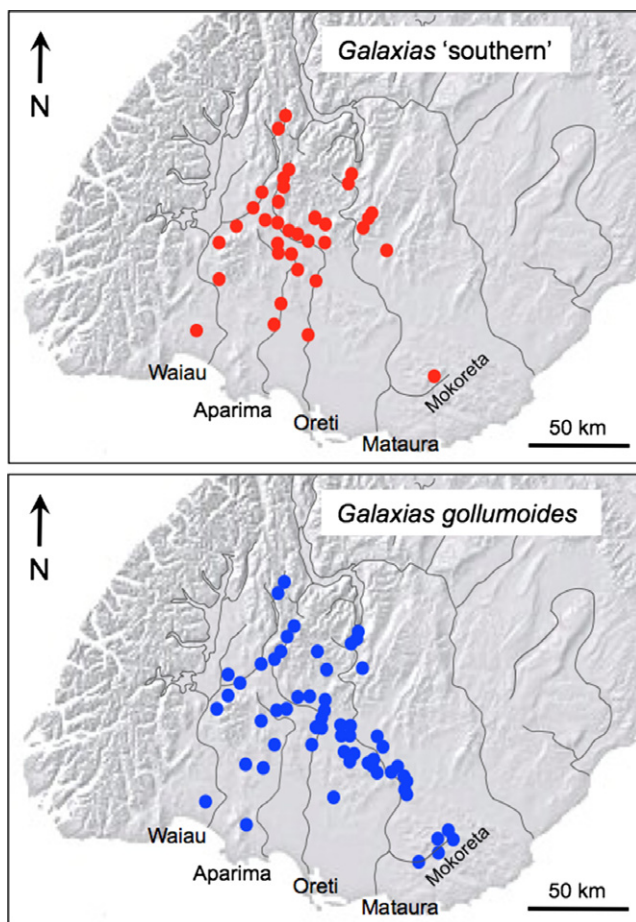


Fig. 1 Sampling localities for the two co-distributed *Galaxias* taxa in four Southland river drainages (Mataura, Oreti, Aparima and Waiau rivers), South Island, New Zealand. Summary details in Table 1.

Methods

Two sympatric native fish species (*Galaxias gollumoides* and *G.* 'southern'; Burridge *et al.*, 2007, 2008; Crow *et al.*, 2010) were sampled from four large rivers (Fig. 1) in Southland, New Zealand. Collections were made with the aid of electric fishing apparatus and/or pole nets, as previously described (Burridge *et al.*, 2007). A total of 429 fish from 95 localities were analysed (Table 1). All of the rivers sampled are similar in length (100–250 km in length; Table 1), and sampling effort was comparable for both taxa. Specifically, around 50 individuals per river

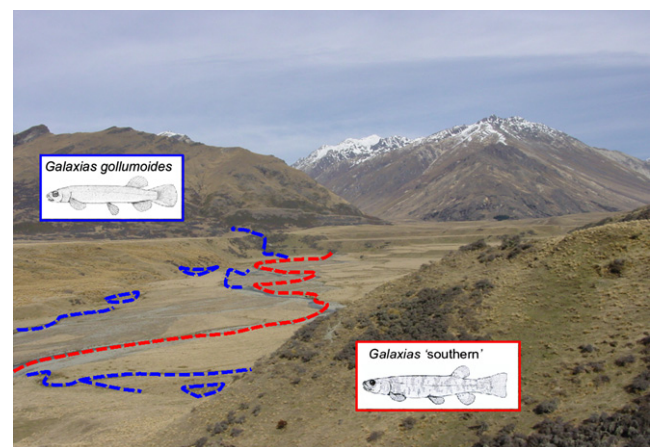


Fig. 2 Photograph of the Von River, Southland, New Zealand, with schematic representation of the contrasting habitat preferences (dashed lines) of *Galaxias gollumoides* (blue: low-velocity, pools, backwaters and swamp habitats) and *Galaxias* 'southern' (red: high-velocity, main-channel riffle habitats).

Table 1 Genetic diversity within Southland populations of freshwater-limited *Galaxias* taxa. Localities are shown in Fig. 1. River length, sample size (*n*), number of haplotypes (hap), mean (avg *d*) and maximum (max *d*) uncorrected genetic divergence values (%) between pairs of sequences, and proportion of genetic variance distributed among sites (ϕ_{ST}) are indicated for each river. Significant differentiation is indicated by bold text, and mean values for each species are given in italics.

Species	River	Length		n	Hap	Avg <i>d</i>	Max <i>d</i>	ϕ_{ST}	<i>P</i>
		(km)	Sites						
<i>gollumoides</i>	Mataura	240	31	103	42	1.2	2.7	0.511	<0.001
	Oreti	203	15	65	25	0.3	1.2	0.198	0.006
	Aparima	102	6	24	10	0.4	1.0	0.712	<0.001
	Waiau	217	8	34	16	0.7	1.5	0.736	<0.001
		<i>191</i>	<i>15</i>	<i>56.5</i>	<i>23.25</i>	<i>0.7</i>	<i>1.6</i>	<i>0.539</i>	
'southern'	Mataura	240	7	45	14	0.2	0.6	0.195	<0.001
	Oreti	203	14	63	29	0.3	1.0	0.109	0.008
	Aparima	102	6	40	11	0.2	0.8	0.009	0.390
	Waiau	217	8	55	22	0.1	0.4	0.256	<0.001
		<i>191</i>	<i>8.8</i>	<i>50.8</i>	<i>19</i>	<i>0.2</i>	<i>0.7</i>	<i>0.142</i>	

were sampled for each species, incorporating approximately 10 localities per river (Table 1, Fig. 1).

Mitochondrial DNA data sets comprised previously published mtDNA sequence data from cytochrome *b* (856 bp) and control region (768 bp) fragments (Burridge *et al.*, 2007), with a maximum of 1624 bp sequenced per individual. All sequences analysed for this study are available from GenBank (details in Burridge *et al.*, 2007). While these data have previously been used for among-river differentiation analyses, they have not previously been used for detailed comparative analyses of within-river differentiation.

We used ϕ_{ST} (Excoffier, Smouse & Quattro, 1992) computed using AMOVA in Arlequin (Excoffier & Lischer, 2010) as a measure of variation attributable to genetic subdivision among population samples. These calculations incorporated genetic distances among haplotypes, estimated using the Kimura (1980) 2-parameter model. Values for *G. gollumoides* and *G. 'southern'* were compared using a two-sample test (unpaired; Sokal & Rohlf, 1981). Uncorrected genetic divergences among sequences within each river were calculated using PAUP 4.0a142 (Swofford, 2015). Numbers of distinct haplotypes in each data set were assessed via UPGMA analysis performed on absolute distances among sequences, using PAUP.

Results

Levels of within-river mtDNA haplotypic diversity were similar for both taxa (e.g. mean 23 haplotypes per river in *Galaxias gollumoides*, versus 19 per river in *G. 'southern'*; Table 1). In contrast, *G. gollumoides* showed larger mean sequence divergences among individuals within rivers (0.7%) than did *G. 'southern'* (0.2%). The taxa also showed contrasting maximum divergence values per river (mean 1.6% in *G. gollumoides*, 0.7% in *G. 'southern'*).

Significant partitioning of genetic variation among sites was detected in seven of eight cases (Table 1). Despite the similar numbers of haplotypes detected in both taxa, the spatial partitioning of this variation within- versus among-sampling localities within rivers showed major contrasts. Specifically, in every river sampled, *G. gollumoides* showed substantially higher among-population differentiation than was evident in *G. 'southern'*. In particular, the mean within-river ϕ_{ST} value for *G. gollumoides* (0.539) was approximately four times larger than the corresponding value for *G. 'southern'* (0.142) (Table 1). An *F*-test failed to reject equality of variances in ϕ_{ST} between species ($F_{3,3} = 5.37$, $P = 0.20$). Means of ϕ_{ST} were significantly different between species ($t_6 = 2.93$, $P = 0.03$). As biogeographic ranges of the taxa are nearly identical, and collections were made over similar (broadly overlapping) spatial scales (Fig. 1), this effect cannot be attributed to differences in the geographic scope of sampling.

Notably, we detected clear regional differentiation among Mataura River samples of *G. gollumoides*, with the Mokoreta subcatchment (Fig. 1) ($n = 28$; five localities) represented by a distinctive phylogeographic clade, with a minimum divergence of 1.1% relative to other Mataura sequences ($n = 75$; 26 sites). No such regional clades were detected within rivers for *G. 'southern'*.

Discussion

This study provides support for the apparent role of habitat preference in shaping genetic diversity within river drainages. Although the vast majority of populations analysed exhibited significant within-river genetic differentiation among sampling sites, *G. gollumoides* showed consistently higher within-river differentiation than was evident in *G. 'southern'*. In particular, the relative proportion of genetic variance explained by

among-population comparisons (versus within-population comparisons) within *G. gollumoides* was approximately four times greater than that detected in *G. 'southern'*. The relatively 'fragmented' genetic structure that typifies *G. gollumoides* could also explain the relatively high mtDNA sequence divergence values detected within this species (Table 1).

As predicted, the high-velocity, main-channel habitat preference of *G. 'southern'* is reflected by relatively low within-river genetic differentiation, whereas populations of the low-velocity species *G. gollumoides* are apparently isolated in discontinuous low-velocity habitats. In addition to increased dispersal potential of *G. 'southern'* mediated by water velocity alone, the increased habitat continuity provided by through-going gravel beds and gravel bars that characterise these systems (Fig. 2) apparently facilitates relatively high connectivity for this species throughout each catchment. These consistent spatial genetic contrasts suggest that subtle ecological differences relating to habitat preference can strongly influence population connectivity patterns. Furthermore, as these comparisons involve closely related taxa (Waters *et al.*, 2010), it is less likely that they have diverged in attributes (other than their habitat preferences) that could also influence population-genetic structuring (Dawson *et al.*, 2002). This study therefore adds to a growing body of comparative phylogeographic literature emphasising the potentially substantial impacts of freshwater ecology and life-history variation on spatial genetic differentiation in fishes (Tibbets & Dowling, 1996; Turner *et al.*, 1996; Whiteley, Spruell & Allendorf, 2004; Hickerson & Cunningham, 2005). Future analyses should incorporate larger numbers of sympatric lineages to further address ecological factors affecting population connectivity within freshwater systems.

The genetic isolation of native galaxiid populations may be partly explained by the competitive and predatory impacts of introduced salmonids (Townsend & Crowl, 1991), as all of the river systems studied here contain abundant salmonid populations. Whereas *G. 'southern'* is apparently able to coexist with salmonids in braided main-channel habitat, *G. gollumoides* has seldom been found coexisting with salmonids. However, the strong phylogeographic differentiation detected within Southland *G. gollumoides* suggests that this structure substantially predates the anthropogenic impacts associated with salmonid introductions 150 years ago (McDowall, 1990). For instance, the substantial genetic differentiation between upper versus lower (Mokoreta; Fig. 1) Maitai *G. gollumoides* (minimum mtDNA sequence divergence 1.1%) is likely to have relatively

ancient geological origins (Burridge *et al.*, 2007). Specifically, under the geologically derived galaxiid mtDNA calibration outlined by Craw *et al.* (2008), this depth of differentiation is estimated to reflect population divergence approximately 100 000 years before present.

In addition to increased levels of genetic diversification, the relatively high genetic fragmentation observed within *G. gollumoides* suggests its populations are likely to be relatively prone to local extinction. This conclusion is echoed by recent conservation status assessments, which list *G. gollumoides* as 'declining', whereas *G. 'southern'* is considered 'not threatened' (Allibone *et al.*, 2010). We suggest that native fish conservation policies in these rivers should take account of this limited connectivity, with isolated *G. gollumoides* populations managed as separate entities. While removal of invasive salmonids would likely facilitate localised population recoveries of *G. gollumoides* in these Southland river systems, intrinsic ecological barriers presented by main channels are likely to continue to constrain within-catchment connectivity in *G. gollumoides*.

Acknowledgments

We thank R. Allibone, L. Chadderton, S. Crow, D. Jack, D. Rowe and G. Wallis for assisting with sampling; D. Craw for discussions of Southland geomorphology; G. Wallis for discussions of genetic analysis; and T. King for assisting with DNA sequencing. This study was funded by the Marsden Fund (Royal Society of NZ), University of Otago and University of Tasmania.

References

- Allibone R., David B., Hitchmough R., Jellyman D., Ling N., Ravenscroft P. *et al.* (2010) Conservation status of New Zealand freshwater fish, 2009. *New Zealand Journal of Marine and Freshwater Research*, **44**, 271–287.
- Allibone R.M., Crowl T.A., Holmes J.M., King T.M., McDowall R.M., Townsend C.R. *et al.* (1996) Isozyme analysis of *Galaxias* species (Teleostei: Galaxiidae) from the Taieri River, South Island, New Zealand: a species complex revealed. *Biological Journal of the Linnean Society*, **57**, 107–127.
- Allibone R.M. & Wallis G.P. (1993) Genetic variation and diadromy in some New Zealand galaxiids (Teleostei: Galaxiidae). *Biological Journal of the Linnean Society*, **50**, 19–33.
- Bănărescu P. (1990) *Zoogeography of Fresh Waters. Vol. 1: General Distribution and Dispersal of Freshwater Animals*. AULA-Verlag, Wiesbaden.

- Burridge C.P., Craw D., Jack D.C., King T.M. & Waters J.M. (2008) Does fish ecology predict dispersal across a river drainage divide? *Evolution*, **62**, 1484–1499.
- Burridge C.P., Craw D. & Waters J.M. (2007) An empirical test of freshwater vicariance via river capture. *Molecular Ecology*, **16**, 1883–1895.
- Craw D., Burridge C., Norris R. & Waters J. (2008) Genetic ages for Quaternary topographic evolution: a new dating tool. *Geology*, **36**, 19–22.
- Crow S.K., Closs G.P., Waters J.M., Booker D.J. & Wallis G.P. (2010) Niche partitioning and the effect of interspecific competition on microhabitat use by two sympatric galaxiid stream fishes. *Freshwater Biology*, **55**, 967–982.
- Crow S.K., Closs G.P., Waters J.M. & Wallis G.P. (2009) Morphological and genetic analysis of *Galaxias* 'southern' and *G. gollumoides*: interspecific differentiation and intraspecific structuring. *Journal of the Royal Society of New Zealand*, **39**, 43–62.
- Dawson M.N., Louie K.D., Barlow M., Jacobs D.K. & Swift C.C. (2002) Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology*, **11**, 1065–1075.
- DeWoody J.A. & Avise J.C. (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, **56**, 461–473.
- Excoffier L. & Lischer H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.
- Excoffier L., Smouse P.E. & Quattro J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Gillespie R.G., Baldwin B.G., Waters J.M., Fraser C., Nikula R. & Roderick G.K. (2012) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology and Evolution*, **27**, 47–56.
- Hickerson M.J. & Cunningham C.W. (2005) Contrasting Quaternary histories in an ecologically divergent sister pair of low-dispersing intertidal fish (*Xiphister*) revealed by multilocus DNA analysis. *Evolution*, **59**, 344–360.
- Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.
- King T.M. & Wallis G.P. (1998) Fine-scale genetic structuring in endemic galaxiid fish populations of the Taieri River. *New Zealand Journal of Zoology*, **25**, 17–22.
- Leathwick J.R., Elith J., Chadderton W.L., Rowe D. & Hastie T. (2008) Dispersal, disturbance and the contrasting biogeographies of New Zealand's diadromous and non-diadromous fish species. *Journal of Biogeography*, **35**, 1481–1497.
- Lomolino M.V., Riddle B.R. & Brown J.H. (2005) *Biogeography*, 3rd edn. Sinauer Associates, Sunderland, MA.
- Mayr E. & Diamond J.M. (2001) *The Birds of Northern Melanesia. Speciation, Ecology and Biogeography*. Oxford University Press, Oxford.
- McCulloch G.A., Wallis G.P. & Waters J.M. (2009) Do insects lose flight before they lose their wings? Population genetic structure in subalpine stoneflies. *Molecular Ecology*, **18**, 4073–4087.
- McDowall R.M. (1990) *New Zealand Freshwater Fishes: A Natural History and Guide*. Heinemann Reed, Auckland.
- Nikula R., Spencer H.G. & Waters J.M. (2011) Evolutionary consequences of microhabitat: population-genetic structuring in kelp- vs. rock-associated chitons. *Molecular Ecology*, **20**, 4915–4924.
- Renner S. (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences*, **165**, S23–S33.
- Sokal R.R. & Rohlf F.J. (1981) *Biometry*, 2nd edn. Freeman, San Francisco, CA, 859pp.
- Strona G., Galli P., Montano S., Seveso D. & Fattorini S. (2012) Global-scale relationships between colonization ability and range size in marine and freshwater fish. *PLoS ONE*, **7**, e49465.
- Swofford D.L. (2015) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0a142*. Sinauer Associates, Sunderland, MA.
- Tibbets C.A. & Dowling T.E. (1996) Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). *Evolution*, **50**, 1280–1292.
- Townsend C.R. & Crowl T.A. (1991) Fragmented population structure in a native New Zealand fish: an effect of introduced brown trout? *Oikos*, **61**, 347–354.
- Turner T.F., Trexler J.C., Kuhn D.N. & Robison H.W. (1996) Life-history variation and comparative phylogeography of darters (Pisces: Percidae) from the North American central highlands. *Evolution*, **50**, 2023–2036.
- Wallis G.P., Judge K.F., Bland J., Waters J.M. & Berra T.M. (2001) Genetic diversity in New Zealand *Galaxias vulgaris sensu lato* (Teleostei: Osmeriformes: Galaxiidae): a test of a biogeographic hypothesis. *Journal of Biogeography*, **28**, 59–67.
- Ward R.D., Woodward M. & Skibinski D.O.F. (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, **44**, 213–232.
- Waters J.M., Craw D., Youngson J.H. & Wallis G.P. (2001a) Genes meet geology: fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. *Evolution*, **55**, 1844–1851.
- Waters J.M., Esa Y.B. & Wallis G.P. (2001b) Genetic and morphological evidence for reproductive isolation between sympatric populations of *Galaxias* (Teleostei: Galaxiidae) in South Island, New Zealand. *Biological Journal of the Linnean Society*, **73**, 287–298.

- Waters J.M., Rowe D.L., Burridge C.P. & Wallis G.P. (2010) Gene trees versus species trees: reassessing life-history evolution in a freshwater fish radiation. *Systematic Biology*, **59**, 504–517.
- Waters J.M. & Wallis G.P. (2001) Mitochondrial DNA phylogenetics of the *Galaxias vulgaris* complex from South Island, New Zealand: rapid radiation of a species flock. *Journal of Fish Biology*, **58**, 1166–1180.
- Whiteley A.R., Spruell P. & Allendorf F.W. (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. *Molecular Ecology*, **13**, 3675–3688.

(Manuscript accepted 20 August 2015)