Otolith microchemistry identifies diadromous populations of Patagonian river fishes

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Abstract

The geographical characteristics of Chile may provide excellent habitat for the establishment of diadromous fish species. The objective of this study was to document migratory life histories of native galaxiids and introduced salmonids from a wide latitudinal range in Chilean Patagonia (39-48°S). Otolith microchemistry data were analysed using a recursive partitioning approach, with the main goal of testing the hypothesis of diadromy for several populations of galaxiids and salmonids. Based on annular analysis of Sr:Ca ratios, a diadromous life history was detected for populations of native *Aplochiton taeniatus*, *A. marinus*, and *Galaxias maculatus*. Residency in freshwater was suggested for populations of *A. zebra* and confirmed for *G. platei*. Among introduced salmonids, populations of *Oncorhynchus tshawytscha* and *O. kisutch* exhibited anadromous migratory patterns consistent with their life history in the native range, whereas the populations of *O. mykiss* examined appeared restricted to freshwater areas. *Salmo trutta* exhibited variable habitat use consistent with the establishment of more than one type of life cycle. Multivariate classification and regression trees (CART) were used to assess changes in ratios of all elements assayed (Sr, Mg, Mn, Ba, Ca) across chronological sequences of otoliths. Changes in Sr had highest relative influence in species that dwell prevalently in saltwater and brackish habitats, whereas changes in Mg and Mn appeared to discriminate fishes that moved exclusively between freshwater environments. Some species (e.g., *O. tshawytscha*) showed equally strong variation in all elements assayed suggesting that time spent between fresh headwaters and the sea jointly influenced otolith microchemistry. As accelerated growth in hydropower capacity further disrupts migratory routes of native and introduced fishes alike, special concern in conservation assessments should be given to diadromous species and populations with dire conservation status or economic importance.

Introduction

Only 47 native and 27 non-native inland fish species are currently recognized in Chile, and about 30% of these exhibit some tolerance for shifting between saline and freshwater habitats (Dyer 2000; Habit and Victoriano 2005; Habit et al. 2006; Vila et al. 2011; Ministerio del Medio Ambiente 2013; Vargas et al. 2015). Furthermore, roughly 15% of these fishes are hypothesized to display diadromous behaviour (Table S1), compared to less than 1% for fishes worldwide (Nelson 2006).

The term diadromy describes regular, predictable, and physiologically mediated movements between freshwater and the sea. Diadromy necessitates profound physiological changes (i.e., osmoregulation) involved in shifting from marine to freshwater and vice versa (Gross et al. 1988), and it is obligatory for many populations within a species, but sometimes facultative. The direction of migration depends on life history stages and habitats where reproductive and feeding events occur. The combination of these factors defines three different types of diadromy: anadromy, catadromy, and amphidromy (Myers 1949; Gross 1987; Gross et al. 1988; McDowall 1992; McDowall 1997; Limburg et al. 2001) (in particular refer to McDowall 1997 for a review of the terminology and a visual aid).

Given the high percentage of fishes in Chile hypothesized to exhibit some form of diadromy, migration might play an important, but not yet recognized role in establishing national priorities of aquatic biodiversity conservation. At present, a high percentage of the continental ichthyofauna in Chile is categorized with some degree of conservation threat by Chilean environmental agencies and other authors, although conservation categories are often incongruent and threats underestimated (Habit and Victoriano 2005; Diario Oficial de la Republica de Chile 2008; Ministerio del Medio Ambiente 2013; IUCN 2015; Vargas et al. 2015).

The geographical characteristics of Chile could facilitate establishment of diadromous species. Andean rivers that flow into the Pacific Ocean include a variety of different habitats in a limited longitudinal distance (average 145 km), spanning from areas of rocky substrates, high gradient, clear waters and low temperatures, to areas of low flow, sandy substrates, and aquatic vegetation (Habit and

Victoriano 2005; Instituto Nacional de Estadisticas 2015). This high spatial heterogeneity could be essential for maintenance and completion of diadromous life cycles, and for maintaining evolutionary potential (i.e., genetic diversity) for life history variation (Dingle 1996; Pulido 2007). Therefore, fragmentation events imposed by human-made barriers may affect fish fitness and restrict movement between habitats more so than in other areas (Waples et al. 2007).

Patagonian fishes offer a unique opportunity to understand migration patterns in relatively pristine habitats and establish a baseline against which future potential impacts associated with river impoundments can be compared. Despite strong economic growth and efforts to develop hydroelectric potential to meet the country's high-energy requirements (Joo et al. 2015), many rivers in southern Chile are still free-flowing, offering opportunities to study pre-impoundment patterns of diadromous migration. For example, galaxiid fishes are distributed across the Southern Hemisphere and diadromy seems to be a recurrent trait among many of them (McDowall 1971; McDowall 1988). Likewise, salmonids are among some of the best-studied diadromous fishes in the Northern Hemisphere and are now well-established in southern Chile (McDowall 2002; Correa and Gross 2008).

Using micro-geochemical data obtained from otoliths, this study sought to determine whether native galaxiids and introduced salmonids exhibit diadromy in Chilean rivers. Otoliths are calcified deposits in the inner ear of fishes that accumulate in ring-like fashion over ontogenetic growth. Elemental analysis of otoliths can help to distinguish origins of marine and freshwater fishes among locations with variable water chemistry. Differing chemical composition of the otolith from the primordium (core) to the edge indicates different environments in which a fish has lived and allows hypothesis tests related to patterns of fish movement. When analysed sequentially across an otolith sagittal section, changes in elemental ratios can indicate fine-scale patterns of movement, connectivity, dispersal, and the location of natal habitats (Halden et al. 2000; Howland et al. 2001; Kraus 2004; Ashford et al. 2005; Campana 2005; Arkhipkin et al. 2009). To quantify these changes, we applied univariate and multivariate recursive partitioning approaches based on Classification and Regression

Trees (CART) to detect discontinuities in ratios that indicate habitat shifts (Vignon 2015).

Methods

Fish Collections

Between 2004 and 2011, adult specimens of *Aplochiton zebra, A. taeniatus, A. marinus, Galaxias maculatus, G. platei, Oncorynchus tshawytscha, O. kisutch, O. mykiss,* and *Salmo trutta* were collected using various methods from 6 locations across a large latitudinal range (39.5–48.0° S) in western Patagonia, Chile (Fig. 1, Table 1). At each location, fish specimens were euthanized by an overdose of anaesthetic solution (tricaine-methanesulfonate MS-222 or clove oil). Genetic data were used to identify individuals in genus Aplochiton to the species level (Alò et al., 2013).

Otolith preparation

Prior to specimen preservation, sagittal otoliths were extracted and either stored dry in test tubes or in 95% ethanol, as elemental compositions and structures of otoliths are not strongly affected by ethanol for the elements assayed (Proctor and Thresher 1998).

In the laboratory, otoliths were polished, cleaned, and mounted individually on clean glass slides using a thermoplastic cement (Crystalbond™). In order to expose growth rings, 3M™ (fine) and Nanolap ® Technologies (coarse) diamond lapping film wetted with deionized water was used to polish otoliths by hand until a satisfactory sagittal section of annuli was visible. For *Aplochiton spp.*, *Galaxias spp.*, *S. trutta*, *O. kisutch* and *O. mykiss* otoliths, a 30-µm and then 3-µm lapping film was used to expose annuli and get a finished polish. *O. tshawytscha* otoliths required larger lapping film (45 and 60 µm) to reach an appropriate view, but were finished with 3 µm film for increased clarity. After the initial polish, and where necessary (largely for *O. tshawytscha*), the adhesive was melted and the otolith flipped for double polishing to produce a thinner section.

Following polishing, the mounting adhesive was dissolved in 100% acetone bath and sonicated for 10 minutes. Larger otoliths were cleaned a second time with acetone as needed. Each otolith was

then sonicated twice in Milli-Q water for 5 to 10 minutes each. Following cleaning, otoliths were rinsed a final time in Milli-Q water, transferred to clean vials and placed in a positive laminar flow hood for 24-48 hours to dry.

Acid-washed porcelain forceps were used to mount clean, dry otoliths on acid-washed microscope slides. Otoliths were grouped according to diameter and mounted 10-28 per slide accordingly. Each otolith was placed within one small drop of fresh Crystalbond melted onto a single slide.

Slides were securely kept in acid-washed, sealed petri dishes for transport to Woods Hole Oceanographic Institute (Woods Hole, MA, U.S.A.). There, laser ablation was conducted from October 8th to 11th, 2012 (*Aplochiton spp., S. trutta, Galaxias spp., O. kisutch,* and *O. mykiss*) and again from February 4th to 5th, 2013 (*O. tshawytscha*). Laser ablation was performed with a large format laser ablation cell on a New Wave UP193 (Electro Scientific Industries, Portland, Oregon) short pulse width excimer laser ablation system. This was coupled with a Thermo Finnigan Element2 sector field argon plasma spectrometer (Thermo Electron Corporation, Bremen, Germany) for elemental analysis. The laser was configured for single pass, straight line scanning at a speed of 5 µm per second. The laser beam spot size was 50 µm at 75% intensity and 10 Hz pulse rate.

Certified standards FEBS-1 and NIES-022 were run before and after each block of 10 to 28 otoliths to account for quality assurance in the measurements (Yoshinaga et al. 2000; Sturgeon et al. 2005). Each otolith was visualized on screen and the intended ablation transect of each sample was plotted digitally and analysed by ablation with a laser beam (refer to Fig. 2 for a visual example). For accuracy of readings, each data point was produced from an average of ten consecutive reads. The ideal double life-history transect run across each sagittal otolith and through the primordium, thus providing two similar (redundant) patterns related to life history variation, one on either side of the primordium. Interpretations were based on the analyses of both sides of each double transect, if possible. However, in a number of cases, transects were imperfect due to damaged otoliths or inaccurate ablation pathways

(Table S2). In those cases, data was analysed as partial transects which can still be used to differentiate diadromous or resident signals (Fig. 2).

Concentrations were determined for Magnesium (Mg), Manganese (Mn), Strontium (Sr), Barium (Ba) and Calcium (Ca). All elemental data is provided in a data dryad file (http://datadryad.org/).

Data Analysis

Classification and Regression Trees (CART, Breiman et al. 1984)) were used in univariate (Sr:Ca ratio as the independent variable) and multivariate analyses (all element ratios) to detect shifts in elemental ratios across the otolith transect. CART was implemented in the Tampo library (version 1.0) for R Statistical Software 3.0.2 (Vignon 2015). CART is an alternative to qualitative methods traditionally used to interpret the chronological signal in otolith microchemistry transects (Vignon 2015). The chronological variation of the otolith transect (predictor variable) is recursively partitioned using regression trees, which differentiate observations according to values of the elemental ratios (response variables). The method proceeds by grouping or splitting elemental concentrations according to their degree of similarity around the mean value of the signal (Breiman et al. 1984; Therneau and Atkinson 1997; De'ath 2002; Strobl 2009).

Univariate Sr:Ca analysis and migratory patterns

First, outliers likely to be caused by recording errors were removed (additive outliers, R Package "tsoutliers" v: 0.6–5, L'opez-de-Lacalle 2016) and summary statistics of Sr:Ca ratios including mean, confidence intervals, percentiles were calculated across all individuals. Second, CART was run on Sr:Ca ratios, as strontium has been identified as a useful trace element to reconstruct environmental history of fishes (Pracheil et al. 2014), especially to discern habitat shifts across salinity gradients and nursery origins of adult fishes (Secor and Rooker 2000; Campana 2005). Since the main goal of this work was to represent movement patterns at a broad scale, CART was used in a semi-supervised manner to identify the presence or absence of sudden discontinuities in the Sr:Ca otolith signal (Vignon

2015). This was done by introducing three progressively relaxed conditions to the splitting procedure, requesting a minimum difference in mean values in order to allow a split in elemental signals. The minimal differences required to allow regression tree pruning were set to condition =1.0, cond.=0.7 and cond. =0.5. The detection of one or more discontinuities or splits in the Sr:Ca signal was interpreted as evidence for diadromy, otherwise, evidence for residency. When diadromy was detected, the direction of ontogenetic movements was inferred from differences in segment means; increasing values indicated movements towards the sea, and vice versa. Further inference about habitat occupancy (freshwaters, estuaries, or the sea) required a visual, heuristic examination of Sr:Ca profiles in relation to published reference values. Finally, all evidence was assembled to make individual inferences about specific migration patterns (amphidromous, catadromous, or anadromous). In this process, otolith transect quality affected our confidence on interpretations; from maximum confidence on inferences from transects that conformed to the model in Figure 2, to uncertain interpretations from incomplete or faulty transects.

Reference values for the three major habitat types (freshwater, estuarine, and marine) were obtained from a meta-analysis of Sr:Ca profiles from otoliths of 41 fish species in three salinity regimes, and were summarised as follows (extracted from Figure 7 in Secor and Rooker 2000):

Freshwater (salinity 0-5 ppt): 10 species screened, mean Sr:Ca (10-90th percentile range) 0.9 (0.3-1.8) mmol mol^{-1}

Estuarine (5-25 ppt): 11 spp., 2.0 (0.9-3.1) mmol mol^{-1}

Marine (>25 ppt): 20 spp., 3.4 (1.9-5.2) mmol mol^{-1}

Multivariate analysis: Relative importance of elements in structuring signals

Other elements can also be useful to trace fish transitions between habitats that differ in water chemistry (Pracheil et al. 2014). Using all elements in a multivariate context may allow the establishment of a fingerprinting protocol useful to reconstruct habitat use and migration trajectories. Therefore, the relative contributions of Sr, Mg, Mn, and Ba were examined in order to detect variation

in elemental influence at the individual level and to discard uninformative elements. In this analysis off-centred (O) and partial transects (P) were omitted to avoid asymmetries in assigning element importance (Table S2). The first step in this analysis was to use boosting as a learning algorithm that classified binary responses, penalized poor predictor variables, and provided the best model estimate for each response variable. This method allowed determination of contributions of each variable and combined outputs into a single prediction. Each element was evaluated by counting how many times it was selected for tree splitting by fitting a generalized boosted model to the data (function 'gbm', R Package "gbm" v: 2.1.3, 1000 iterations, bagging fraction = 0.7) (De'ath 2007; Vignon 2015; Ridgeway 2017). The relative contribution of each element was scaled so that the sum of all the elements in an individual was 100. A stronger influence of an element was indicated by a higher value and a variable was considered important if its contribution exceeded 5% of the total variance. The influence of an element increases when its changes are correlated and synchronous with other elements, defining large and unique zones in the time series.

Non-parametric comparison of the relative contribution of all elements was used to test whether there were significant differences among groups of species, locations, and migratory strategy (R Package "npmv" v: 2.4.0, 10,000 permutations) (Ellis et al. 2017).

As a final step, using the matrix of elements' relative importance obtained by boosting, the inter-individual and inter-specific variation of the multivariate profiles was illustrated in a two-dimensional plot by Nonmetric Multidimensional Scaling (NMDS) based on the Euclidean dissimilarity matrix (stress = 0.13) using the function 'metaMDS' from the package vegan (R Package "vegan" v: 2.4–3) (Oksanen et al. 2017). Graphical representation of these distances allowed for intuitive comparison of each individual relative to others in relation to the importance of elements structuring the profiles (Manly 1994).

Results

Variation in Sr:Ca ratios generally corresponded to reference ranges associated with freshwater (FW), brackish (B) and saltwater (SW) (Fig. 3). CART analysis identified patterns of change in Sr:Ca elemental ratios consistent with different migratory life histories across species (Fig. 4).

Details on the splitting results under different stringencies are given in Fig. 5. Table S2 reports details of the mean and standard deviation at each split.

Contributions of Sr, Ba, Mg and Mn in the chronological sequences of otoliths revealed that the importance of elements for discriminating changes is significantly dependent on the species and on the migratory strategy adopted (Table 2 and Fig. 6).

The null hypothesis of no difference between locations could not be rejected, as in this case all elements contributed to a similar extent to the chronological sequence of otolith chemical composition (Table 2).

A posterior non parametric tests quantified the relative effects for each of the four elements (Mg, Mn, Sr, Ba as response variables) and the two factors (Species and Inferred Migratory Strategy). The relative effect quantifies the tendencies observed in the data as probabilities (Ellis et al. 2017). For example, the probability that one amphidromous individual would exhibit higher importance in Mn compared to the rest of all individuals equals 83% (Table 3). Also, the probability that juvenile *O. kisutch* would have higher Mg relative importance compared to the rest of the populations is 88% (Table 3).

All four elements seemed to be equally important in anadromous *O. tshawytscha*, juveniles of several species and individuals exclusively in freshwater (e.g. *G. platei*) (Table 3 and Fig. 6).

Mg was more important in individuals that use freshwater throughout their lives but display higher mobility within the freshwater habitat (e.g. *S. trutta*). Sr had higher relative influence in individuals that predominantly used saltwater environments (e.g. *A. marinus*). More random fluctuations were observed in individuals of *G. maculatus* and *A. taeniatus*, indicative of more variable habitat use over their life histories. For example, amphidromous *A. taeniatus* and *G. maculatus* showed

a higher than average relative importance for Mn at Santo Domingo and Tocoihue (Fig. 6).

Discussion

This study quantitatively identified significant transitions across otolith profiles using univariate and multivariate regression trees approaches. Sr:Ca ratios were most influential for populations that spent significant time at sea whereas other elements were important for distinguishing more subtle movement across freshwater and brackish habitats. Native galaxiids showed considerable variation in habitat shifts compared across species, with some species exhibiting population and individual differences and a high degree of plasticity. Nonnative salmonids have established populations with a broad array of migratory life histories, reflective of those found in their native ranges. Some results may be influenced by maternal effects or induced by local temporal variation in water chemistry.

Of five native galaxiids examined, evidence was found for one catadromous (*A. marinus*) and two facultatively amphidromous or catadromous species (*G. maculatus* and *A. taeniatus*). Likewise, among exotic salmonids, patterns consistent with anadromy were present in three (*O. tshawytscha, O. kisutch, S. trutta*) of four species included in this study. Overall, several species appear to regularly use habitats with different salinity levels. This suggests that these species either need a heterogeneous environment (spatial or temporal variability) to complete their life-cycles, have physiological adaptations or plasticity that allows for osmoregulation in a wide range of saline and freshwater environments (euryhaline life history), or both.

Essentially, otolith profiles that showed variation under the most restrictive analytical conditions were those most likely to exhibit large-scale habitat shifts between different environments and salinity levels (*A. taeniatus, A. marinus, G. maculatus, O. tshawytscha*). Otolith profiles that varied under less stringent conditions provided information about more subtle shifts within habitat types.

Native galaxiids. Large elemental shifts in otolith profiles indicated a catadromous life-history for most *A. taeniatus* analysed (Table 1 and Fig. 5). Both univariate and multivariate data suggest that *A.*

taeniatus shows broader habitat plasticity and more heterogeneity compared to all other galaxiid species examined, as it clearly crosses hypothetical threshold boundaries between freshwater, brackish and saltwater (Fig. 3 and 5). Even when confined to strictly freshwater habitats, as in Lake Caro, *A. taeniatus* juveniles show patterns of habitat mobility (Fig. 5, cond.= 0.5), as contrasted with *A. zebra* or *O. mykiss* in the same environment.

Findings based on otolith microchemistry are consistent with previous genetic and morphological data (Alò et al. 2013) showing that *A. taeniatus* exhibits considerable phenotypic variability within populations. Therefore, even in presumably landlocked populations, morphological variability could be related to differential propensity for movement within freshwater (Northcote 1997).

A. marinus (Eigenmann 1927), which for a long time was synonymous with *A. taeniatus*, (Alò et al. 2013), was originally described as a diadromous fish. *A. marinus* is the largest (maximum standard length 361 mm; McDowall and Nakaya 1988) amongst freshwater and diadromous endemic species in Chile. Collection records show a restricted geographic distribution, although its apparent rarity is attributable, in part, to inadequate sampling (Alò et al. 2013; C. Correa unpublished data). Otolith profiles (Fig. 4 and 5) suggested that *A. marinus* copes with high levels of salinity variation in the Baker River system. Otolith primordia of all specimens of *A. marinus* showed evidence of higher Sr:Ca ratios at early stages of growth before the fish entered the estuary (site of capture). Taken together, these data suggest that *A. marinus* is catadromous and a habitat generalist.

Otolith profiles indicated that *A. zebra* uses a chemically uniform habitat at both collection localities (Fig. 4), although results should be corroborated by future studies because *A. zebra* individuals assayed were juveniles. Nevertheless, specimens from Tocoihue River appear to have been exposed to higher salinity levels than those from Lake Caro (Table S2), suggesting preference for residency, but capacity for osmoregulation at higher salinity levels. According to historical records, this species is more abundant and widely distributed than *A. marinus* and *A. taeniatus*. However, DNA barcoding studies coupled with morphological analysis revealed that some individuals previously

thought to be *A. zebra* were actually *A. taeniatus* (Vanhaecke et al. 2012; Alò et al. 2013). Taxonomic confusion between *A. zebra*, *A. taeniatus* and *A. marinus* has impeded full understanding of life-history variation in this species complex. For example, earlier researchers classified them all as diadromous simply because taxonomic and life-history distinctions had not been discovered or appreciated.

G. maculatus individuals were sampled from the same site as some specimens of *A. zebra* (Tocoihue). Among the four specimens analysed, three showed exposure to higher salinity than any other galaxiid species analysed (Fig. 3) with evidence of both catadromous and amphidromous transitions from brackish to saltwater (Table 1). The *G. maculatus* specimen in Fig. 4 was caught in the lower reach of Tocoihue River, an area with exceptionally high tides. It shows an amphidromous life-cycle with intermediate salinity influence in the primordium followed by a period of residency in higher salinity and subsequent migration back into brackish and freshwater. This observation mirrors McDowall's (1968) description of their migratory history where larvae hatch in estuaries, are washed to sea to grow and develop, and return to continental waters as juveniles. A fourth specimen of *G. maculatus* showed no major Sr:Ca fluctuations across the otolith transect (Table S2), suggesting that this individual did not drift out to the ocean during its larval stage and it was likely a resident of the estuarine area in Tocoihue. These data support a hypothesis of facultative diadromy for *G. maculatus* which coincides with an increasing number of studies reporting flexibility in diadromous patterns for numerous fishes (Hicks et al. 2010; Górski et al. 2015).

G. maculatus is widespread in the Southern Hemisphere with low levels of genetic differentiation across geographically disjunct populations (e.g. Chilean versus Falkland Islands mtDNA haplotypes; Waters and Burridge 1999), suggesting high levels of gene flow. Facultative amphidromy and its associated potential for ocean dispersal help explain this observation (McDowall 1968; Berra et al. 1996). Landlocked populations of *G. maculatus* in Chile exhibit higher levels of genetic structure when compared to ocean-migrating populations of the same species, therefore, non-migrating populations are exposed to higher degrees of genetic isolation, possibly leading to a depauperate local

gene pool (Waters and Burridge 1999; Zattara and Premoli 2005; González-Wevar et al. 2015).

Finally, *G. platei* is one of the most widespread, endemic freshwater fish in Patagonia, with adaptations to glacial and postglacial lakes and rivers (Ruzzante et al. 2008; Zemlak et al. 2008). Results of this study support freshwater residency of *G. platei* from the low-elevation coastal Palos Lake as revealed by uniformly low Sr:Ca ratios across the entire otolith transect (Fig. 3 and 4).

Introduced Salmonids

Introduction efforts of salmonids in Chile began over 100 years ago, with a second wave of exotic salmonid imports in the 1970s for ocean ranching and net pen aquaculture (Pascual et al. 2007; Correa and Gross 2008; Arismendi et al. 2014). As a result, several self-sustaining species of salmonids are now established in the country (Correa and Gross 2008; De Leaniz et al. 2010; Górski et al. 2016). Successful establishment of anadromous exotic salmonids in Chile suggests that biotic and abiotic conditions required for diadromy to be maintained are present in Chilean waters.

This study supports established anadromy in *O. tshawytscha*, as previously shown by other authors (Ciancio et al. 2005; Correa and Gross 2008; Arismendi and Soto 2012; Araya et al. 2014). Data are consistent with regularly timed changes in Sr:Ca concentration levels that suggest hatching in freshwater, migration to areas with higher salinity concentrations and return to inland, freshwater areas to spawn (Fig. 4). *O. tshawytscha* specimens collected from the Simpson River do not show as much variation as other *O. tshawytscha* from this study as these fish were all juveniles that had not yet migrated.

Migratory behaviour in *O. tshawytscha* showed less variation in element importance among individuals compared to other species. As adult run timing, time of spawning and juvenile migration can shape the importance of the multi-elemental signatures in the otolith matrix, it is possible that these fish are moving across freshwater and saltwater chemical gradients at intervals that differ from the other species analyzed.

The two parr *O. kisutch* analysed revealed one large scale habitat shift between birth and time of

capture. Most of their otolith profile was characterized by a relatively high Sr:Ca signature around the core which diminished substantially towards the edges (Fig. 4). These specimens where caught during the summer, c. 55 km upstream of the Baker River's mainstream. It was, therefore, assumed that the observed patterns were induced by maternal effects (Kalish 1990; Volk et al. 2000; Zimmerman and Reeves 2002) and these specimens were interpreted as anadromous.

O. mykiss exhibited a pattern consistent with freshwater residency and minor salinity fluctuations within their habitat (Fig. 3 and 5), but this study was limited to two individuals collected from Caro Lake, with restricted connectivity to the sea. Future studies should include other populations of *O. mykiss* in Patagonia, with the goal of better understanding the potential interference of this species with migratory and/or resident populations of native fishes within different habitats. The species has become the most common freshwater fish in Patagonia, and in Chile it has been identified in at least 25 Araucanian lakes (Pascual et al. 2001; Arismendi et al. 2011). Although most established populations of *O. mykiss* in Patagonia are reported as residents, at least one population in Argentinean Patagonia is known to have both resident and migratory fish (Pascual et al. 2001). Arismendi et al. (2011) described that the species is adaptable to different types of freshwater environments with key differences between lake and stream dwellers.

Evidence of at least two different life-cycle patterns emerged for *S. trutta* specimens caught at three different locations. The Sr:Ca transect of juveniles from Lake Caro and adults from Lake Palos showed a pattern consistent with continuous residency in freshwater (Fig. 3 and 5) whereas *S. trutta* from Baker River showed higher values at the primordium (Fig. 4). These results provide some evidence in support of various life history strategies of *S. trutta* in southern Chile. In its native range, *S. trutta* displays an extremely high ecological plasticity. Several large habitat shifts are possible during its life-cycle (Mortensen 1977) and after its first year *S. trutta* can undertake different life-cycle

strategies[1] as: 1) fluvial-adfluvial 2) lacustrine-adfluvial; 3) anadromous migrations; and 4) landlocked populations (Northcote 1997; Klemetsen et al. 2003). Sr:Ca ratios suggest that Lake Caro and Lake Palos individuals are consistent with sole residency in the lake (strategy 4). Multi-elemental analysis showed a higher relative importance of Mg for two individuals from Lake Palos and a higher contribution for Mn in one juvenile from Lake Caro (Fig. 6). As some experimental studies have shown, a positive relationship of Mg with temperature and a correlation of Mn with fish living in hypoxic conditions (Barnes and Gillanders 2013; Limburg et al. 2015), it is possible that these results represent the chemical footprints of movements across different FW environments (strategies 1 and 2). On the other hand, the only specimen of *S.trutta* analysed from the Baker Estuary showed evidence of anadromy (strategy 3), as already documented in Atlantic Patagonian basins (Ciancio et al. 2005; Ciancio et al. 2010).

Otolith microchemistry for introduced salmonids reinforces that some species have established movement strategies similar to those in their native ranges and additionally shows that the extent of their environmental and spatial preference may differ from native fishes.

Further studies are required to determine if higher Sr:Ca ratios in primordia observed for *O. kisutch*, *O. tshawytscha* and *S. trutta* should be attributable to maternal effects or other causes. For example, although the mechanisms are not completely understood, early ontogeny could increase the rate of Sr absorption into the calcium carbonate matrix of the otolith (de Pontual et al. 2003). Also, as the Baker corridor is influenced by a large ice field (Campo de Hielo Norte), high amounts of glacier flour (suspended solids) can contribute to increased salinity levels in water that flows into the estuary (Vargas et al. 2011; Marín et al. 2013). These seasonal salinity changes may promote the uptake of Sr

[1] 1) fluvial-adfluvial migrations occur when juveniles migrate from a natal river to a larger river to feed and grow, and then move back into tributaries to reproduce; 2) lacustrine-adfluvial migrations occur when juveniles migrate from natal river inlets or outlets into a lake; 3) anadromous migrations, when juveniles migrate to the estuary or coastal areas for feeding and return to freshwater for spawning or wintering; and 4) naturally or artificially landlocked populations where fish complete their entire life-cycle in a single habitat (Northcote 1997; Klemetsen et al. 2003)

into the otolith matrix and confound the assumption of low Sr in freshwater environments (Zimmerman 2005).

Conservation Issues

This study should help refine conservation priorities for freshwater fishes in southern Chile. Given extremely high endemism and the likelihood of dependence on diadromous behaviour, potential threats to fishes from fragmentation of river to estuary networks are correspondingly high.

Hydroelectric power development causes loss of hydrological connectivity and alteration of river flows, disproportionately affecting fishes with migratory life histories. Otolith microchemical results underscore the importance of considering species' life-history strategies when planning to manipulate water-flow for hydroelectric developments as the reproductive biology and feeding habits of diadromous fauna rely heavily on the habitat diversity created by unobstructed watersheds. Anthropogenic barriers and alterations to water flow (e.g., hydropeaking) could also negatively affect landlocked populations because such structures disrupt successful reproduction, recruitment and habitat quality (Alò and Turner 2005; Fullerton et al. 2010; Garcia et al. 2011).

Current hydropower capacity in Chile (~6.000 mega watts of energy connected to the central grid) is expected to increase (~ 11.000MW by the year 2020, Fig. 1D) by approximately 900 additional hydroelectric power plants due to legislative privileges given to hydroelectric investors (Prieto and Bauer 2012; Santana et al. 2014; Toledo 2014). Most of the hydroelectric potential is in the south-eastern sector of the country, in the sub-basins with high elevations and discharge (Santana et al. 2014). These basins harbour the majority of native diadromous fish species diversity (Fig. 1).

The ongoing spread of exotic species also potentially imposes negative interactions due to predation, competition, behavioural inhibition and homogenization (Correa and Gross 2008; Penaluna et al. 2009; Correa et al. 2012; Correa and Hendry 2012; Habit et al. 2012; Arismendi et al. 2014; Vargas et al. 2015). In particular, establishment of migration runs of *O. tshawytscha* and *O. kisutch* could trigger additional threats such as the shift of significant amounts of marine-derived nutrients to previously

oligotrophic environments (Helfield and Naiman 2001; Arismendi and Soto 2012) and increased competition for limited resources with the native diadromous species. It is ironic that non-native salmon and trout are also likely to be negatively affected by future hydroelectric dams. A development that could threaten a flourishing tourism industry supported by salmonid recreational fisheries (Arismendi and Nahuelhual 2007; Vigliano et al. 2007).

The evolutionary processes that allowed dispersal and colonization of Patagonian fishes are likely to be influenced by the region's unique geography, climate, and geological processes. To ensure proper conservation of native freshwater, diadromous, and commercially relevant sport fisheries, managers will have to carefully designate and protect critical habitats, and in many cases mitigate obstruction of river flows imposed by dams with appropriate fish passage structures. Long-term monitoring should also be a priority to understand the broad impacts of hydropower development on aquatic biodiversity.

Supporting Information

The following additional tables and figures are supplemented as part of this work and are available for review:

Table S1: A list of Continental Native Fishes, including life histories and/or habitat.

Table S2: Description of the samples analysed and CART Splitting means and standard deviations.

S3: Pictures of each otolith associated with a graph reporting Sr:Ca transect data.

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Figure Legends

Fig. 1 Estimated distribution range for native Chilean galaxiids (shaded and dash lined polygons) and the sampling locations of specimens used in this study (dots) for a) genus *Aplochiton*, b) genus *Galaxias*, c) non-native fishes examined in this study, and d) locations of hydroelectric power plants above 0.5 MW that are operating, approved for construction and projected (Ministerio de Energia 2016; SEIA 2016)

Fig. 2 The uppermost image depicts a schematic representation of an otolith, showing how growth rings accrue over time around the core and culminate at the edge. The number of distinct layers in the otolith depends on the age of the individual. The images below represent idealized time series data obtained by repeatedly measuring (via laser ablation and spectrometry) elemental strontium to calcium (Sr:Ca) ratios across the otolith. Each box shows an expected time series for each life-history strategy. The blue dashed line represents the average Sr:Ca for freshwater as 0.9 $mmol \cdot mol^{-1}$, the orange dashed line for estuarine areas at 2.0 $mmol \cdot mol^{-1}$, and the black dashed line is drawn at 3.4 $mmol \cdot mol^{-1}$ as the saltwater mean (Secor and Rooker 2000)

Fig. 3 a) Violin plot of Sr:Ca values for otoliths grouped by species. Black dot = median, White line = first to third quartile. Grey areas = kernel density plot. b) Extracted reference values from Figure 7 in Secor and Rooker 2000. Average values of Sr:Ca ratios for freshwater, brackish and marine water are represented as dashed blue, orange, and black lines, respectively. Fading shades around each mean correspond to 10-90th percentiles for each average value

Fig. 4 Detection of discontinuities by semi-supervised CART employed on Sr:Ca ratios for representative individuals of native and exotic fishes in southern Chile. Numbers after taxonomic names refer to the individual ID of each fish. The mean and standard deviation are

delimited for each cluster in a grey box. Vertical dashed lines indicate splitting points induced by the condition used to fit the regression trees, which is reported for each individual graph as "cond". Reference values from Secor and Rooker (2000) are reported on the right-hand side of the graph; see legend of Fig. 3

Fig. 5 Total number of splits obtained by semisupervised CART on univariate Sr:Ca otolith data for all the species included in the study. Original data for native *A. taeniatus, A. marinus, and G. maculatus* and introduced *O tshawytscha* was divided in more than one homogenous cluster by semisupervised regression trees and led to rejection of the hypothesis of freshwater residency. Details for each individual and split reported in this graph are available in the supplementary material Table S2. "Trans" refers to the quality of the otolith transect, that is: "F" is a full or good quality transect, edge – core – edge, “H” is a half transect, edge – core; “P” is a partial transect, edge – core – extra data without reaching the next otolith edge; “O” is a flagged transect which failed to go through the core and may have some missing data. "St" refers to each fish’s ontogenetic phase at the time of capture, where "J" is for juveniles and "A" for adult specimens; "Loc" indicates the sampling locality where AC: Aysén-Caro, CT: Chiloé-Tocoihue, VS: Valdivia-Santo Domingo, VL: Valdivia-Lingue, AP: Aysén-Palos, BE: Baker-estuary, AN: Aysén-Ñireguao, BJ: Baker-Jaramillo, PP: Petrohué-Patos, TP: Toltén-Peuco, TT: Toltén-Truful, AS: Aysén-Simpson, BV: Baker-Vargas; "ID" is the unique identification of each fish

Fig. 6 Nonmetric Multidimensional Scaling on individual variation for element importance. Each point represents an individual whose relationship with other individuals is measured by the distance in the two-dimensional space based on their similarity in element importance. Individual flagged as “O” and “P” in Figure 5 were not included in this analysis. 95% confidence dispersion ellipses drawn when there are more than three data points

Table 1 Description of the samples studied and summary of results of migration pattern determination. Values correspond to the percentage of individuals assigned to one of five possible patterns: freshwater resident (FW), brackish water resident (B), anadromous (ANA), catadromous (CAT), amphidromous (AMPH) or else omitted from interpretation due to uncertainties in the otolith transects (O). For individual results, see Table S2

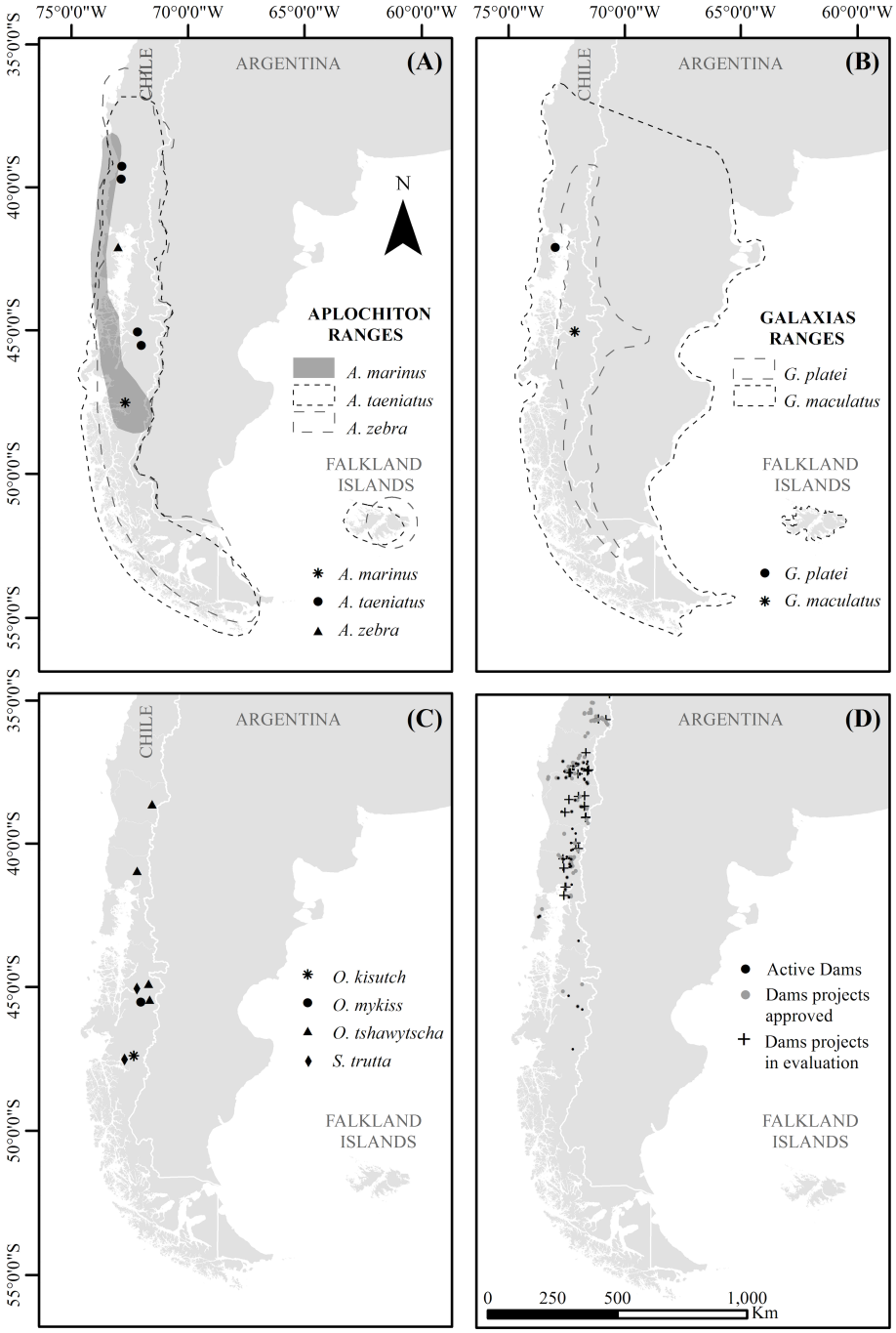
							Inferred Migratory Strategy (%)					
	Species	N	Location	Year	Latitude	Longitude	FW	B	ANA	CAT	AMPH	O
Native Galaxiids	*A. zebra* (Jenyns, 1842)	5	Aysén-Caro	2009	-45.80	-72.55	100					
	A. zebra	3	Chiloé-Tocoihue	2011	-42.30	-73.44		100				
	A. taeniatus (Eigenmann, 1928)	8	Valdivia-Santo Domingo	2010	-39.91	-73.14		12.5		50	25	12.5
	A. taeniatus	2	Valdivia-Lingue	2011	-39.46	-73.09				100		
	A. taeniatus	3	Aysén-Palos	2007	-45.32	-72.70				66.7		33.3
	A. taeniatus	2	Aysén-Caro	2009	-45.80	-72.55	100					
	A. marinus (Jenyns, 1842)	7	Baker-estuary	2007	-47.79	-73.52				100		
	G. platei (Steindachner, 1898)	2	Aysén-Palos	2007	-45.32	-72.70	100					
	G. maculatus (Jenyns, 1842)	4	Chiloé-Tocoihue	2011	-42.30	-73.44		25		50	25	
Introduced Salmonids	*S. trutta* (Linnaeus, 1758)	4	Aysén-Palos	2007	-45.32	-72.70	100					
	S. trutta	4	Aysén-Caro	2009	-45.80	-72.55	100					
	S. trutta	1	Baker-estuary	2007	-47.79	-73.52			100			
	O. tshawytscha (Walbaum, 1792)	8	Aysén-Ñireguao	2004	-45.17	-72.12			75			25
	O. tshawytscha	8	Baker-Jaramillo	2004	-47.70	-73.05	12.5		50			37.5
	O. tshawytscha	6	Petrohué-Patos	2004	-41.18	-72.46			66.7			33.3
	O. tshawytscha	4	Toltén-Peuco	2004	-38.85	-71.76			50			50
	O. tshawytscha	4	Toltén-Truful	2004	-38.85	-71.67			50			50
	O. tshawytscha	5	Aysén-Simpson	2008	-45.73	-72.10	80					20
	O. kisutch (Walbaum, 1792)	2	Baker-Vargas	2007	-47.68	-73.04			100			
	O. mykiss (Walbaum, 1792)	3	Aysén-Caro	2009	-45.80	-72.55	100					

Table 2 Global nonparametric test statistic Wilks Lambda and its permutation test analogue. Each of the four elements' relative importance was used as a response variable while the factors "Species", "Location" and "Inferred Migratory Strategy" were selected as explanatory variables. "df1" and "df2" are numerator and denominator degrees of freedom, respectively. Significant p-values (F approximation) and Permutation Test p-values (randomization) are reported in bold.

	Wilks Lambda	df1	df2	P-value	Permutation Test p-value
Species	1.740	32.000	204.4252	**0.012**	**0.018**
Location	1.003	48.000	198.4960	0.476	0.473
Inferred Migratory Strategy	2.057	16.000	180.8856	**0.012**	**0.017**

Table 3 Numerical description fitting the nonparametric global test of Table 2. A list of the relative effects for the two groups that significantly departed from the null hypothesis of no differences among groups: Group 1. "species" and Group 2. "inferred migratory strategy". Column abbreviations are Mg: Magnesium, Mn: Manganese; Sr: Strontium, Ba: Barium.

Group 1	Mg	Mn	Sr	Ba
A. marinus	0.49403	0.16269	0.68507	0.12388
A .taeniatus	0.376	0.58266	0.54363	0.47933
A. zebra	0.48294	0.54264	0.4936	0.60235
G. maculatus	0.39179	0.73881	0.39552	0.29478
G. platei	0.48507	0.6194	0.47015	0.60448
O. kisutch	0.8806	0.35075	0.19403	0.41045
O. mykiss	0.46517	0.28109	0.16169	0.64925
O. tshawytscha	0.51221	0.50882	0.54003	0.59091
S. trutta	0.64428	0.48673	0.47512	0.47512
Group 2				
amphidromous	0.32587	0.83831	0.52985	0.26119
anadromous	0.56377	0.50543	0.48168	0.56038
brackish	0.43134	0.68806	0.1806	0.52687
catadromous	0.43532	0.35771	0.62637	0.38259
freshwater	0.51967	0.50271	0.50068	0.54613


75°0'0"W
70°0'0"W
65°0'0"W
60°0'0"W
35°0'0"S
40°0'0"S
45°0'0"S
50°0'0"S
55°0'0"S
(A)
ARGENTINA
CHILE
N
APLOCHITON RANGES
A. marinus
A. taeniatus
A. zebra
FALKLAND ISLANDS
A. marinus
A. taeniatus
A. zebra
(B)
GALAXIAS RANGES
G. platei
G. maculatus
G. platei
G. maculatus
(C)
O. kisutch
O. mykiss
O. tshawytscha
S. trutta
(D)
Active Dams
Dams projects approved
Dams projects in evaluation
0
250
500
1,000
Km

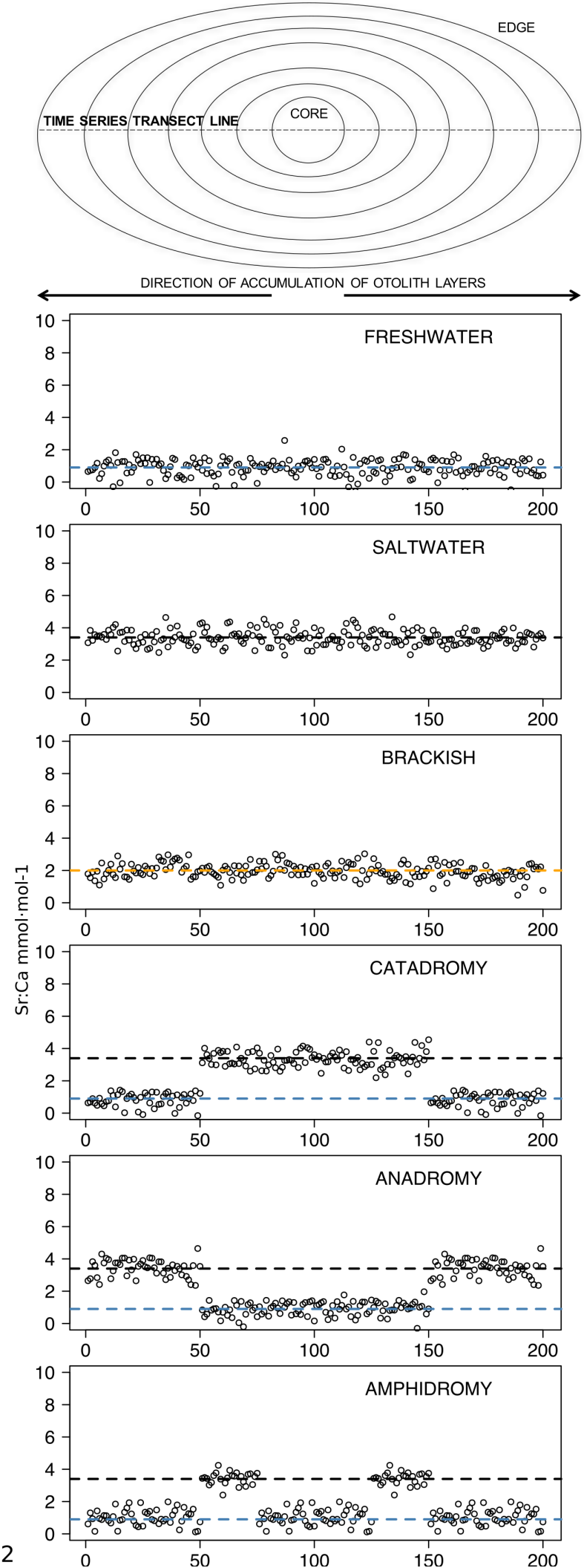
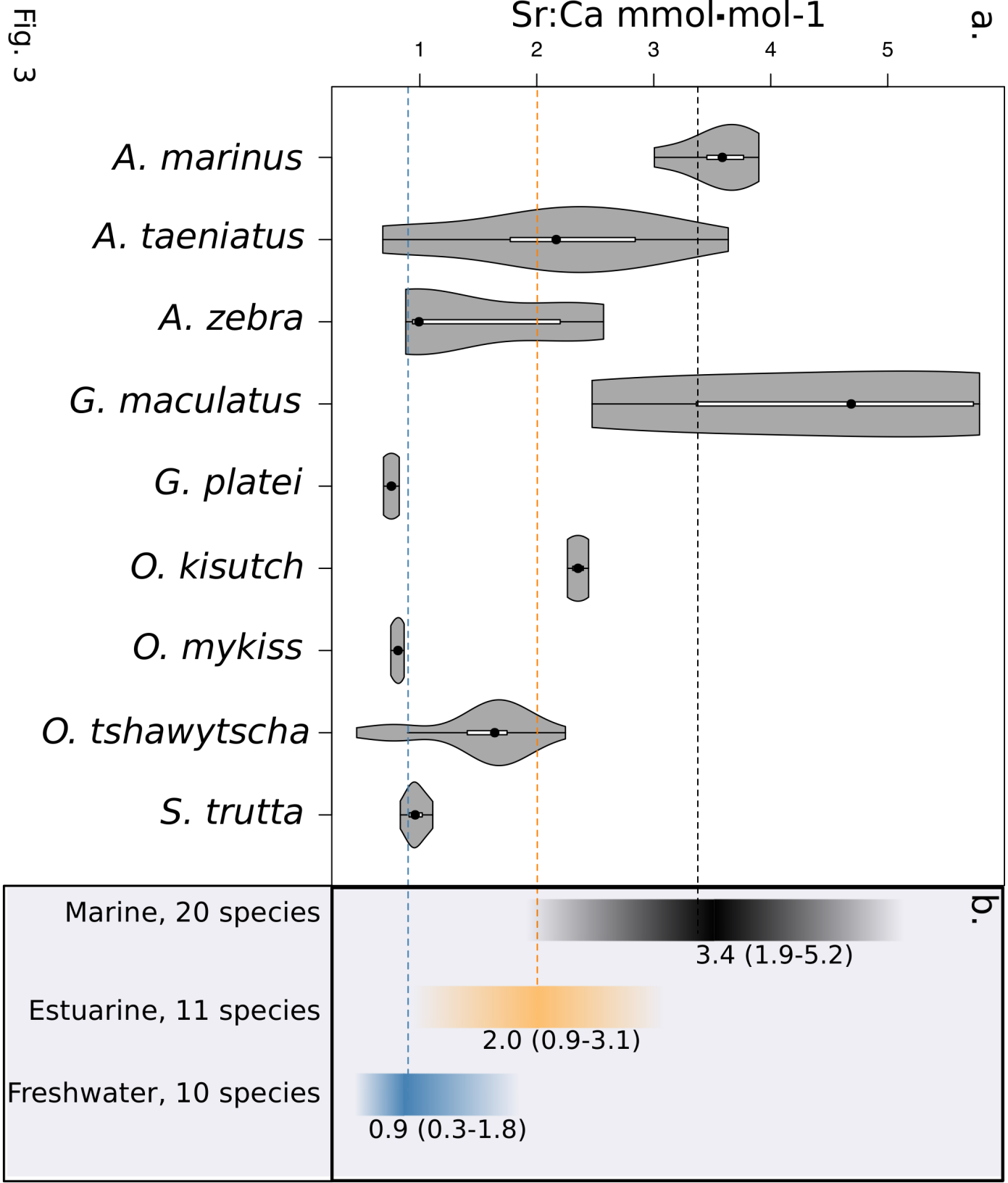

EDGE
TIME SERIES TRANSECT LINE
CORE
DIRECTION OF ACCUMULATION OF OTOLITH LAYERS
FRESHWATER
SALTWATER
BRACKISH
CATADROMY
ANADROMY
AMPHIDROMY
Sr:Ca mmol·mol-1
0
2
4
6
8
10
0
50
100
150
200

Fig. 2

Fig. 3


a.
Sr:Ca mmol·mol-1
1
2
3
4
5
A. marinus
A. taeniatus
A. zebra
G. maculatus
G. platei
O. kisutch
O. mykiss
O. tshawytscha
S. trutta
b.
Marine, 20 species
3.4 (1.9-5.2)
Estuarine, 11 species
2.0 (0.9-3.1)
Freshwater, 10 species
0.9 (0.3-1.8)

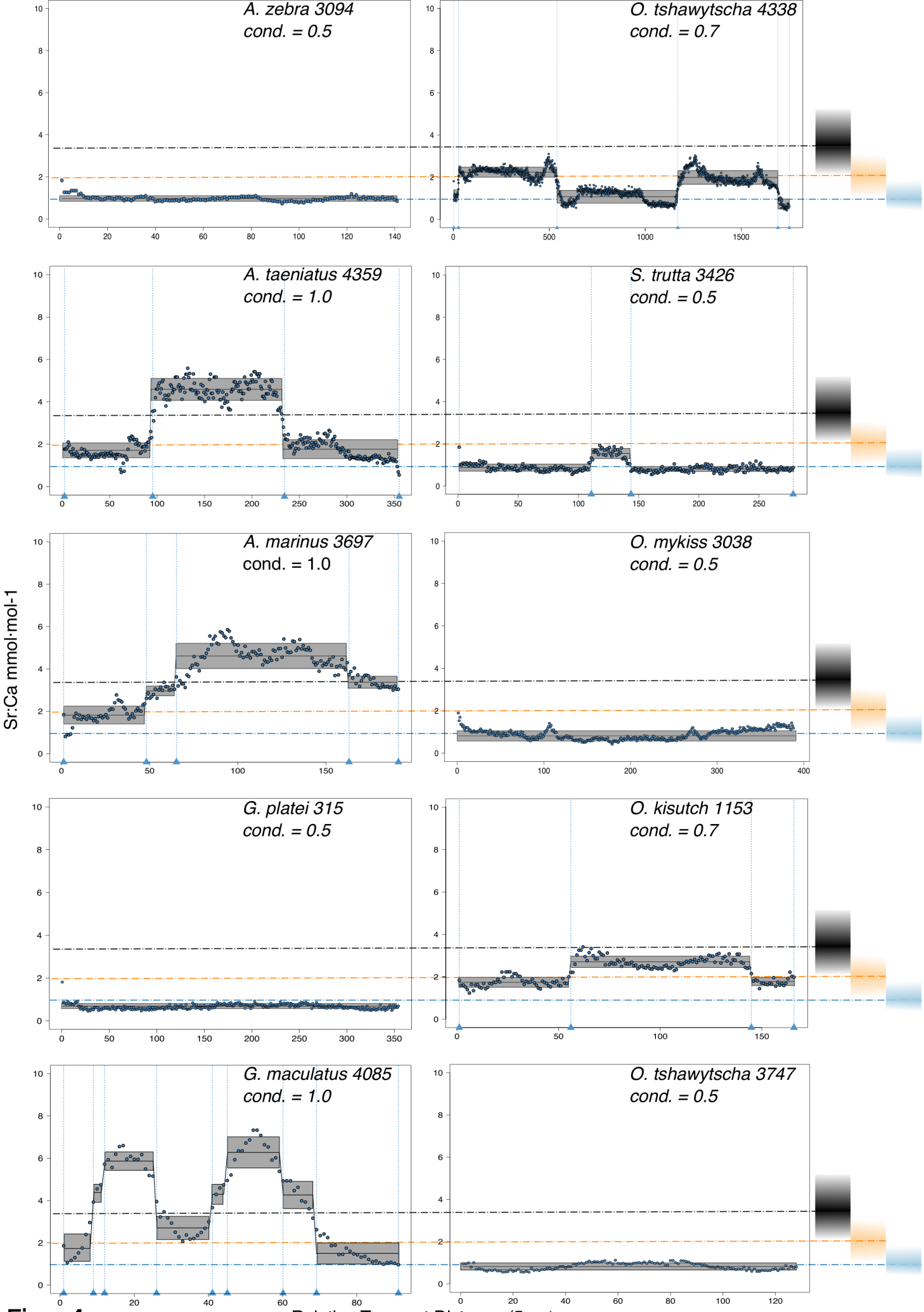

A. zebra 3094
cond. = 0.5
O. tshawytscha 4338
cond. = 0.7
A. taeniatus 4359
cond. = 1.0
S. trutta 3426
cond. = 0.5
A. marinus 3697
cond. = 1.0
O. mykiss 3038
cond. = 0.5
G. platei 315
cond. = 0.5
O. kisutch 1153
cond. = 0.7
G. maculatus 4085
cond. = 1.0
O. tshawytscha 3747
cond. = 0.5
Sr:Ca mmol·mol-1
Relative Transect Distance (5µm)

Fig. 4

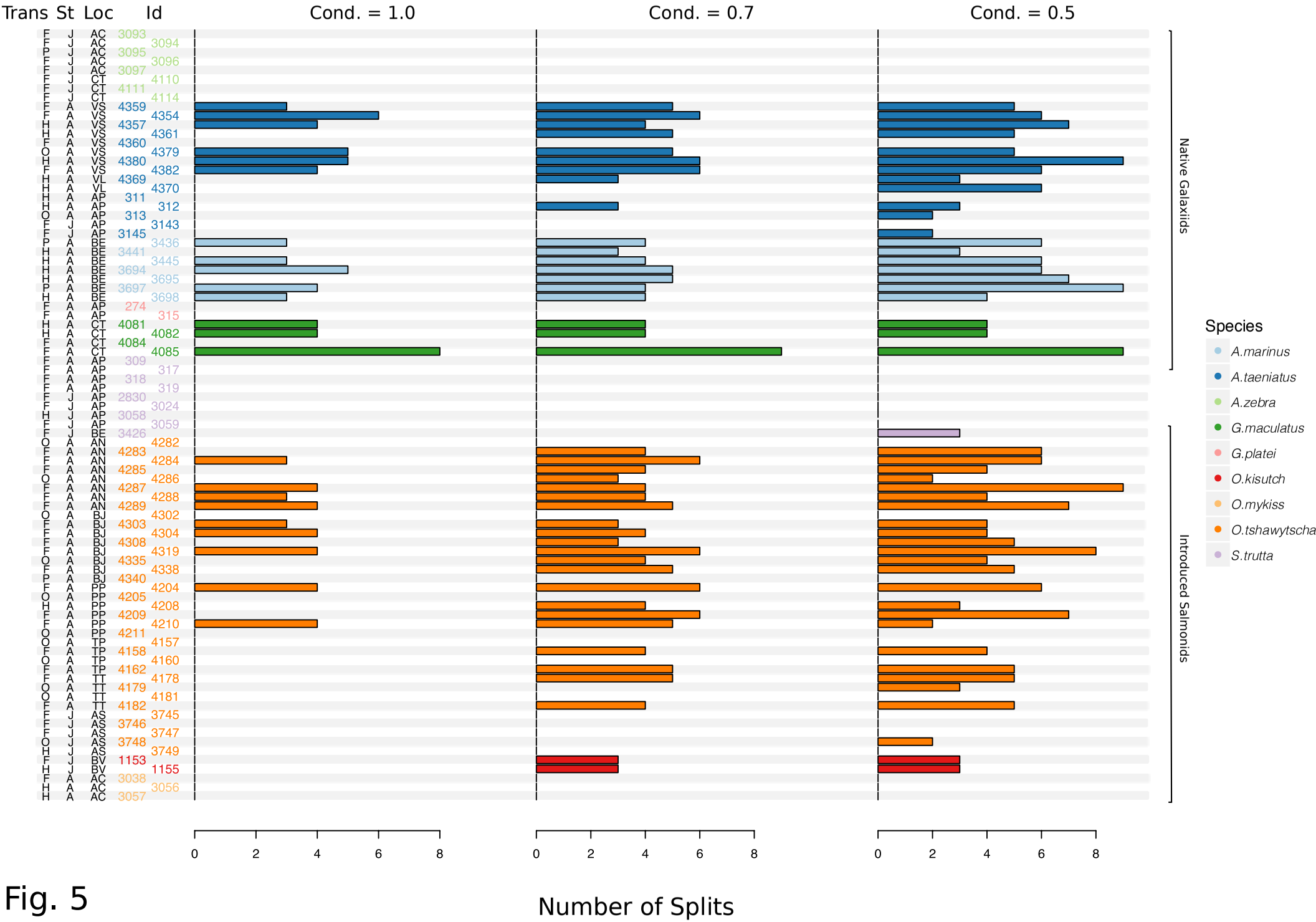

Trans St Loc Id
Cond. = 1.0
Cond. = 0.7
Cond. = 0.5
Native Galaxiids
Introduced Salmonids
Species
A.marinus
A.taeniatus
A.zebra
G.maculatus
G.platei
O.kisutch
O.mykiss
O.tshawytscha
S.trutta
0 2 4 6 8
Number of Splits

Fig. 5

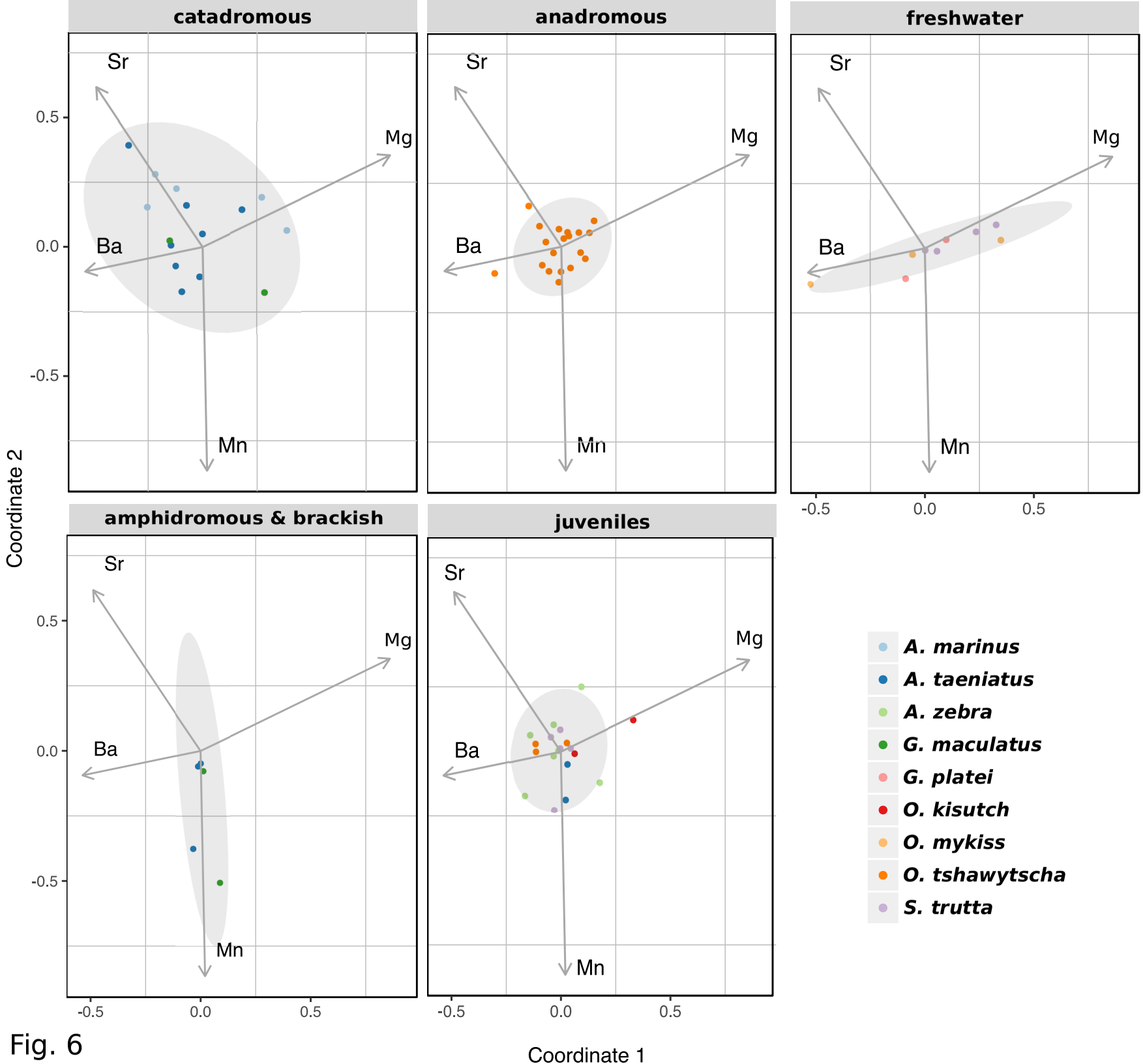

catadromous
anadromous
freshwater
amphidromous & brackish
juveniles
Sr
Mg
Ba
Mn
0.5
0.0
-0.5
Coordinate 2
Coordinate 1
A. marinus
A. taeniatus
A. zebra
G. maculatus
G. platei
O. kisutch
O. mykiss
O. tshawytscha
S. trutta

Fig. 6