Shifts in δ^{15}N signature following the onset of exogenous feeding in rainbow trout *Oncorhynchus mykiss*: importance of combining length and age data

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The δ^{15}N isotopic change of recently emerged rainbow trout *Oncorhynchus mykiss* due to diet shift from yolk sac to exogenous feeding was evaluated in a field study. The fit of a general model including both fish length and age in days as co-variables indicates that the specific δ^{15}N of individual fish at any given time along the ontogeny is determined by its growth trajectory. The results suggest that estimations based on fish size alone could bias data interpretation and maternal origin determinations in partially migratory salmonids. © 2013 The Authors

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Stable isotope analysis is a powerful tool to assess energy flow and nutrient sources in food webs (Finlay *et al*., 2002), estimate trophic interactions (Post, 2002), track animal movements (Hobson, 1999; Rasmussen *et al*., 2009) and study diet shifts (Vander Zanden *et al*., 1998). In order to study ecological processes through isotope signatures, ecologists have devoted much effort in constructing models that describe the pattern of isotopic change in different tissues (Boecklen *et al*., 2011). To date, there are four major classes of isotope incorporation models: growth-dependent models which track changes in isotopic ratios as a function of the growth of the consumer (Fry & Arnold, 1982; Maruyama *et al*., 2001), time-dependent models which track changes in isotopic ratios as a function of time (Hobson & Clark, 1992; O'Brien *et al*., 2000; Martínez del Rio & Wolf, 2005), growth-and-time-dependent models which are the latest modelling attempts at examining joint contributions of growth and metabolism to isotopic turnover (Hesslein *et al*., 1993; Carleton & Martínez del Rio, 2010) and multi-compartment models which use distinct isotope pools to examine isotopic turnover (Ayliffe *et al*., 2004; Cerling *et al*., 2007; Carleton *et al*., 2008).

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In spite of attempts to include both body mass and time perspectives into isotope dynamics through modelling approaches, many field studies still base their analyses on growth-dependent model formulations. In aquatic systems, for instance, the study of δ^{13}C and δ^{15}N signatures during fish emergence has allowed researchers to infer the maternal organic environment of individual fish, which is inherited from eggs, and ultimately by emerging alevins (Doucett *et al.*, 1999; Curry, 2005; Jardine *et al.*, 2008). This technique has been particularly useful for partially migratory salmonids (*i.e.* only a fraction of the population migrates to the sea to feed) to fingerprint the progeny of migratory and resident mothers. As recently emerged juveniles feed and grow in natal streams, maternal isotope ratios are diluted. Fishes with migratory mothers will eventually adopt the isotope ratios of resident fishes and at that time the origin of individual fishes will become indistinguishable (Doucett *et al.*, 1999).

Characterizing this process of maternal isotope signature dilution is key to the ability to determine the maternal origin of individual fishes from snapshots of their isotopic signatures. In this study, the dilution pattern of δ^{15}N for rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) fry is described as a function of body length and age from first feeding in order to include both body mass and time perspectives into isotope dilution patterns. The aim of this study was to assess how reliable isotopic signature measurements are for discriminating the maternal origin of fish in natural settings. In addition, the reliability of assignments of maternal origin based only on isotopic signatures and body length, without accounting for fish age, a more difficult variable to estimate, was investigated.

Young *O. mykiss* were captured with electrofishing gear at five sites within three anadromous spawning locations along the Santa Cruz River (Santa Cruz Province, Argentina, 50° S; 70° W, total river length *c.* 380 km) previously identified through field and telemetry studies (Riva Rossi *et al.*, 2003). Site 1 covered a large spawning area in the middle reach (170–190 km from mouth) and sites 2–5 covered two spawning areas in the upper reach (320–330 km from mouth). Seventy-five fish were analysed. Fish were killed with MS222 and frozen immediately at −20° C until laboratory preparation. Fish were measured (fork length L_F, to the nearest mm), guts removed and otoliths were dissected. The carcass was then prepared for isotope analysis according to the method described by Teece & Fogel (2004) and analysed in a mass spectrometer at the Geophysical Laboratory of the Carnegie Institution of Washington. Stable isotope ratios are expressed as δ^{15}N values, measures of ‰ (ppt) difference between the nitrogen isotope ratios of a sample relative to standard N_2 of the atmosphere (Mariotti, 1983). This study focused on the dilution pattern of δ^{15}N because in the Santa Cruz River system nitrogen isotope ratios allow a better distinction between anadromous (15·2 ± 1·0‰, mean ± S.D.) and resident fish (8·8 ± 1·1‰) than carbon isotope ratios (anadromous: −19·2 ± 1·3‰, resident: −23·2 ± 2·5‰) (Ciancio *et al.*, 2008).

In order to estimate age and time since the onset of exogenous feeding, daily increments in otoliths were counted (Campana, 1983). One otolith per individual was cleaned, mounted sulcus side down and polished until all increments between the core and the outer edge were visible. Otoliths were visually examined under a transmitted light microscope (×400 magnification) with an image analysis system. All otoliths exhibited a hatching check, which consisted of a dark prominent mark surrounding the fused primordium. Some otoliths exhibited a first-feeding check which was followed by a transitional area from broad or indistinct increments of

the post-hatch alevin to the well-defined daily incremental banding of emergent fish (Zhang *et al*., 1995; Morales-Nin, 2000). Total number of increments between hatch mark and the edge of the otolith (total age) and total increments from first-feeding mark to the edge of the otolith (exogenous age) were counted. All measurements were conducted along the longest axis.

Changes in population δ^{15}N as a function of exogenous age, L_F and site of capture were modelled with linear functions. Seven alternative models of different complexities (different number of parameters) were considered (Table I). The level of complexity of a given model is given by whether or not δ^{15}N concentration decline as a function of exogenous age and L_F (dilution) and the dilution rate is site specific. For instance, the most complex model (M7; Table I) considers that starting with an initial δ^{15}N concentration common to all sites (N_0), dilution occurs as a function of both time (age) and growth (L_F), and the rate of dilution with respect to each of the two variables is site dependent. One of the simplest models (M1) also assumes that dilution occurs as a function of both age and L_F, but with the same overall dilution rates for all sites. Models with intermediate complexity (M5 and M6) and with less complexity (M2 and M3) were also fitted, as well as a variation of M1 where the initial δ^{15}N concentration was assumed to be site dependent. This collection of seven models encompasses all the different levels of complexity possible for variations in isotopic concentration and dilution as a function of time, size and location of the fish.

Point estimates for all parameters in each model were estimated through maximum likelihood estimation, assuming a simple normal error structure, using the bbmle package in R software (R Development Core Team; www.r-project.org). The seven models were statistically evaluated in a search for the best model in two ways. First, the AIC was computed for each model. The model with the smallest AIC value gives the most parsimonious description of the data. In general, models with AIC (AIC differences) of >10 have essentially no support and can be omitted from further consideration, models with AIC < 2 have substantial support, while models with 4 < AIC < 7 have considerably less support (Burnham & Anderson, 2002). Second, a sequential stepwise comparison of pairs of models was performed based on the likelihood ratio test (LRT). Given a pair of nested models of different complexity (different number of parameters) which are fitted to the data, LRT allows statistical consideration of whether the data support rejecting the simpler model in favour of the more complex one (Kimura, 1980). The collection of sequential pair-wise comparisons leads to a 'best' model which is favoured in all contrasts over simpler or more complex models.

The value of L_F ranged from 24 to 35 mm, total ages from 25 to 82 days (Fig. 1) and δ^{15}N from 9 to 17·7‰ (Fig. 2). Fish showed a wide range of ages for similar L_F with different L_F and age relationships across the sampled sites, probably associated with site-specific variation in size at hatching and emergence. For example, no significant relationships between age and L_F were found for sampling sites 1, 2 and 4 ($P > 0.05$, $r^2_1 = 0.008$, $r^2_2 = 0.01$, $r^2_4 = 0.01$, d.f.$_1 = 19$, d.f.$_2 = 8$, d.f.$_4 = 8$; Fig. 1), where most fish were still relying on endogenous nutrients. A significant effect of age on L_F was found, however, for sites 3 and 5, where fry had already begun exogenous feeding, with fry from site 3 being larger for any given age than those from site 5 (ANCOVA, site, d.f. $= 27$, intercept $P < 0.001$; Fig. 1). Further, the beginning of the exogenous feeding in the population was

TABLE I. Model selection for $\delta^{15}N$ dilution patterns in *Oncorhynchus mykiss*. Seven models (M1–M7) of different complexity (different number of parameters) are statistically evaluated in two ways: (a) based on their AIC, which provides a direct measure of goodness-of-fit, with lower values indicating better fit and (b) a stepwise comparison of pairs of nested models based on the likelihood ratio test (LRT)

Model	Model structure	Model description: effects on isotopic signature	Number of parameters	(a) AIC	LogLik	(b) LRT Pair-wise contrast
M1	$\delta^{15}N = N_0 + \alpha\ \text{age} + \beta\ \text{length}$	Effects of both age and length on dilution Effects are non-site specific Same initial isotopic concentration among sites	3	282·8	−136·2	
M2	$\delta^{15}N = N_0 + \alpha\ \text{age}$	Only effects of age on dilution Effects are non-site specific Same initial isotopic concentration among sites	2	285·2	−138·5	M1 *v.* M2; $P < 0{\cdot}05$
M3	$\delta^{15}N = N_0 + \beta\ \text{length}$	Only effects of length on dilution Effects are non-site specific Same initial isotopic concentration among sites	2	343·0	−163·1	M1 *v.* M3; $P < 0{\cdot}001$
M4	$\delta^{15}N = N_{0(1-5)} + \alpha\ \text{age} + \beta\ \text{length}$	Effects of both age and length on dilution Effects are non-site specific Initial isotopic concentration varies among sites	7	286·5	−134·0	M4 *v.* M1; $P > 0{\cdot}05$
M5	$\delta^{15}N = N_0 + \alpha\ \text{age} + \beta_{(1-5)}\ \text{length}$	Effects of both age and length on dilution Effects of length are site specific Same initial isotopic concentration among sites	7	286·0	−133·7	M5 *v.* M1; $P > 0{\cdot}05$

TABLE I. continued

Model	Model structure	Model description: effects on isotopic signature	Number of parameters	(a) AIC	(b) LRT	
					LogLik	Pair-wise contrast
M6	$\delta^{15}N = N_0 + \alpha_{(1-5)}$ age $+ \beta$ length	Effects of both age and length on dilution; Effects of age are site specific; Same initial isotopic concentration among sites	7	284.9	−133.2	M6 $v.$ M1; $P > 0.05$
M7	$\delta^{15}N = N_0 + \alpha_{(1-5)}$ age $+ \beta_{(1-5)}$ length	Effects of both age and length on dilution; Both effects of age and length are site specific; Same initial isotopic concentration among sites	11	285.6	−131.8	M7 $v.$ M1; $P > 0.05$

LogLik, the log-likelihood value for each model; P values provide the probability of rejecting the simpler of the two models being compared (the one with fewer parameters) when true; lower P values favour the selection of the more complex of the two models. For simplicity, parameters indexed 1–5 (e.g. $\alpha_{(1-5)}$) represent five different parameters, one for each site.
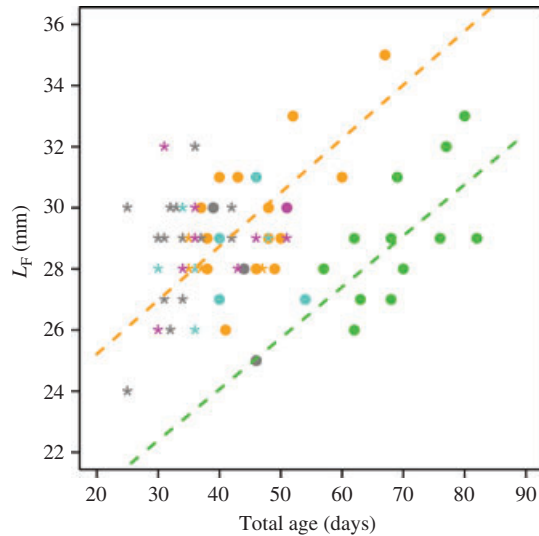
FIG. 1. Fork length (L_F) and total age for sites 1 ($^*\bullet$), 2 ($^*\bullet$), 3 ($^*\bullet$), 4 ($^*\bullet$) and 5 ($^*\bullet$). Site-specific regressions were fitted for sites 3: $y = 21 \cdot 7 + 0 \cdot 17x$ ($r^2 = 0 \cdot 41$) and 5: $y = 17 \cdot 4 + 0 \cdot 17x$ ($r^2 = 0 \cdot 40$); *, fish with no evidence of exogenous feeding; $\bullet$, fish with evidence of exogenous feeding.

not clearly associated with a length limit. Eighty-seven percent of fish older than 41 days and 100% of fish older than 55 days, however, had begun exogenous feeding (Fig. 1).

A clear pattern of isotope change was observed for $\delta^{15}N$ against exogenous age, with $\delta^{15}N$ decreasing with increasing age [Fig. 2(a)]. On the other hand, two patterns were evident for $\delta^{15}N$ against L_F, fish from sites 1, 2 and 4 did not vary isotopically with increasing L_F, whereas those from sites 3 and 5 displayed decreasing $\delta^{15}N$ with increasing L_F [Fig. 2(b)]. The site-specific differences in the relationship between $\delta^{15}N$ and L_F are clearly associated with the onset of exogenous feeding, as older fish from sites 3 and 5 have begun the exogenous feeding and therefore have already started to dilute their maternal signature. The range in $\delta^{15}N$ values in fish with no change (*i.e.* with no dilution) corresponded well with isotopic values observed for anadromous individuals (15–17·6 for fry and 13·4–17·1‰ for adults; Ciancio *et al.*, 2008), whereas those with change (*i.e.* diluted) spanned observed isotopic values for both anadromous and resident adults (6·8–10·5‰ for resident fish; Ciancio *et al.*, 2008), making it difficult to classify them considering their maternal origin (Fig. 2).

On the basis of both LRT and AIC selection methods, the best fit was provided by a model with dilution depending on both L_F and exogenous age, and without site-specific dilution rates (M1; Table I and Fig. 3). This model was always favoured when contrasted with more complex versions of site-specific dilution rates (M5, M6 and M7), indicating that there is a common pattern of nitrogen isotope change as a function of L_F and age for all individuals and sites sampled (Table I; see M4–M7 against M1, Fig. 3). Also, reduced models only accounting for age (M2) or L_F effects (M3) were rejected ($P < 0\cdot05$ and $P < 0\cdot001$; ΔAIC *c.* 2·6 units and ΔAIC
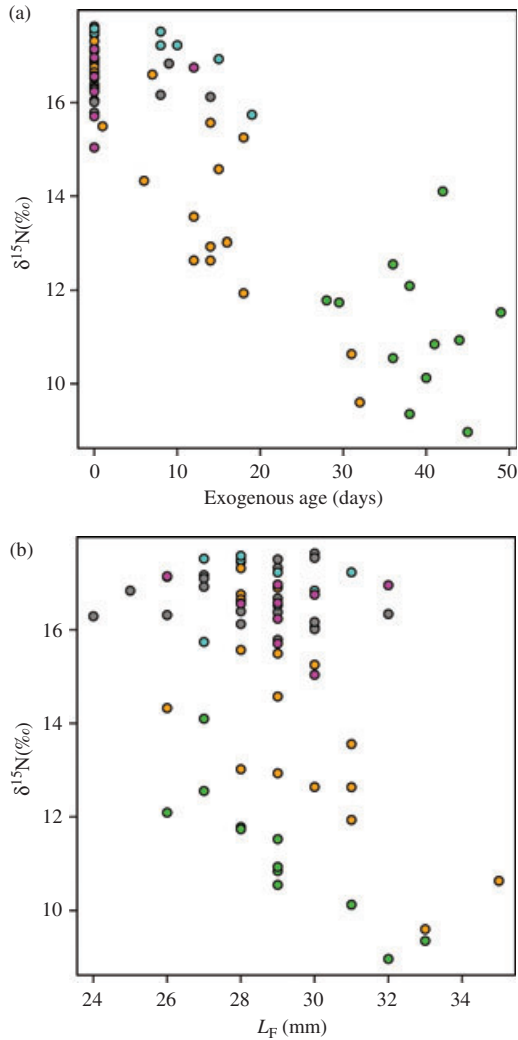
FIG. 2. δ^{15}N isotopic change and (a) exogenous age and (b) fork length (L_F). Symbols represent the five sites sampled: 1 (⬤), 2 (⬤), 3 (⬤), 4 (⬤) and 5 (⬤).

c. 60 units, respectively; Table I). M1 was also favoured over a variation with site-specific intercepts (M4), indicating that initial nitrogen isotope signals do not vary among sites.

Selection of the full model with both length and age predictors implies that the particular δ^{15}N signature of a fish can be explained by its specific growth trajectory, not by time or size alone (Fig. 2). Particularly, the poor fit of M3 (Table I) implies that the evaluation of isotope change against length alone can clearly bias data interpretation, as length and time from the onset of exogenous feeding are not strongly linked. Because the Santa Cruz *O. mykiss* population is partially migratory, progeny of two alternative maternal origins are expected to occur. Analysis of δ^{15}N data alone across
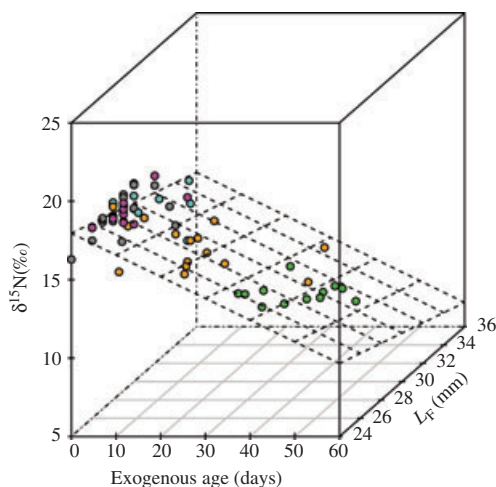
FIG. 3. Bi-dimensional plot of δ^{15}N (y) and fork length (L_F) and exogenous age (A). Symbols represent the five sites sampled: 1 (●), 2 (●), 3 (●), 4 (●) and 5 (●). The plane represents the multiple regression ($y = 24.18 - 0.14A - 0.26 L_F$).

the length dimension would assign fish of similar size, but with contrasting δ^{15}N signatures to different maternal origins; for example, most fish from sites 3 and 5, which show depleted δ^{15}N values in comparison to fish from remaining sites, would have been assigned as resident progeny. When the exogenous age of fish is accounted for in the model, however, a single dilution pattern fits well to fish from all sites, giving stronger support to the hypothesis of a common anadromous maternal origin.

This study shows that the maternal origin from isotope signatures of fish with large variability in growth and different size at hatching and emergence cannot be assigned based on isotope readings and length data alone. Both dimensions of isotopic change (size and time) should be considered as complementary rather than as alternative approaches for identifying the maternal origin of juvenile fish with high predictive power. When the nature of the study or the field methodology does not allow following individuals through time in order to apply existing models, all possible efforts should be made to include age estimations in the isotope change evaluation.

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