

Ontogenetic shifts in terrestrial reliance of stream-dwelling brown trout

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ABSTRACT

This study focuses on terrestrial reliance of brown trout (Salmo trutta) and compared it to the potential prey available (macrozoobenthos and drifting invertebrates) in three temperate rivers (Galicia, NW Spain), with special emphasis on variations in terrestrial energy intake through the ontogeny of brown trout. Additionally, we paid particular attention to individual variation of terrestrial resource use within and between age classes. Prey items were grouped in four categories: i) aquatic invertebrates; ii) imagoes of aquatic invertebrates; iii) terrestrial invertebrates; and iv) fish prey. Next, energy composition was measured according to dry weight-energy equations for each individual in line with above-mentioned prey categories. Our findings illustrate that terrestrial invertebrates appeared to be scarce in the environment, whereas aquatic food resources were rather abundant and accessible. The use of terrestrial invertebrates tended to increase with age, but with a high degree of inter-individual variation in resource use. In fact, the individual reliance of brown trout on terrestrial invertebrates may vary considerably (between 0% and 76.9%). Besides, the frequency of terrestrial foragers, i.e., individuals with terrestrial invertebrates in their stomachs, increased with age, except in one population which had the maximum value in the age-2 class. The acquisition of terrestrial invertebrates thus appears to be a process strongly dependent upon the actual food availability in the environment, but with a high degree of individual variance in resource use within the same age class. Finally, we discuss that terrestrial invertebrates may largely contribute to cover the energy intake of the species, highlighting the interface between terrestrial and aquatic ecosystems, and thereby the importance of riparian canopy cover as a key factor for food supply of stream-dwelling salmonids species.

Key words: Allochthonous resources; feeding; inter-individual variation; ontogeny; prey accessibility.

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INTRODUCTION

The terrestrial-aquatic linkages are well known, the most plausible cause for this being the research on allochthonous resource use (mainly terrestrial leaves and terrestrial invertebrates) by organisms inhabiting stream food webs (Nakano *et al.*, 1999a; Nakano and Murakami, 2001; Alvim *et al.*, 2015; Fenoglio *et al.*, 2015). In fact, inputs of terrestrial invertebrates from riparian canopy cover may represent an important food resource for stream-dwelling fish species (Kawaguchi and Nakano, 2001; Utz and Hartman, 2007; Syrjänen *et al.*, 2011). Therefore, the importance of terrestrial invertebrates in riverine systems is unquestionable, such importance has been identified in different ways; that is, in terms of energy subsidy for fish species (Edwards and Huryn, 1996; Nakano *et al.*, 1999b; Utz and Hartman, 2007), annual fish production (Edwards and Huryn, 1995), food resource partitioning between sympatric fish species (Dineen *et al.*, 2007; Sánchez-Hernández *et al.*, 2013) or ecosystem functioning (Nakano *et al.*, 1999c). That said, it should be kept in mind that the terrestrial invertebrate input to the riverine systems greatly depends on riparian canopy cover (Edwards and Huryn, 1996; Kawaguchi and Nakano, 2001; Ryan and Kelly-

Quinn, 2015), but this allochthonous prey supply occurs primarily during summer, when aquatic invertebrate biomass is usually low (Nakano and Murakami, 2001). In addition, food resources may broadly vary in nature, but stream-dwelling salmonid species can adapt their forage mode according to prey abundance and accessibility in the environment (Nakano *et al.*, 1999a; Sánchez-Hernández and Cobo, 2013).

Brown trout *Salmo trutta* Linnaeus, 1758 has a worldwide distribution inhabiting both running waters and lentic systems. The species exhibits ontogenetic shifts in their diet and habitat use (Montori *et al.*, 2006; Ayllón *et al.*, 2010; Sánchez-Hernández and Cobo, 2012). Although aquatic invertebrates are usually the main food resource for brown trout, terrestrial invertebrates and fish prey are more frequently consumed by older trout (Fochetti *et al.*, 2008; Sánchez-Hernández and Cobo, 2012). The present study addresses prey availability in the environment as well as the terrestrial energy subsidy for brown trout over its ontogeny in three temperate rivers, with special emphasis on variations at individual level. We hypothesized that the use of terrestrial invertebrates by brown trout may be highly dependent on fish age. We further hypothesized that the energy contribution of terrestrial invertebrates to the total diet might be high in older age classes.

METHODS

Sampling sites were established in three rivers of Galicia, NW Spain (Anllóns, Furelos and Lengüelle) (Supplementary Fig. 1). The study area includes a mixture of agricultural and relatively undisturbed areas, with small rural areas interspersed. The vegetation structure comprises a series of extended grazing lands with Monterey pine (*Pinus radiata*) and eucalypt (*Eucalyptus globulus*) forests. The climate is typically Atlantic, with higher differences between extreme temperatures in summer and winter, and deciduous riparian vegetation in all sampling sites was principally composed of alder (*Alnus glutinosa*) and willow (*Salix* spp.).

Sampling was carried out in three wadeable riffle sections in September 2007. Prior to electrofishing, samples of potential prey (macrozoobenthos and drifting invertebrates) were collected to study the prey availability in the environment. Macrozoobenthos were collected from riffles using a 0.1 m² Surber sampler (n=3). Brundin nets (250 µm mesh size, 1 m long, 30 cm mouth diameter) were used to collect two drifting samples (one at the water surface and the other on the bottom). Drift nets were set at sunrise (8:00 a.m.) and retrieved after at least 2.5 hours. After collection, we fixed samples using 4% formalin and stored them for later processing. We estimated drift density per 100 m³ of water using the following equation provided by Allan and Russek (1985):

$$\text{Drift density} = 100 \times [(\text{number of organisms per net hour}) / (\text{m}^3 \text{ filtered per net hour})] \quad (\text{eq. 1})$$

Brown trout were collected using pulsed D.C. backpack electrofishing equipment (Hans Grassl GmbH, Schönaun am Königssee, Germany; ELT60II). Fish were killed immediately by an overdose of anaesthetic (benzocaine) and scales were taken only in individuals > 10 cm. Young-of-the-year (age-0) were identified using Petersen's length-frequency method (Bagenal and Tesch, 1978) and according to published literature for brown trout in nearby territories (Alonso-González *et al.*, 2008; Sánchez-Hernández and Cobo, 2012). With regard to scale reading, regenerated scales were discarded for age determination. Scale annuli were identified using standard criteria based on spacing and continuity of circuli along the anterior-posterior axis of a scale (see for example Richard and Baglinière, 1990). The number of analyzed brown trout was 138 ($n_{\text{age-0}}=45$, $n_{\text{age-1}}=50$, $n_{\text{age-2}}=30$ and $n_{\text{age-3}}=13$).

In the laboratory, fish were dissected and the stomachs were removed for diet analysis. Prey items were sorted, identified and weighed for latter weight-energy equations. The prey items were grouped in four categories: i) aquatic invertebrates; ii) imagoes of aquatic invertebrates; iii) terrestrial invertebrates; and iv) fish prey. Next, prey items were converted into energy composition using dry weight-

energy equations (Cummins and Wuycheck, 1971; Cobo *et al.*, 1999, 2000), and the total energy for each prey category in the stomach was summarized for each fish. Finally, we quantified the frequency of terrestrial foragers for each age class, that is, the frequency of specimens feeding on terrestrial invertebrates based on the presence/absence of this prey category in their stomachs. Normality of distribution and homogeneity of variances were tested through Shapiro-Wilk and Levene's tests, respectively. Kruskal-Wallis tests for non-normal data were used for detecting differences in energy composition of each prey category among age classes. Next, the differences between pairs of age classes were tested by Student's *t*-test. Prior to Student's *t*-test, homogeneity of variances was tested through Levene's tests (*F*-test), that is when the P-value for the *F*-test was <0.05, we used the *t*-test based on unequal variance, whereas when P-value for the *F*-test was >0.05, we assumed that the variances were equal (*t*-test based on equal variance). A significance level of P=0.05 was used in all analyses. Statistical analyses and graphical outputs were performed using R 3.2.0 (R Core Team, 2012) which can be freely obtained at <http://www.r-project.org/>.

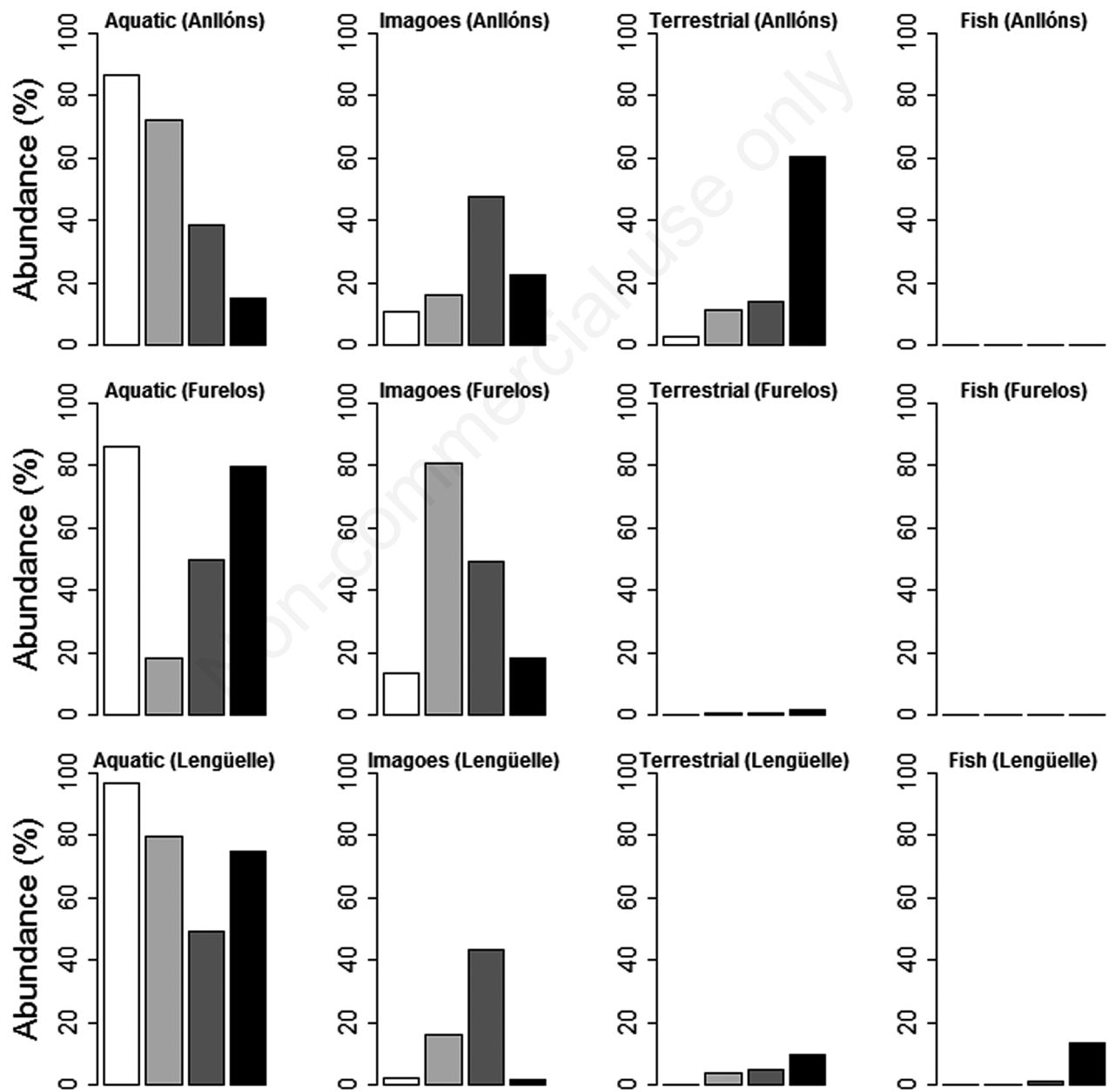
RESULTS

The abundance of potential prey for brown trout (macrozoobenthos and drifting invertebrates) broadly differs among riverine systems (Tab. 1). Benthic invertebrate abundances varied among sampling sites from 4086 ind m⁻² to 10170 ind m⁻². With regard to drifting samples, aquatic invertebrates were taken in large numbers at each sampling site and formed a considerable proportion of the surface drift. In fact, the terrestrial invertebrates constituted < 11% of the surface drift samples, whereas the imagoes component of the surface drift was much more important in two out of three studied rivers (Tab. 1).

No empty stomachs were observed. The diets of the different age classes consisted primarily of aquatic invertebrates, except age-1 class of River Furelos and two age classes of River Anllóns (age-2 and age-3), which imagoes of aquatic resources (age-1 and age-2) and terrestrial invertebrates (age-3) were the most abundant prey category (Fig. 1). The individual reliance on terrestrial invertebrates, in terms of energy, may vary considerably within and between age classes (between 0% and 76.9%), but tended to increase with age (Fig. 2). The age-3 class had the highest reliance on terrestrial invertebrates, whereas juveniles (namely age-0 and age-1 classes) showed a clear reliance on aquatic invertebrates. Imagoes taken from the surface of the water column, were of obvious importance for some age classes, such as age-2 class (all rivers) and age-1 class (River Furelos). Detailed energy contribution of each prey category and the outputs of the statistical comparisons among age classes are given as supplementary material (Supplementary Tabs. 1 and 2). In addition,

Tab. 1. Potential prey availability (macrozoobenthos and drift) for each sampling site.

	Anllóns	Furelos	Lengüelle
Macrozoobenthos density (ind m ⁻²)	8111	10170	4086
Surface drift density (ind 100 m ⁻³ hour ⁻¹)	336.5	887.2	764.1
Terrestrial invertebrates in the surface drift (%)	5.9	3.7	10.5
Imagoes in the surface drift (%)	7.4	21.6	6.2
Benthic drift density (ind 100 m ⁻³ hour ⁻¹)	890.9	545.7	307.6

**Fig. 1.** Abundance (%) of different prey categories for each age class of brown trout. Data are presented for each river (names in brackets). Age-0 (white bars), age-1 (light grey bars), age-2 (dark grey bars) and age-3 (black bars).

the frequency of terrestrial foragers, *i.e.* individuals with terrestrial invertebrates in their stomachs, increased through the ontogeny (Fig. 3).

DISCUSSION

Our estimation of potential prey availability, in terms of abundance (macrozoobenthos and drift density), in all

three studied rivers were higher than values reported by other researchers in the Iberian Peninsula (Rieradevall and Pratt, 1986; Almodóvar *et al.*, 2006; Sánchez-Hernández, 2011). Despite the fact that our macrozoobenthos abundances varied widely among sampling sites, abundances were by far higher than values reported by other workers, who found abundances between 598 ind m⁻² and 4381 ind m⁻² in other Iberian rivers (Almodóvar *et al.*, 2006;

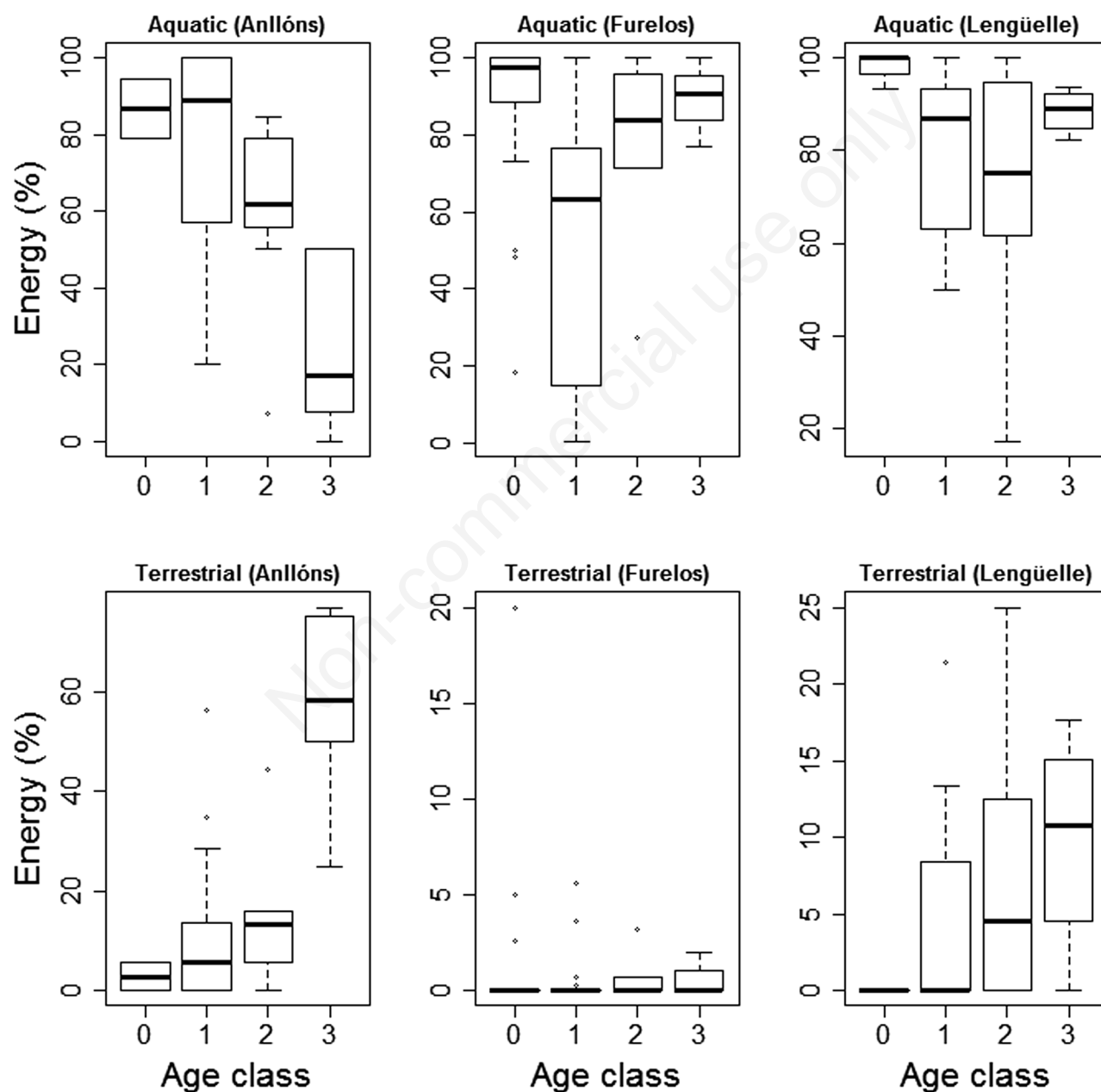


Fig. 2. Energy contribution (%) of aquatic and terrestrial invertebrates to the diet of brown trout. Data are offered by age classes and sampling sites. The boxes show 25th to 75th percentile and median (the inner horizontal line in each box). Whiskers above and below the box indicate the 90th and 10th percentiles.

Sánchez-Hernández, 2011; Sánchez-Hernández *et al.*, 2011). Likewise, even though our drift densities varied largely among sampling sites, values found in this study were higher than other previous studies carried out in the Iberian Peninsula (Rieradevall and Pratt, 1986; Rincón and Lobón-Cerviá, 1997; Sánchez-Hernández, 2011). Nevertheless, in this study the contribution of terrestrial invertebrates to the surface drift sample was very low. In this context, it is important to note that stream-dwelling salmonids are generally considered drift feeders (Rader, 1997), but salmonids are able to adapt their forage mode (benthic or drift foraging) according to drift availability; that is, when the abundance of drifting prey drops, the frequency of benthic foraging increases (Nakano *et al.*, 1999a). That said, our findings illustrate that the diet of brown trout was chiefly dominated by aquatic invertebrates, most likely because of aquatic invertebrates appeared to be accessible and abundant in the environment.

Our study confirms the hypothesis that terrestrial reliance increases across the ontogeny of brown trout. In general, juveniles (age-0 and age-1 classes) fed mostly on aquatic invertebrates, and terrestrial invertebrates were more frequently consumed by older trout. It has been concluded that on average the abundance of terrestrial resources in the diets of salmonids is around 17%, being much higher in large fish than in small ones (Syrjänen *et al.*, 2011). Except the age-3 class in one out of three studied rivers (Fig. 1), our values were in general lower than those reported by Syrjänen *et al.* (2011), but with a high degree of inter-individual variation within and between age classes. As it has been noticed by other researchers, our outcomes show that the abundance of terrestrial invertebrates tended to increase through the ontogeny of brown trout (Montori *et al.*, 2006; Fochetti *et al.*, 2008;

Sánchez-Hernández and Cobo, 2012), except in one population (River Furelos) in which brown trout fed massively on aquatic resources (both macrozoobenthos and imagoes of aquatic invertebrates) regardless of age class (Fig. 1). This riverine system showed the highest abundance of macrozoobenthos and the lowest ratio of terrestrial invertebrates drifting, therefore the diet of brown trout can be highly depend on the type of prey available in the environment (here aquatic resources). As noted above, our findings illustrate that terrestrial invertebrates appeared to be scarce in the environment. The acquisition of terrestrial invertebrates thus appears to be a process strongly dependent upon the actual prey availability, but with a high individual variance in resource use within the same age class. In fact, the inter-individual variation in terrestrial reliance may vary considerably within the same age class (Fig. 2), but the frequency of terrestrial foragers increased through the ontogeny.

CONCLUSIONS

Overall, the reliance of brown trout on terrestrial invertebrates tended to increase with age. Noteworthy, our findings can exemplify the interface between terrestrial and aquatic ecosystems through the feeding of top predators in riverine food webs (here brown trout). As noted in the Introduction, other workers have found that terrestrial invertebrates may enhance fish production, food resource partitioning among competing species and stream benthic community (Edwards and Huryn, 1995; Nakano *et al.*, 1999a; Dineen *et al.*, 2007). That said, planning of restoration works or land-use practices on river basins should take into account the input of terrestrial invertebrates from the riparian canopy as a key factor for food supply of stream-dwelling salmonids species, as well as other advantages in ecosystem processes such as resource partitioning or trophic cascades.

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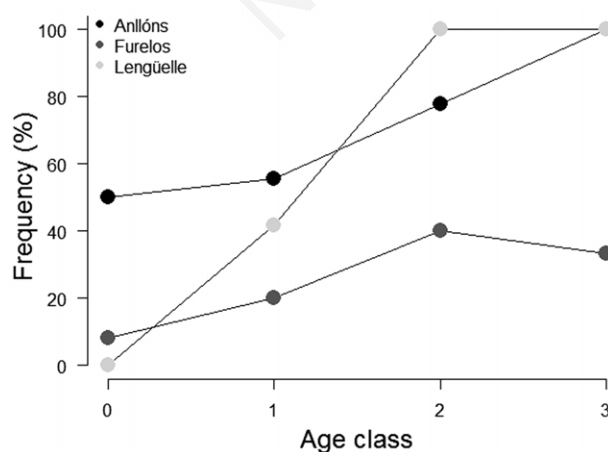


Fig. 3. Ontogenetic shifts in the frequency of terrestrial foragers (individuals with terrestrial invertebrates in their stomachs). Data are displayed for each age class and river.

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