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Individual diet variation in *Salamandra salamandra* larvae in a Mediterranean stream (Amphibia: Caudata)

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Studying the diet of amphibians is useful to understand their ecological role and define the ecosystem processes in which they are involved (DAVIC & WELSH 2004). While many studies have focused on the trophic ecology of adult amphibians, both in aquatic and terrestrial ecosystems, fewer studies investigated the trophic ecology of amphibians at larval stage (WELLS 2010). Moreover, individuals within a population can exploit different resource categories, because they have different food requirements related to different morphologies, different foraging strategies or different acquired feeding behaviours (e.g., ARAÚJO et al. 2011). Therefore, each individual can contribute differently to the observed trophic niche of the entire population and, in fact, it has been demonstrated that many generalist populations are composed of a pool of specialized individuals, with each one feeding on a relatively different pool of resources (BOLNICK et al. 2002, SNOWBERG et al. 2015). In the case of salamanders, there have been few studies that analysed trophic specialization at individual level (e.g., COLLINS et al. 1993, MAERZ et al. 2006, DA ROSA et al. 2011, SALVIDIO et al. 2015) and in only one case, to our knowledge, the larval stage was included into this kind of investigation (SCHRIEVER & WILLIAMS 2013).

In this paper we focus on the trophic strategy and intra-population diet variation of the larvae of the fire salamander, *Salamandra salamandra gigliolii* (EISELT & LANZA, 1956), living in a fish-free freshwater habitat, in which they rank as top predators (OBERRISSER & WARINGER 2011). Even if data of the trophic ecology of *S. salamandra* larvae are available in the literature, they refer mainly to larvae inhabiting stagnant water bodies (BRESSI et al. 1996, REINHARDT et al. 2013, but see WEITERE et al. 2004 for data on stream-dwelling larvae). Therefore, our aims were (1) to

identify the emerging trophic strategy of a stream population of *S. salamandra* larvae; (2) to assess prey selection, taking into account the availability of food resources in the environment; and (3) to test for the presence of trophic specialization at individual level and, if present, correlate it to individual body size.

We conducted a study along the first-order Apennine stream Rio Bronzino within the protected Parco Regionale delle Capanne di Marcarolo, in Piedmont, Italy (44°37'00" N, 8°43'21" E). The sampling site is located at 250 m above sea level and surrounded by a riparian forest dominated by poplar (*Populus* sp.) and alder (*Alnus* sp.). Salamanders and invertebrate sampling was conducted along a 300-m stretch; salamanders were found in four pools along the stream and invertebrates were sampled in the same pools where salamanders were captured.

Larvae were sampled in June of 2013 during a single night, at the peak of their daily activity on the stream bottom, using hand nets. Stomach flushing was performed in situ (FRASER 1976) in order to prevent prey digestion (SOLÈ et al. 2005), using a 5-ml syringe and a flexible plastic tube, and stomach contents were preserved in 70% ethanol. For each individual larva, we measured snout-vent length (SVL) using a digital calliper (precision 0.1 mm). After stomach flushing, all larvae were held for a few hours before being released and no mortality was observed.

The potential prey items available to the salamanders were sampled using a 60-cm diameter tube that was pushed firmly on the streambed in order to isolate a water column and then we sampled both the water column and the substrate with a rigid frame net (0.03 mm mesh). Sampling was continued until no more invertebrates were cap-

tured for at least ten consecutive times; this procedure was repeated eight times for a total of 2.3 m².

Invertebrates in the stomach contents and the environment were sorted using a dissecting microscope and identified at order level using taxonomic keys; prey with similar morphologies and ecologies (e.g., Plecoptera and Ephemeroptera) were pooled together in the analysis. Prey selection was evaluated by means of VANDERPLOEG & SCAVIA'S (1979) Relativized Electivity Index (E*) that ranges between -1 (avoidance) and 1 (positive selection) and supposes a zero value for random feeding. E* was calculated only for prey categories with at least 5 individuals sampled both in diet and environment, while the significant threshold value was calculated as the 5th percentile of the absolute value of E* (RAMOS-JILIBERTO et al. 2011, SALVIDIO et al. 2012). The population foraging strategy was then assessed using COSTELLO'S (1990) modified graphical method (AMUNDSEN et al. 1996), which plots prey frequency of occurrence [FO – frequency of occurrence of predators feeding on prey (i)] against specific prey abundance [Pi – relative abundance of prey item (i) calculated on the total items found only in those individuals that fed on (i) prey category] and gives a graphical interpretation of the population foraging strategy (e.g., generalist or specialist). Concerning inter-individual diet variation, we analysed the population diet using the R package RinSp (ZACCARELLI et al. 2013) calculating the Shannon-Weaver index as a proxy for the total niche width (TNW) of the population (ROUGHGARDEN 1972, BOLNICK et al. 2002). The TNW was split into two components: the within individual component (WIC) and the between-individuals component (BIC). The ratio WIC/TNW is a measure of individual specialization and ranges from 0 to 1, assuming lower values in the case of low diet overlap between individuals and high levels of inter-individual variation within the population (BOLNICK et al. 2002). As an alternative measure of individual specialization, we also employed the proportional similarity index (PSi), which describes the overlap of any individual diet with the diet of the entire population (BOLNICK et al. 2002). PSi ranges from 0 to 1, assuming values near 0 in a case of individual specialization. The statistical significance of both WIC/TNW and PSi was assessed by generating through Monte Carlo resampling 999 simulated populations from the original dataset. Each simulated population had a number of individuals equal to the number observed in the real population, and each individual had a random sample of prey items. This procedure yields a null model distribution, consistent in 999 populations composed entirely of generalist individuals (BOLNICK et al. 2002). At last, in order to evaluate if individual specialization is related or not to morphological variation (BOLNICK & PAULL 2009), we calculated the diet overlap of each individual's diet with the diet of other individuals in the population, obtaining a pairwise diet overlap matrix; the same pairwise matrix was built for the distance calculated for SVL and we employed a regression analysis in order to test if inter-individual resource variation was related to body size variation.

Table 1. Complete data from environmental sampling (2.3 m² of stream bed and water column) and salamander larva diet (43 individuals). Values of individual specialization indices. Values of electivity index E*, calculated only for categories with at least five individuals both in environment and diet.

Prey categories	Environment		Diet	
	Abundance (n)	%	Abundance (n)	%
Ephemeroptera + Plecoptera	653	53.9	90	37.0
Diptera adults	4	0.5	10	4.1
Diptera larvae	215	17.7	101	41.5
Oligochaeta	187	15.5	2	0.8
Trichoptera adults	–	–	2	0.8
Trichoptera larvae	100	8.3	13	5.3
Heteroptera	12	1.0	1	0.4
Coleoptera adults	12	1.0	4	1.6
Coleoptera larvae	9	0.7	16	6.6
Hirudinea	4	0.3	–	–
Mollusca	4	0.3	–	–
Collembola	3	0.2	–	–
Crustacea	2	0.2	3	1.2
Odonata	1	0.1	–	–
Araneae	1	0.1	–	–
Acarina	1	0.1	–	–
Hymenoptera	–	–	1	0.4
Total	1208	100	243	100
Shannon H	1.363		1.446	
Confidence limits 95%	1.295–1.496		1.292–1.553	

Larval salamander body size ranged from 23 to 31 mm (mean 28.1 ± 2.6) and the population consisted of a single cohort (data not shown). The environmental sampling provided 1,208 macroinvertebrates, classed in 15 taxa, while the stomach contents from 43 salamander larvae provided 243 invertebrates representing 11 taxa (Table 1) and resulting in a mean prey number per individual of 5.8 ± 4.0. The analysis of the relativized electivity index E* showed that salamanders positively selected only Diptera and Coleoptera larvae, while all other prey categories were apparently ignored (Table 2). The graphical analysis of the foraging strategy showed that the larval population behaved broadly as a generalist predator (no prey taxa were observed in the upper half of the diagram in Fig. 1), taking prey on the water surface as well as on the streambed. The dominant prey groups were Diptera and Ephemeroptera + Plecoptera larvae, with a high within-phenotype contribution in the overall trophic niche (Fig. 1). Regarding the analysis of individual diet variation, the TNW (1.45) was equally partitioned in WIC and BIC, resulting in a WIC/TNW ratio of 0.50 (p < 0.001) that, together with a mean PSi of 0.49 (p < 0.001), indicated that the proportion of specialised individuals within the population was significantly higher than expected from the null model (Fig. 2). The regression analysis, performed between the pairwise diet overlap and

Table 2. Values of individual specialization indices and values of electivity index E^* , calculated only for categories with at least five individuals both in environment and diet.

Individual specialization Indices		Electivity Index E^*	
Index	Value	Prey category	Value of E^*
TNW	1.446	Diptera larvae	0.39
WIC	0.723	Trichoptera larvae	-0.22
BIC	0.723	Ephemeroptera + Plecoptera	-0.19
WIC/TNW	0.500	Coleoptera larvae	0.37

the pairwise body size dissimilarity, revealed a highly significant correlation (Pearson's correlation coefficient -0.13 , $p < 0.0001$; slope -0.52 ± 0.12 , $p < 0.0001$). This result clearly shows that individuals with more similar diets were also more similar in body size and vice versa (Fig. 3).

This study considerably improves our understanding of the trophic strategy of a larval population of the fire salamander inhabiting a stream habitat. In particular, the larval population broadly behaved as generalist predators that were able to feed in the entire water column. This is

in accordance with WEITERE et al. (2004), while the trophic strategy was characterised by a high within-phenotype component, suggesting that most individuals consumed different arrays of prey types simultaneously (see Fig. 3e in AMUNDSEN et al. 1996). The dominant prey groups in the diet of the salamander larvae were Diptera larvae, which were positively selected, along with Coleoptera larvae; while larvae of Plecoptera + Ephemeroptera, which were the most abundant category in the environment, were apparently ignored. These results may be explained by differences in prey mobility and microhabitat, because Diptera larvae are slow-moving invertebrates abundant in slow-flowing stretches of streams (with a few exceptions corresponding to the families Blephariceridae and Simuliidae), while the larvae of Plecoptera + Ephemeroptera are fast swimmers that are able to evade predators (OBERRISER & WARINGER 2011) and concentrate in fast-flowing microhabitats of streams (DE CRESPIN DE BILLY & USEGLIO POLLATERA 2002). Furthermore, salamanders might prefer Diptera and Coleoptera larvae, because, being soft-bodied arthropods, they are more easily digested than other macroinvertebrates (COSTA et al. 2014).

Concerning the individual trophic specialization, both the WIC/TNW and the PSi indexes confirmed that the

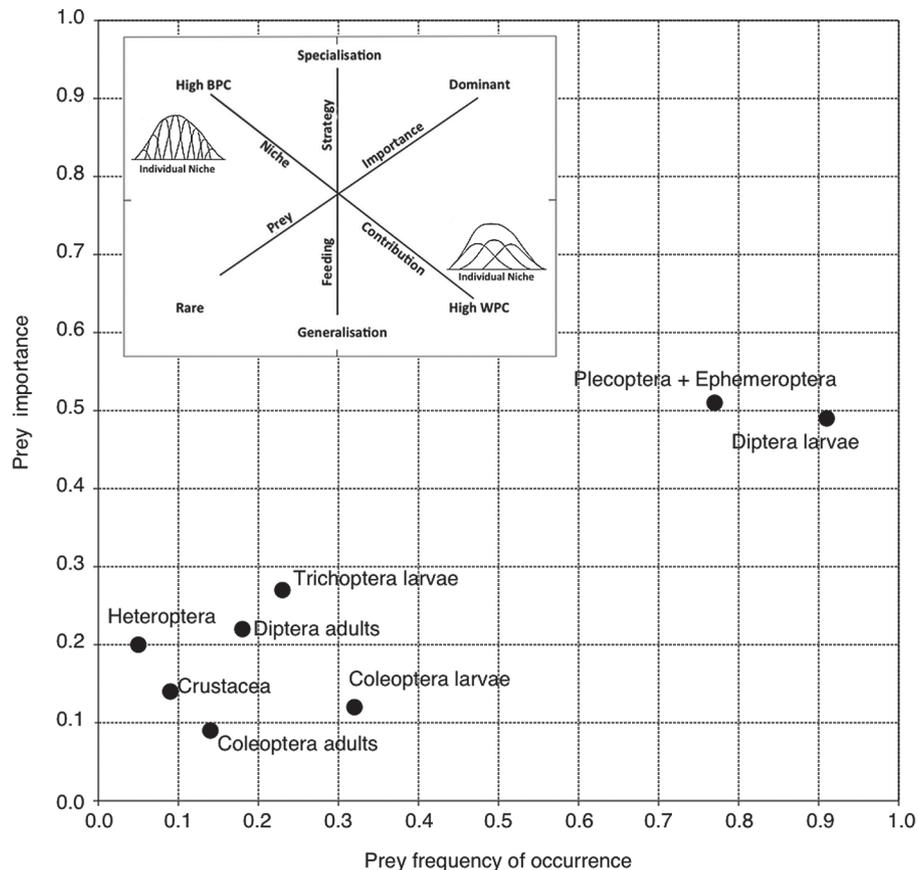


Figure 1. Graphical interpretation of a larval *Salamandra salamandra* population feeding strategy (AMUNDSEN et al. 1996). Only prey categories with at least 5 individuals in the diet are shown in the analysis.

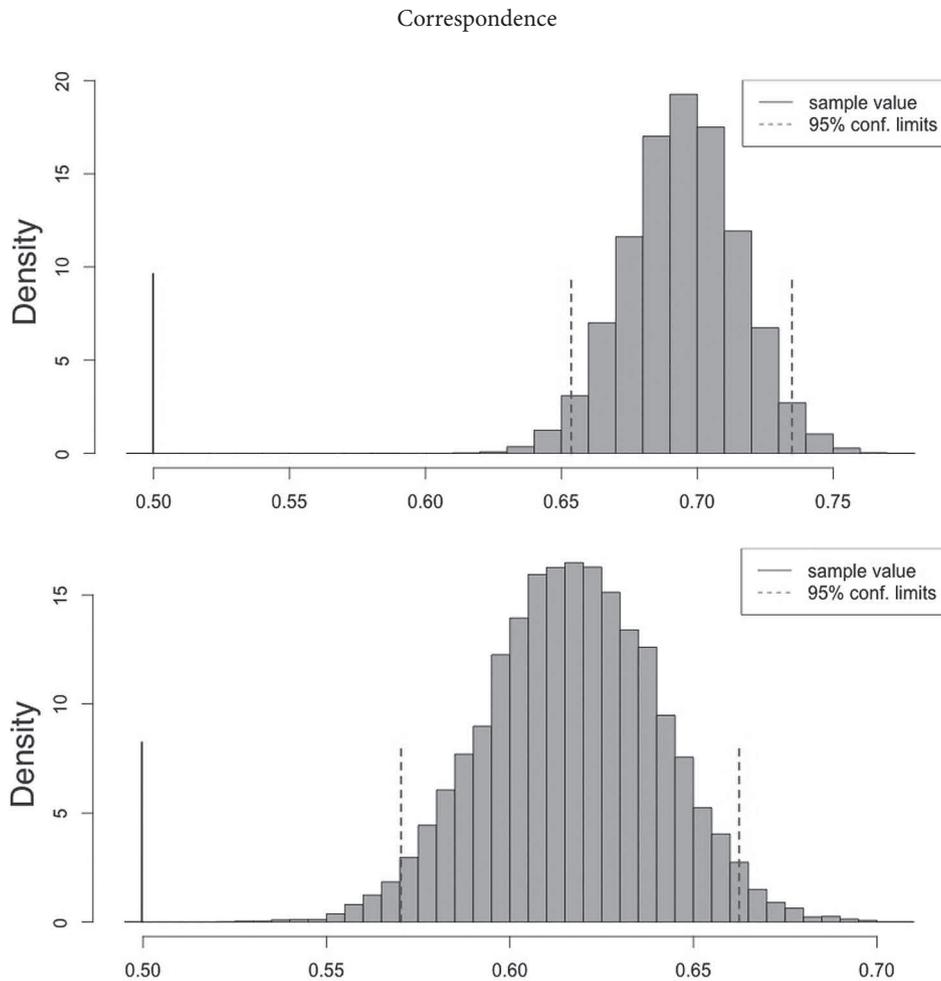


Figure 2. Histograms of distributions of within-individual component/trophic niche width (WIC/TNW) ratio and individual specialization (SI; i.e., mean PSi) indices obtained through the Monte Carlo resampling procedure. Vertical broken lines show the 95% confidence limits of the simulated distribution, while the vertical solid line shows the actual index value for the original data.

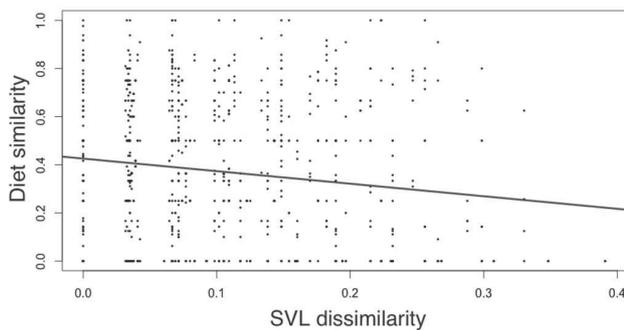


Figure 3. Linear regression between pairwise diet similarity and pairwise size dissimilarity.

study population was composed of many relatively specialized individuals that consumed different subsets of the entire prey population. This result confirms previous studies that analysed this issue in different animal groups (BOLNICK et al. 2002, 2003, ARAÚJO et al. 2011) and in particular in amphibians (COLLINS et al. 1993, MAERZ et al. 2006, SCHRIEVER & WILLIAMS 2013, SALVIDIO et al. 2015).

Finally, a relevant result was the highly significant negative relationship between salamander diet similarity and body size dissimilarity (Fig. 3). This finding clearly indicates that more similarly sized individuals had also more similar diets and thus may compete more intensively in the case of limited resources. This correlation between diet and morphology is predicted by the niche evolution theory (ROUGHGARDEN 1972, BOLNICK & PAULL 2009), but in fact has rarely been tested in natural populations and in particular in amphibian larvae. Larval growth rate and metamorphosis size are correlated, amongst others, to intraspecific larval competition, which is density-dependent (VITT & CALDWELL 2009), and different larval size cohorts probably reduce their feeding competition by adopting different diets.

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