Seasonal variation in the trophic niche of a heterochronic population of *Triturus alpestris apuanus* from the south-western Alps

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The aim of this study was to analyse the diet composition and feeding strategy of a heterochronic population of the Alpine newt, *Triturus alpestris apuanus* (Bonaparte, 1839), in a high-altitude pond in the south-western Alps, characterized by a reduced aquatic habitat and low environmental heterogeneity. The co-existence of both adult newt morphs in such a non-complex habitat with short suitable hydroperiod (water surface free of ice) may not be governed by different microhabitat selection or temporal activity, but could be due to diet composition differentiation among morphs. The diet of the newt was examined and compared with prey availability in summer and autumn. Both numerical and volumetric analyses of the gut contents showed that: 1) metamorphs represented a homogeneous group clearly linked to epineustonic prey categories (exogenous arthropods), whereas paedomorphs showed a prey selection mainly towards planktonic food categories; 2) evident seasonal variations in diet composition not connected to aquatic prey availability were observed in the studied forms; 3) metamorphs and paedomorphs may possibly avoid competition (if there is any) through food resource partitioning rather than microhabitat selection.

Key words: alpine newt, Amphibia, feeding strategy, high-altitude pond, Salamandridae

INTRODUCTION

In the absence of fish and snakes, newts are often the aquatic top predators in lentic temperate water bodies (e.g. Schabetsberger & Jersabek, 1995). In playing this ecological role in aquatic trophic webs, the food habits of newts are considered relevant in freshwater ecology and this topic has been investigated in depth by several authors (e.g. Joly, 1979; Griffiths, 1986; Griffiths & Mylotte, 1987; Joly & Giacoma, 1992; Schabetsberger & Jersabek, 1995). These studies were aimed at characterizing the diet of newt species as well as the interspecific relationships among related species assemblages, which are common in many pond habitats throughout Europe (Avery, 1968; Stoch & Dolce, 1984; Griffiths, 1986; Griffiths & Mylotte, 1987; Arntzen & De Wijer, 1989; Fasola & Canova, 1992; Joly & Giacoma, 1992; Braña et al., 1996). There is much evidence for the paraphyletic origin of the genus *Triturus* Rafinesque, 1815 and, although it has recently been subdivided into three genera (García-París et al., 2004), namely *Triturus*, *Lissotriton* Bell, 1839 and *Mesotriton* Bolkay, 1927, the taxonomic status of the genus is still controversial. Hence in this paper *Triturus* was maintained for all "*Triturus*" species. *Triturus alpestris* (Laurenti, 1768) is a polytypic species with a fragmented range (Zuiderwijk, 1997). The subspecies *apuanus* (Bonaparte, 1839) is widespread from the Ligurian Alps, along the whole Northern Apennines, and with discontinuities in the Rieti province and the central Tuscan hills (Andreone & Tripepi, 2006).

Mixed populations, with both metamorph and paedomorph individuals, were reported for some of the twelve European species of newts (Whiteman, 1994; Denoël et al., 2005; Denoël, 2006). Paedomorphosis is frequent in southern European populations of the Alpine newt, *T. alpestris*, including the subspecies *apuanus* (see Denoël et al., 2001 for a recent review). The maintenance of facultative paedomorphosis in this species is said to be favoured by both pond and surrounding habitat characteristics (abundance of prey and high energy intake; Denoël et al., 2001; Denoël & Poncin, 2001), as well as by eco-ethological features (resource partitioning among morphs, reproductive activity, early maturity; Denoël et al., 1999; Denoël & Joly, 2001; Denoël et al., 2001).

The trophic niche of *T. alpestris apuanus* populations, as single species or in syntopy with other newt species, has already been studied in distinct areas of the Northern Apennines (Ferracin et al., 1980; Fasola & Canova, 1992; Fasola, 1993; Denoël & Andreone, 2003). In particular, Fasola & Canova (1992) and Denoël & Andreone (2003), compared female and male food preferences of both paedomorphic and metamorphic individuals. As other newt species, *T. alpestris* is a polyphagous forager and its prey consists of a large variety of both aquatic and terrestrial animals, particularly arthropods (see Ambrogi & Gilli, 1998 for a synthesis).

In the Ligurian Alps and Prealps (Cuneo and Savona provinces – its extreme western range), the Italian alpine newt has been observed from near sea level to 1328 m a.s.l. (Andreotti, 1994), but occurs up to 1560 m a.s.l. (this study). In the present paper, the food habits of this newt were investigated in a population inhabiting a small alpine lake, which represents an uncommon habitat for this subspecies, usually distributed at a lower elevation.

It is generally believed that, in the presence of limited resources, the coexistence of different, phylogenetically related and morphologically similar organisms (as is the case with the two heterochronic morphs of the alpine newt) is possible through the use of different strategies that are not mutually exclusive: 1) microhabitat resource partitioning, 2) trophic resource partitioning and 3) temporal segregation. Alternatively, if habitat and trophic availability exceed the requirements of the organisms, no resource partitioning is present (e.g. see Schoener, 1974, 1977; Pianka, 1975; Toft, 1985; Griffiths, 1986). At the elevation of the study site (1560 m a.s.l.), it is likely that the reduced availability of suitable climate conditions (ice-free water surface is available to newts for only a few months per year) may represent a relevant constraint for newt activity in comparison with that of other *apuanus* populations. In theory, the coexistence of both adult newt morphs in heterogeneous habitats is made possible through different microhabitat selection or by different diel or seasonal activity time between morphs (Denoël & Joly, 2001; Denoël & Schabetsberger, 2003). In non-complex habitats, such as our study site, the coexistence of metamorphs and paedomorphs might not be governed by any resource partitioning (Denoël & Andreone, 2003), or it may be due to a certain degree of food resource partitioning among morphs, reflecting their capability for feeding on different prey types (Denoël et al., 2004).

The aim of this paper is to analyse the food habits of an alpine population of the *apuanus* subspecies, and to compare the food preferences of metamorphs of both sexes and of paedomorphs throughout the summer and autumn periods. We also wish to highlight the importance of considering possible seasonal variations in the diet composition of coexisting newt heterochronic morphs, a topic that has been poorly investigated up to now. Moreover, an estimate of the food availability at the pond site in the different seasons is also provided, given that an adequate knowledge of the temporal distribution and abundance of prey is relevant in drawing sound conclusions on the dietary strategies of these two types of newts (Avery, 1968; Griffiths, 1986).

MATERIALS AND METHODS

Study area

Lao Lake, 1560 m a.s.l., is positioned in the upper Tanaro valley (Cuneo province) (44°08'24"N, 07°51'32"E). This pond is fed by a small spring, about 300 m² in surface, with a maximum depth of about 1.5 m during the flood period (late spring season), and constitutes a mixed freshwater system together with a moss, some isolated small pools and a rivulet. This system is surrounded by a secondary alpine meadow in continuity with primary alpine prairies. The aquatic vegetation of the pond is very scarce with isolated shrubs of *Carex* and *Juncus* along the shoreline. Water is transparent only in the shallow areas; the chemical-physical characteristics recorded in August 1998 were: pH=7.8, water T=18 °C, redox potential=172 mV, conductivity=41 μS. From May to October (the study period), the water depth underwent minor fluctuations of about 1 m. The pond surface usually remains iced from about November to April, but the air and water temperature could occasionally reach 0 °C even in late October and in May (pers. obs.). This freshwater system is isolated from other suitable natural or artificial habitats, and the nearest known newt population is more than two linear kilometres away. The Italian alpine newt has never been found in the rivulet, while it is extremely abundant in the pond and uncommon in the small pools. No fish or other newt species are present in this aquatic system, and the grass snake, *Natrix natrix*, has rarely been observed, while the common frog, *Rana temporaria*, breeds in the small pools and in the stream.

Sampling methods

Six sampling sessions were carried out during periods when the pond surface was free of ice, on 20 May 2002, 31 July 2000, 3 August 1999, 7 August 2004, 10 October 2002 and 22 October 2004. Four newt morphs were recognized in Lao Lake during the sampling period: 1) male and 2) female metamorphs, 3) paedomorphs (genders were not differentiated in the analysis because of the difficulty in sexing with certainty) and 4) gilled larvae (not considered in the analysis of trophic niche). Newts were sampled with a fishing net (authorization from the Italian Ministry of Environment, DPN/2D/2003/2267), dragging the pond along the bottom from the centre to the shoreline. Sampling was conducted in order to collect, where possible, at least 10 specimens each of male and female metamorphs and 10 paedomorphs. Specimens (paedomorphs and metamorphs) were collected selecting a similar size (4.5–5 cm SVL) to avoid size-dependent biases in diet analysis. All the newts, immediately after capture, were subjected to a non-invasive stomach flushing procedure repeated until no further content came out (Legler & Sullivan, 1979; Joly, 1987; Leclerc & Courtois, 1993). Any food items still present in the oral cavity after flushing were carefully removed with entomological tweezers. Specimens were released in the pond after half an hour, once their regular activity was checked. The stomach contents were then preserved in ethanol solution (70%) and subsequently examined in the laboratory using a stereomicroscope. Food items were identified to the lowest taxonomical level possible using macrobenthos identification manuals (Sansoni, 1988; Campaioli et al., 1994), then photographed with a digital camera. Pictures of items were analysed by Image Tool 3.00 software (University of Texas Health Science Center, San Antonio) and measured, approximating their volume to that of a prolate spheroid [$V=4/3\pi$ (prey length/2)·(prey width/2)²] or of a cylinder [$V=$(prey width/2)² π (prey length)], according to the item's shape (Dunham, 1983). We included all the newts collected in the diet analysis. Pseudoreplication biases were alleviated by collecting all the specimens in the same day in each session. Frequency of occurrence (FO), and numeric and volumetric relative abundance (Pn and Pv respectively) were calculated for each prey category. A complete sampling of the aquatic fauna present in the pond was carried out in August 2004 and October 2004. Macro- and micro-invertebrates were collected in order to estimate the trophic availability and evaluate the degree of selectivity in foraging activity of the different newt morphs.

Samplings were conducted using a 0.335 mm (21/cm) mesh dragnet covering half of the pond's surface area proceeding from the shallow to the deep areas, applying the "kick sampling" method (see Rosenberg, 1978 and Storey et al., 1991 for a review) in order to collect bottom dweller invertebrates.

Data analysis

Cumulative-diversity curves (prey diversity plotted against the number of stomachs) were developed for both newt morphs to test whether the collected data were representative of the dietary spectrum of the population and to avoid intra-specific comparison being influenced by the effect of the sample size. All curves reached a plateau at a number of individuals less than those sampled.

The food niche breadth was calculated using Levins' (1968) index (B) and its standardized form (B_A) (Hurlbert, 1978):

$$B = \frac{1}{\left[\sum_i^n p_{ji}^2 \right]}$$

$$B_A = \frac{(B-1)}{(n-1)}$$

where p_{ji} is the proportion of morph j using prey i and n is the number of food categories found.

Prey niche overlap was estimated applying 1) Schoener's (1970) and 2) Pianka's (1973) indices in order to make our study comparable to previous studies on the same topic (Griffiths, 1986; Griffiths & Mylotte, 1987; Fasola, 1993; Denoël & Andreone, 2003):

1)
$$C = 1 - 0.5 \left(\sum_i |p_{xi} - p_{yi}| \right)$$

2)
$$O_{xy} = \frac{\sum_{i=1}^n p_{xi} \, p_{yi}}{\left(\sum_{i=1}^n p_{xi}^2 \sum_{i=1}^n p_{yi}^2 \right)^{1/2}}$$

where p_{xi} is the proportional utilization of prey i by morph x and p_{yi} the proportional utilization of prey i by morph y. Both indices range from 0 (no prey in common in diet spectrum) to 1 (same diet spectrum). Because of limitations of the niche overlap indices for arbitrary cut-offs (Feinsinger et al., 1981), we also compared the observed overlap values to an appropriate null model. The distribution of the null model was created using Ecosim software (version 7.68) (Gotelli & Entsminger, 2001), developing two simulations with 1000 randomized replications of the data set. The simulations were generated by using the randomization algorithm RA3 (the "scrambled-zeros"

randomization algorithm proposed by Winemiller & Pianka, 1990), whereby the entries in each row of the utilization matrix were randomly reshuffled for each iteration retaining the niche breadth of each species but randomizing which particular resource states are utilized. Statistical significance was determined by comparing the observed overlap value to the null distribution; an observed value greater than 95% of the simulated values indicates significant overlap at the α<0.05 level (Winemiller & Pianka, 1990).

The graphical technique developed by Costello (1990), modified by Amundsen et al. (1996), was used to represent the feeding strategy of the analysed morphs, as well as the inter-individual shifts in niche utilization. This method enabled us to graphically interpret the importance of dietary items (the first diagonal [/] represents abundance increase along with prey importance), the types of feeding specializations (the vertical axis represents predator strategy going from generalist to specialist), and the dietary niche-width of the morphs analysed (the second diagonal axis [\] represents resource use changing from between phenotype component, BPC – high diversity among individuals of population – to within phenotype component, WPC – tending towards the same resource use), by dividing the diet into its constituent components (Amundsen et al., 1996). These components are then graphed plotting frequency of occurrence (F.O.) on the X-axis and prey-specific abundance on the Y-axis. Prey-specific abundance (P_i) is defined as the proportion a prey item (i) includes all prey items in only those individuals in which prey i occurs (Amundsen et al., 1996).

Statistical analyses were performed to compare food habits among newt morphs. Because most of the data did not fit the normal distribution even following log and arcsin transformations, we used Monte Carlo analysis to compare numeric and volumetric data on food items recovered from the stomachs (in each comparison, the standard F-ratio is calculated as a value not determined indirectly, by looking up the theoretical value in a statistical table, but directly, through simulation – Gotelli & Entsminger, 2001). Factorial Analysis of Correspondence (FAC) was performed on numeric and volumetric data to evaluate how the different morphs differentially exploit the food resources. Food items were grouped into four homogeneous assemblages based on ecological characteristics (benthos, epineuston, neuston, plankton), in order to optimize the analyses (Joly & Giacoma, 1992). Moreover, a Multi Response Permutation Procedure (MRPP) was applied on the data distribution produced by FAC in order to evaluate the significance of inter-morph differences. The strategy of MRPP is to compare the observed intra-group average distances with the average distances that would have resulted from all the other possible combinations of the data under the null hypothesis. In order to evaluate the relationships between prey use and prey availability, Spearman rank correlations were performed. Obviously, positive values in Spearman correlation coefficients would mean a prey use by a given morph depending on its relative availability in the environment, whereas negative values would indicate the opposite trend. Statistical analyses, all tests being two-

Table I. Numeric and volumetric data for 24 prey categories eaten by the 240 specimens analysed. Relative abundances (P) of prey are shown based on numeric (N) and volumetric (V) data. Main food categories in italic. Symbols: ACA = Acara; AME = Amphibia eggs; ANE = Anellida; ANI = Anisoptera larvae; ARA = Aranea; BRA = Brachicera; BRL = Brachicera larvae; CLA = Cladocera; COA = aquatic Coleoptera larvae; COL = Collembola; COP = Copepoda; COT = terrestrial Coleoptera; EXA = undetermined Exapoda; FOR = Hymenoptera Formicidae; HEA = aquatic Heteroptera; HET = terrestrial Heteroptera; HOM = Homoptera; HYM = Hymenoptera; LEP = Lepidoptera; NEL = Nematocera larvae; NEM = Nematoda; NEP = Nematocera pupae; OST = Ostracoda.

	Males Pn (Pv)		Females Pn (Pv)		Paedomorphs Pn (Pv)		Availability[1] Pn	
	Summer	Autumn	Summer	Autumn	Summer	Autumn	Summer	Autumn
NEM	-	-	-	-	0.003 (0.003)	-	0	0.0188
ANE	-	-	-	-	0.003 (0.001)	-	0.0940	-
CLA	*0.674 (0.058)*	-	*0.561 (0.034)*	-	*0.665 (0.217)*	*0.211 (0.012)*	*0.7240*	*0.7393*
COP	-	0.286 (0.025)	-	0.046 (0.001)	0.005 (0.001)	0.046 (0.001)	0.0630	0.1081
OST	-	-	-	0.005 (0.001)	0.021	0.006	0.1140	0.1028
ARA	-	0.004 (0.006)	-	-	0.003 (0.003)	0.006 (0.001)	-	-
ACA	0.004 (0.001)	0.013 (0.001)	-	0.019 (0.001)	-	0.029 (0.001)	-	0.0053
COL	-	0.169 (0.008)	-	0.167 (0.002)	-	0.120 (0.001)	0.0005	0.0007
ANI	0.029 (0.141)	-	0.037 (0.007)	0.005 (0.109)	0.050 (0.1049	0.034 (0.455)	0.0009	0.0004
HEA	0.004 (0.023)	0.002 (0.015)	0.018 (0.022)	0.028 (0.015)	0.005 (0.012)	0.006 (0.338)	0.0020	0.0014
HET	0.007 (0.006)	0.002 (0.008)	0.043 (0.024)	0.009 (0.207)	0.003 (0.018)	0.006 (0.003)	-	-
HOM	0.044 (0.011)	*0.459 (0.650)*	0.012 (0.014)	*0.630 (0.313)*	0.008 (0.005)	*0.417 (0.080)*	-	0.0064
COT	0.015 (0.016)	0.007 (0.057)	0.024 (0.063)	0.019 (0.075)	0.005 (0.013)	0.029 (0.011)	-	0.0004
COA	0.004 (0.153)	0.002 (0.056)	-	-	-	-	-	-
LEP	-	-	0.018 (0.560)	-	-	-	-	-
NEP	0.044 (0.037)	0.002 (0.006)	0.055 (0.013)	0.014 (0.008)	0.131 (0.243)	0.011 (0.035)	0.0005	-
NEL	0.059 (0.039)	-	0.061 (0.009)	0.005 (0.001)	0.052 (0.105)	0.006 (0.001)	0.0005	0.0153
BRL	0.001 (0.056)	-	0.001 (0.006)	-	-	-	-	-
BRA	0.029 (0.001)	0.002 (0.008)	0.012	-	0.008 (0.008)	-	-	-
HYM	0.051 (0.327)	0.042 (0.062)	0.116 (0.171)	0.051 (0.266)	0.010 (0.032)	0.069 (0.046)	-	-
FOR	0.007 (0.024)	0.004 (0.046)	0.006 (0.002)	-	0.013 (0.181)	-	-	-
EXA	0.029 (0.110)	0.004 (0.051)	0.037 (0.074)	0.005 (0.004)	0.013 (0.052)	0.006 (0.018)	0.002	-
AME	0.001 (0.001)	-	-	-	-	-	-	-

[1]Terrestrial arthropods composing the epineustonic prey category are underestimated (see Methods).

tailed and with alpha set at 5%, were done using Statistica (StatSoft, Inc., 2001 – Version 6).

RESULTS

During the entire sampling period, 240 specimens were flushed: sampling session 1 – 30 specimens (10 of each morph); session 2 – 30 specimens (10 of each morph); session 3 – 30 specimens (15 male and 5 female metamorphs, 10 paedomorphs); sessions 4 and 6 – 60 specimens each (20 of each morph analysed); session 5 – 30 specimens (10 of each morph analysed). A total of 3291 prey items, belonging to 24 prey-type categories, were collected. A summary of the numerical and volumetric data for each prey category is given in Table I.

The Factorial Analysis of Correspondence (FAC) performed on total numerical and volumetric data grouped into four comprehensive food categories (excluding prey with frequency of occurrence <5%; see caption of Fig. 1 for food categories composition), based on the first two factors (71.63% of the explained variance), allowed us to spatially arrange the three newt morphs (for the sake of brevity, only an analysis of volumetric data is shown in

Fig. 1). The distribution of metamorphs and paedomorphs largely overlapped, although two different patterns can be discerned: the paedomorph plot distribution was influenced predominantly by planktonic food categories, whereas both metamorph sexes were linked primarily to prey belonging to epineuston and benthos. MRPP analysis performed on the distribution generated by FAC highlighted that newt morphs were grouped in different parts of the plot (δ_{obs}=1.60, P<0.001; MRPP analysis). Males and females were clustered together and constituted a homogeneous group (δ_{obs}=1.65, P=0.285; MRPP analysis), whereas paedomorphs represented a separate group, P (δ_{obs}=1.60, P<0.0001; MRPP analysis). In consideration of the FAC and MRPP analysis results, males and females were henceforth considered as a single group (M).

The stomachs of paedomorphs contained a significantly higher number of prey items (mean=26.58, n=62, SD=38.81) than metamorphs (mean=12.90, n=129, SD=16.07, pseudo-F=11.91, P<0.001; Monte Carlo procedure for ANOVA). In terms of prey volume, no differences were observed between newt morphs (M: mean=2.70 mm³, SD=5.33; P: mean=2.08 mm³, SD=13.57; pseudo-F=0.308, P=0.37).
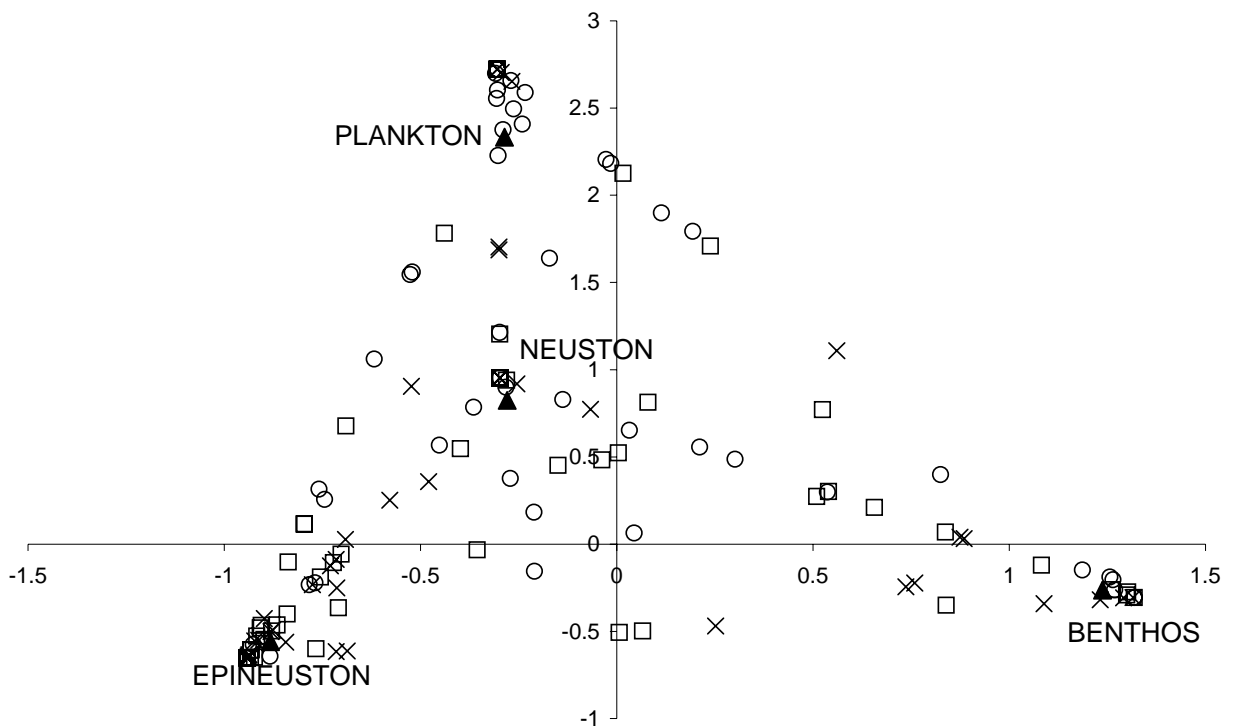
Fig. 1. Factorial Analysis of Correspondence (performed on the first two axes, explaining 71.6% of the total variance) analysing food spectrum of males (squares), females (crosses) and paedomorphs (circles) based on total volumetric data. Food items, represented by black triangles, are grouped in four categories on the basis of ecological characteristics (Benthos: Amphibia eggs, Anellida, Anisoptera larvae, aquatic Coleoptera larvae, Nematocera larvae, Nematoda; Epineuston: Acara, Aranea, Brachycera larvae and adults, terrestrial Coleoptera, Collembola, Formicidae, Homoptera, Hymenoptera, Lepidoptera, Nematocera adults, Neuroptera, Thysanoptera; Neuston: aquatic Heteroptera, Nematocera pupae; Plankton: Cladocera, Copepoda, Ostracoda).

Seasonal variations in diet strategy were observed in both metamorphs and paedomorphs. All the newts sampled in spring (May) presented empty stomachs. Metamorphs showed a significant trend in the increasing number of prey found in stomachs from summer to autumn (pseudo-$F_{1,78}$=5.713, $P<0.05$), whereas paedomorphs showed the opposite trend (but just above statistical significance) (pseudo-$F_{1,38}$=3.943, $P=0.06$). As regards the prey volume, both newt morphs showed the inverse seasonal variation trend observed for numeric data (for all tests: pseudo-$F\geq4.043$, $P<0.001$). In summer, the newt morphs fed on prey of different size (M>P; M: mean=5.44 mm³, $n=453$, SD=34.94; P: mean=0.80 mm³, $n=406$, SD=2.46; pseudo-F=6.877, P<0.001), whereas in autumn, metamorphs fed on prey smaller in size than paedomorphs (M: mean=0.94 mm³, $n=677$, SD=4.29; P: mean=4.44 mm³, $n=157$, SD=21.30; pseudo-F=15.624, $P<0.001$).

Costello graphics (based on numerical and volumetric data; Fig. 2) showed seasonal differences in both metamorph and paedomorph feeding habits. In summer (Fig. 2A), the dominant prey for metamorphs were terrestrial arthropods (particularly Homoptera), and three other food categories (Nematocera pupae and larvae, Cladocera) constituted a secondary exploited resource; metamorphs' niche breadth based on numeric and volumetric data was quite narrow (B_A=0.11 and 0.05 respectively). Paedomorphs showed a more generalist diet composition, feeding mainly on planktonic crustaceans and on four secondary food categories (terrestrial arthropods, immature nematocerans and dragonfly larvae), and thus their niche breadth was wider, particularly as regards to volumetric data (B_A number=0.10; B_A vol-

Table 2. Degree of seasonal diet spectrum overlap between adults and paedomorphs based on numeric and volumetric data. *P* values within brackets for Pianka's and Schoener's indices represent the tail probability that the observed overlap was greater than or smaller than expected by chance (average niche overlap in the simulated communities). N = number, V = volume.

Data		Pianka (O)		Schoener (C)	
		Observed	Simulated	Observed	Simulated
Summer	N	0.965	0.209	0.832	0.221
			(P<0.001)		(P<0.001)
	V	0.713	0.235	0.463	0.193
			(P<0.05)		(P<0.05)
Autumn	N	0.936	0.148	0.742	0.143
			(P<0.05)		(P<0.05)
	V	0.337	0.164	0.249	0.135
			(P=0.190)		(P=0.177)

ume=0.31). The remaining aquatic food categories were characterized by low scores of P_i and Freq. for both morphs. In autumn (Fig. 2B), both metamorphs (feeding almost exclusively on aphids fallen on the water surface) and paedomorphs fed mainly on terrestrial arthropods; however, cladocerans and dragonfly larvae represented a minor portion of the paedomorph diet. For both newt morphs, the autumnal niche breadth was extremely narrow, both numerically and volumetrically, and ranged from 0.03 to 0.09. The distribution of the remaining aquatic prey (high P_i score and low Freq.) showed a high diversity among individuals of the same morph (BPC pattern) in resource use.

The application of Pianka (O) and Schoener (C) indices showed a broad overlap in the diet spectra of metamorphs and paedomorphs (Table 2). The observed degree of overlap was significantly higher ($P<0.05$) than expected (mean values obtained from simulations) in summer (O and C indices, numeric and volumetric data) and in autumn (O and C indices, numeric data).

Aquatic prey availability did not change from summer to autumn (df=22; χ^2=6.650; P=0.998; chi-square test), whereas the presence of exogenous terrestrial arthropods found on the water surface increased from summer to autumn (pers. obs.). Spearman rank correlation analysis performed on prey utilization frequency of the newt morphs against the prey categories' availability revealed that in both seasons metamorphs exploited aquatic food resource independently of prey availability (for both analyses $r\leq0.35$; $P\geq0.31$), whereas paedomorphs were
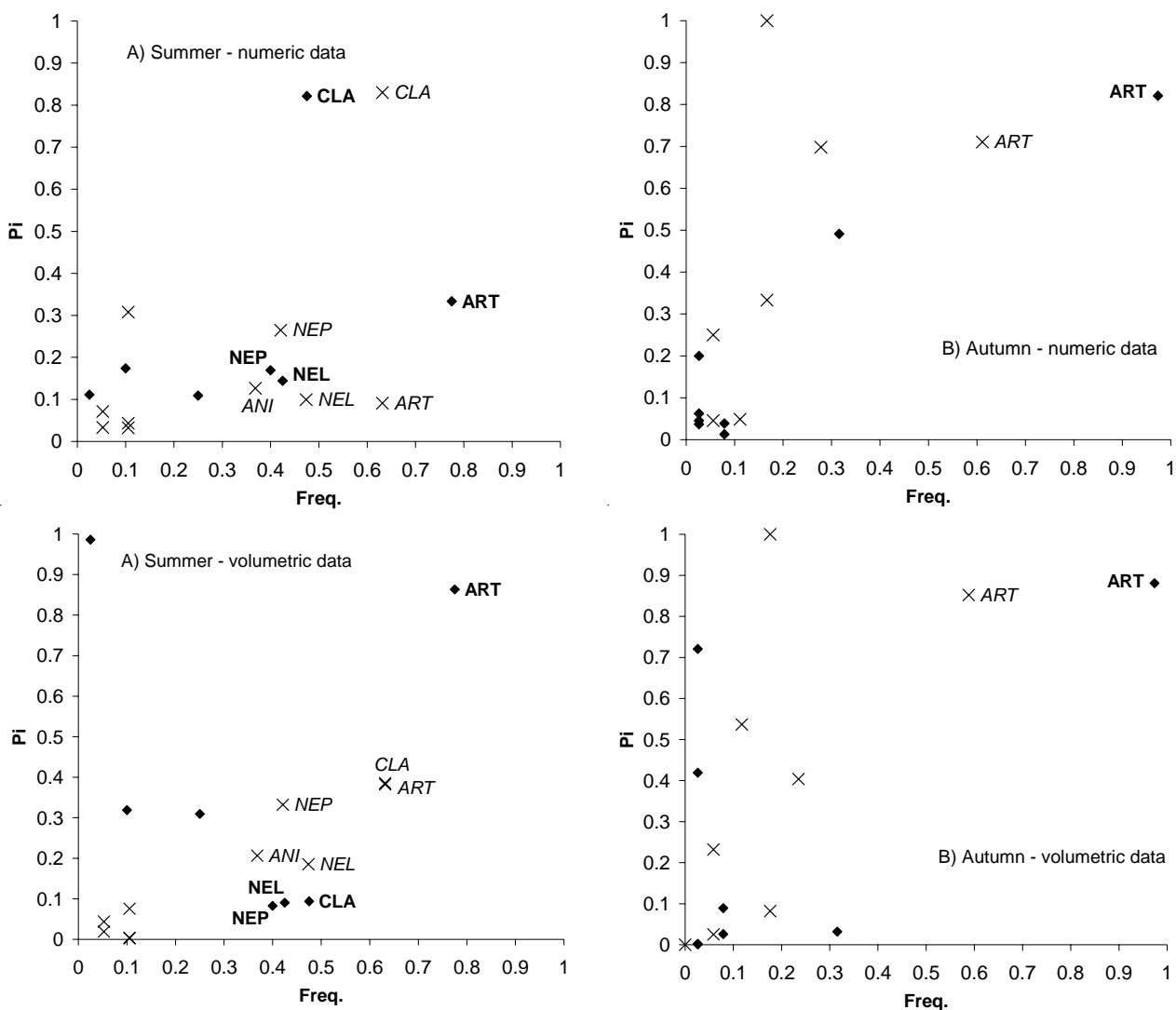

Fig. 2. Costello graphic visualization modified by Amundsen et al. (1996) showing trophic strategies of metamorphs (black diamonds and bold labels) and paedomorphs (crosses and italic labels). Analysis was performed on summer (A) and autumn (B) sampling sessions using both numeric (n) and volumetric (v) data. Pi = prey relative importance; Freq = frequency of occurrence. See Materials and Methods for the interpretation of the graphics. Food categories with low frequency of occurrence or Pi not shown for clarity. ANI = Anisoptera larvae; ART = terrestrial arthropods (Aranea, Acara, Collembola, Thysanoptera, Neuroptera, terrestrial Heteroptera, terrestrial Coleoptera, Lepidoptera, Hymenoptera, Nematocera adults, Brachicera); CLA = Cladocera; NEL = Nematocera larvae; NEP = Nematocera pupae.

marginally non-selective ($r \geq 0.59$; $P \leq 0.07$) and had a propensity to devour aquatic prey according to their relative abundance.

Assuming that the study pond lacks microhabitat heterogeneity at the scale of newt foraging range, the only hypothesis that could explain the observed intra-morph variability (BPC pattern in Costello analysis too high to be addressed exclusively to the normal inter-individual variability) in individual exploitation of aquatic resources is a non-homogeneous distribution of these prey in the pond. Considering the average eco-field (Farina & Belgrano, 2004) of aquatic prey (significantly lower than that of the newts), the study pond may represent a heterogeneous environment that allows for a different microhabitat use (i.e. shallow waters vs. open waters) determining their lumped distribution. Thus, newt diet composition (excluding terrestrial arthropods that were actively preyed on water surface) could be influenced by the individual position in the pond that determined differences in aquatic prey category accessibility and in inter-individual food resource exploitation. To test this hypothesis, PCAs analyses (based on log(1+x)-transformed total and seasonal prey volumetric data) were performed on each newt morph. The analyses allowed the spatial arrangement of the individuals of both morphs in multi-clustered distributions (at least three clusters per morph) (for all analyses: explained variance on the first two factors $\geq 59.8\%$; eigenvalues ≥ 8.75 and 3.26 respectively), indicating different shared patterns of diet composition among individuals within the same morph.

DISCUSSION

In terms of prey number, the comparison among morphs indicated a higher average prey number per stomach in paedomorphs than in metamorphs, as observed by Denoël & Andreone (2003) in an Apennine population. This difference is related to the selection by paedomorphs of prey types of smaller size (particularly cladocerans). Male and female metamorphs represented a homogeneous group clearly linked to epineustonic non-aquatic prey, whereas paedomorphs tended to forage on planktonic food categories. No main prey categories were exclusive to the diet of one morph. Both morphs showed a mixed feeding strategy, with high inter-individual variations where some individuals were stenophagous and others presented a more generalized diet.

If we consider the eco-field (Farina & Belgrano, 2004) of a newt, the structural characteristics of the study pond represent a simple and non-diversified aquatic habitat. In these conditions, the coexistence of different morphs may not be determined by different microhabitat selection, but could be related to diet composition differentiation among morphs. Our null model analysis highlighted significant overlap in the diet composition of the study morphs. Moreover, despite the observed overlap of the food spectra, metamorphs and paedomorphs exploited some prey categories in a different way (i.e. epineustonic and planktonic food categories respectively). This evidence could be interpreted as an overall shared food resource use with quantitative differences in the exploitation of some prey categories. The dietary discrepancies between metamorphs and paedomorphs appear to be related to the different feeding mechanisms of the two alternative morphs, being linked to different morphological traits (Joly, 1981; Denoël, 2004; Denoël et al., 2004) that sustain their coexistence and maintain heterochrony. Similar results were obtained by Denoël (2003, 2004), Denoël & Schabetsberger (2003) and Denoël et al. (1999, 2001, 2004), who studied several *T. alpestris* populations of different subspecies inhabiting deep high-altitude lakes. On the contrary, when specifically considering the *apuanus* subspecies, other authors did not find any significant prey strategy differentiation between morphs, but only differences in prey-size selection (Fasola & Canova, 1992; Fasola, 1993) or in number of prey per stomach (Denoël & Andreone, 2003). All these studies carried out in the Apennines involved *T. alpestris apuanus* populations inhabiting very small and non-complex ponds at low or middle altitudes. Denoël & Andreone (2003) inferred that in *T. alpestris* populations inhabiting deep high-mountain lakes (Denoël & Joly, 2001; Denoël et al., 2004), the presence of a vertical habitat component permits microhabitat partitioning and a consequent differentiated foraging strategy between morphs. At our study site, an alpine lake (1560 m a.s.l.) with reduced and homogenous aquatic habitat, probably devoid of a vertical component and where the climate represents a relevant constraint for newt activity, newt morphs could not avoid competition (if there were any) through microhabitat partitioning. However, a significant difference in the food spectrum between morphs was observed. As reported above, the main discrepancy in the diet spectrum among morphs is due to the preference of metamorphs for terrestrial arthropods, whereas in the pond studied by Denoël & Andreone (2003) this food category was very rare. This probably determined the lack of specialization of metamorphs towards non-aquatic prey and the high degree of overlap in food spectrum between morphs.

Few studies on the alpine newt have investigated diet composition throughout the seasons (Fasola & Canova, 1992; Denoël & Joly, 2001), but no one has discussed the seasonal variations and comparisons between morphs in detail. At our study site, in spring (May) newts had not yet started their feeding activity. At that time, the water surface had just thawed completely, and possibly the beginning of newt feeding activity had been delayed after the ice had melted. Clear seasonal variations in diet composition and strategy were observed in both newt morphs: the diet of metamorphs shifted towards a smallness–numerousness diet strategy from summer to autumn due to increased consumption of homopterans, which in October represented 50.5% of the metamorphs' diet spectrum in terms of prey volume. Although the average size of aphid individuals is smaller than that of planktonic organisms, they constituted a more energetic food than cladocerans, copepods and ostracods (Sage, 1982; Ward & Robinson, 2005), and the metamorph seasonal diet change could be related to the increased availability of exogenous arthropods and the need for storing up energy for over-wintering. Paedomorphs have the same need, but their oral morphology did not allow

them to prey easily on epineustonic organisms (Denoël, 2004), and thus they adopted a mixed feeding strategy: paedomorphs augmented their aphid consumption in autumn according to increased availability, and at the same time intensified their selection on aquatic benthic insect larvae (i.e. Anisoptera and Nematocera), whereas small crustaceans were consumed at a lower rate.

At the study site, predation on newt eggs was not observed (only one case), as reported for *T. alpestris* populations studied by Schabetsberger & Jersabek (1995) and Denoël & Joly (2001), whereas this food category assumed a relevant proportion in the diet composition in other alpine newt populations (Denoël & Andreone, 2003; Denoël & Schabetsberger, 2003). It was hypothesized that this food habit could be regulated by population density (Denoël & Andreone, 2003), and consequently it might be considered as an adaptation linked to the peculiar condition of single populations.

As regards aquatic food categories, the observed newt diet composition and seasonal differences in the feeding strategy were either slightly linked (paedomorphs) or not linked at all (metamorphs) to prey availability. Aquatic prey distribution in Costello visualization and intra-morph PCA analyses revealed an individual stochastic diet composition for both morphs, strongly influenced by the foraging position in the pond and determined by a non-homogeneous aquatic prey distribution.

The overall observed diet composition patterns suggest distinct feeding strategies for the two newt morphs: 1) metamorphs are highly specialized towards exogenous prey categories actively captured on the water surface, whereas other prey are consumed occasionally and stochastically, revealing an opportunistic use of those resources; 2) paedomorphs are characterized by a less specialized diet, consuming crustaceans (being morphologically adapted to prey on them) and terrestrial arthropods (more energetic but less easy to capture) in a proportion depending on the abundance of the latter, and showed an opportunistic strategy as regards the remaining aquatic categories.

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