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A Midsummer Night's Diet: Snapshot on Trophic Strategy of the Alpine Salamander, *Salamandra atra*

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Abstract: Information on the trophic ecology of the Alpine salamander, *Salamandra atra*, is scattered and anecdotal. We studied for the first time the trophic niche and prey availability of a population from an area located in Italian Dolomites during the first half of August. Considering that *S. atra* is a typical nocturnal species, we collected food availability separately for diurnal and nocturnal hours. Our aims were: (i) to obtain information on the realized trophic niche; (ii) to provide a direct comparison between trophic strategy considering only nocturnal preys or considering all preys; (iii) to study trophic strategy of this species at the individual level. In two samplings nights we obtained prey from 50 individuals using stomach flushing technique. Trophic strategy was determined using the graphical Costello method and selectivity using the relativized electivity index. During the short timeframe of our sample, this salamander showed a generalized trophic strategy. The total trophic availability differed significantly from nocturnal availability. Interindividual diet variation is discussed in the light of the optimal diet theory. Finally, we highlighted that considering or not the activity time of the studied taxon and its preys may lead to a conflicting interpretation of the trophic strategies.

Keywords: amphibians; feeding ecology; individual specialization; resource selection; salamanders

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1. Introduction

The ecological role of salamanders is often overlooked, despite the fact they can act as top predators in certain trophic webs [1]. They may represent a significant part of vertebrate biomass in North American ecosystems [2], and can reach high densities in North American [3] and European forests as well [4,5]. Salamanders mainly prey on invertebrates and play a key role in nutrient cycling [6]. Furthermore, those species characterized by a biphasic life cycle are also an important energy exchange vector among different habitat types [1]. For these reasons, dietary studies on salamanders are an indispensable tool for assessing their ecological role, and for planning future conservation measures [7]. At population level, the effect of the diet, together with other ecological factors and variables such as climate, predation, human pressure, stress, and disease (e.g., [8–11]), is considered of paramount importance to determine animals' abundance [12]. Considering the use of the trophic resources, populations may be assessed as generalist if they are composed by individuals feeding to the environmental availability of prey proportionally or as specialist if individuals select only a limited array of the available resource categories [13]. Amphibians in general, and salamanders in particular, are often seen as generalist, opportunistic predators that feed on a large range of prey [14]. However, some studies highlighted how salamanders actively select prey and may show diet specialization, at least under particular environmental conditions [15,16]. Moreover, while the realized trophic niche may result as the outcome of a generalist feeding strategy in a given population, it could be actually composed of specialist individuals consuming different resources [13,17]. Patterns of individual specialization, or interindividual diet variation, occur in hundreds of cases and in many taxa [18]. Interindividual diet variation indeed was observed in salamanders too, both at the postmetamorphic [16,19–21] and at the larval stage [22]. Individual specialization in salamanders is usually inferred by cross-sectional data, but also with longitudinal studies [20].

The Alpine salamander, *Salamandra atra* Laurenti 1768, is a widespread terrestrial salamander occurring in the Central and Eastern Alps, and in the Dinaric Alps where some isolated populations may be found [23,24]. Salamanders are abundant vertebrates in some environments and contribute to ecosystem resilience–resistance in several ways. One of these concerns their role as predators. The goal of this paper is to elucidate some aspects of the *Salamandra atra* as a predator in an alpine environment. There are few and mainly observational studies [25–28] on the feeding habits of the focal species, which were reviewed by Kuzmin [29]. Therefore, information on its trophic strategy is scattered and anecdotal [23,25–29]. So far, no fully quantitative information on the Alpine salamander diet comes from a single study [30]; that, however, was performed without taking into account trophic availability and feeding strategies. In the present paper, although during a short time frame about in the midseason of activity, we focused on the trophic strategy of this species at three different levels. At the top level, we defined the realized trophic niche by analyzing the mutual proportion of the preyed taxa. At the second level, we studied the trophic strategy in terms of prey selection, considering relationships between preyed taxa and their environmental availability, also taking into account the mainly nocturnal behavior of the Alpine salamander. Finally, we studied the trophic strategy of this species at the individual level.

2. Materials and Methods

2.1. Study Species

The Alpine salamander, *Salamandra atra*, is a fully terrestrial and viviparous salamander [24]. Mixed coniferous and deciduous forests, alpine meadows, and rocky tundra-like areas, mainly on limestone substrates, are the typical habitats. Activity pattern is concentrated in the warmest months (April–October) while in the rest of the year the salamanders are inactive at the ground surface [28].

2.2. Study Area

The study area (about 4000 m^2) is located in the Paneveggio–Pale di San Martino Natural Park (Northern Italy), at about 1850 m a.s.l., near the locality Malga Venegiotta (municipality of Tonadico; 46°18′48″ N, 11°48′53″ E). At macroscale, the area is characterized by open habitats (pastures, other grasslands, and rocky areas) mixed with coniferous woodland, which is dominated by European larch (*Larix decidua*) and Norway spruce (*Picea abies*). Following the fourth level Corine Land Cover nomenclature, the study area is classified as "Coniferous forests with discontinuous canopy on mire" (habitat 3.1.2.4). At smaller scale, the study area, where the sampling of salamanders and invertebrates was performed, is homogeneous and characterized by some coniferous trees mixed with small open habitat, such as rocks, grass, and dwarf shrub cushions dominated by *Erica carnea*. This site is especially suitable for sampling predators since at this place the detection probability of salamanders is high, as shown by a previous study [5].

2.3. Prey Availability (Potential Trophic Niche)

Ground-dwelling invertebrates were sampled by 20 pitfall traps. Each pitfall trap (500 cm^3) was partially filled with a killing/preserving solution (salty water with and addition of 500 mg of benzoic/acetic acid) [31,32]. Pitfall traps were active for four days immediately after salamander sampling. They were divided in two typologies: 10 diurnal traps (DT, which were active from 7:00 a.m. to 7:00 p.m.) and 10 nocturnal traps (NT; which were active from 7:00 p.m. to 7:00 a.m.). DT and NT were inactivated by covering them with a plastic lid when they did not have to capture invertebrates. Pitfall traps are widely used to measure diversity and abundance of ground-dwelling invertebrates, e.g., [33]. These traps, although they may overestimate mobile fauna [33,34], it is reasonable to assume that such bias would not be misleading in the assessment of prey availability since prey mobility increases the detection probability by amphibians [35,36]. Traps were placed in 10 different trapping points within the salamander sampling area separated by a minimum distance of 30 m. In each trapping point, a pair of two pitfall traps (one DT and one NT) was placed at 20–50 cm from each other. Thus, in each trapping point we were able to sample diurnal and nocturnal invertebrate separately. To prevent the accidental fall of the salamanders in the traps, a 20 mm-mesh rigid plastic net was placed at the entrance of the traps when they were activated. Invertebrates obtained from environmental sampling and from stomach contents were sorted, identified, and counted using a dissecting microscope and taxonomic keys. Since invertebrates obtained with stomach flushing are partly digested, all invertebrates, both from stomach contents and from environmental sampling, were generally determined at the Order level or higher, annotating the life stage (i.e., we distinguished larvae from adults) and radical differences in locomotion type (e.g., flying Hymenoptera were distinguished from walking ones, e.g., ants).

2.4. Sampling Predators

Sampling of salamanders occurred within an area of about 4000 m^2 in the first half of August 2018. Salamanders were sampled following rain and while active on the floor during two consecutive nights. They were transported to the laboratory, 5.5 km from the sampling site. Stomach contents were obtained by stomach flushing [37,38] performed by a single person using a 5 mL syringe [one injection per salamander] and a flexible soft plastic tube and preserved in 70% ethanol. Since there is a significant increase in digestion rate with increasing temperature [39], salamanders were stored at 5 °C in a refrigerator and they were flushed within three hours from capture [40,41]. A removal approach was used to avoid recaptures of the same individuals. Salamanders were photographed with a digital camera situated perpendicular to the dorsal surfaces of the animals. Digital photographs of salamanders were imported into the ImageJ® software program to measure their total length (TOTL, distance from the tip of the snout to the end of the tail). Sexes were distinguished by analysis of external secondary sexual characters; adult males have a prominent, swollen cloaca and are more slender than females. According to Klewen [27], we considered as "juveniles", which were excluded in the present study, those individuals without evident external secondary sexual characters and a TOTL smaller than 90 mm. All salamanders were returned to their original site within a maximum of 30 h from their capture.

2.5. Data Analysis

2.5.1. Realized Trophic Niche

The sex differentiation in diet was analyzed by means of analysis of similarity [ANOSIM], based on Bray–Curtis distance [42]. The diversities of prey taxa in salamander stomachs and in the environment, as well as the diversity of prey taxa in salamander stomachs of our population and the two populations studied by Fachbach et al. [30], were estimated through Simpson's index [1-D] and 95% confidence limits calculated by bootstrapping [43]. In fact, although our method (stomach flushing) and that used by Fachbach et al. ([30], stomach dissection) are different, these two methods provide comparable results [44]. Analyses were performed in the statistical package PAST [45]. Considering that prey availability is generally calculated on invertebrates captured within 24 hours for a few days, we compared the results of diurnal and nocturnal traps and the results of nocturnal traps versus the pooled results (i.e., diurnal plus nocturnal preys) by means of the diversity permutation test [9999 permutations].

2.5.2. Trophic Strategy

The use of prey types in relation to their abundance in the environment was estimated by means of the Vanderploeg and Scavia [46] relativized electivity index (E^*), which is strongly supported by comparative evaluations [47]:

$$E^* = (W_i - 1/n)/(W_i + 1/n)$$

where $W_i = (r_i/p_i)/(\Sigma\ r_i/p_i) - 1$, r_i is the relative abundance of prey i in the diet, p_i is the relative abundance of prey i in the environment, and n is the number of prey types. This index ranges from +1 (positive selection) to −1 (avoidance), while $E^* = 0$ indicated that prey items were consumed according to their availability. Since the index is particularly sensitive to the categories of prey with reduced environmental availability, and considering the low number of individuals within each prey taxon in our samples, the threshold electivity value (u) was considered only for prey type with more than three trapped individuals, calculating the fifth percentile of the absolute values of E* [15,48]. The trophic strategy of the Alpine salamander was also analyzed with a modification of Costello's graphical representation [49,50]. According to this method, each prey type is plotted on a graph in which the x-axis is the prey frequency of occurrence (FO) in the predators' stomachs, and the y-axis is the prey-specific abundance (P_i), defined as the proportion of prey items (i), considering only all the prey items found in the individuals that consumed that specific prey type [50]. This graphical approach gives insights on the population feeding strategy: specialized (when some prey taxa have high P_i values and are projected in the upper part of the plot) vs. generalist (when all prey taxa have low P_i values and are projected in the lower part of the plot).

2.5.3. Interindividual Diet Variation

Interindividual diet variation for the study population was assessed by means of network analysis [18,51]. Within this approach, the interactions between individuals and resources are interpreted as a bipartite network where two sets of nodes, one representing individual salamanders and one representing prey types, are connected by links reflecting the utilization of each prey type by individuals [52–54]. Individual specialization with bipartite network is often investigated with qualitative data [52,54] that only represent the use of a resource. The use of weighted networks where the frequency of use of each resource is retained, however, may give better estimates of some network metrics; therefore, we decided to use this approach [53,55]. Within our network approach, we employed the degree of diet variation E, as proposed by Araujo et al. [51] to quantify the presence of interindividual diet variation. This index is based on the pairwise diet overlap between individuals and increases from 0 to 1 in presence of individual specialization [18,51]. Two other network metrics were calculated: nestedness and modularity. Nestedness is observed when individuals with the narrowest trophic niche consume a subset of the prey types used by the more generalist individuals. The latter is recorded when, within a population, it is possible to segregate some individuals in groups (modules) that share the same resources. We used a metric of nestedness based on overlap and decreasing fill (NODF; [56]), which ranges from 0 to 100 (minimum and maximum nestedness, respectively). Modularity Q was measured and modules within the population were identified (function computeModules in the R package Bipartite) using the Beckett's algorithm [57], which ranges from 0 to 1 (minimum and maximum modularity, respectively). Since some level of both specialization degree, nestedness and modularity may arise from stochastic processes and sampling bias, significance of these metrics was tested by comparing the observed value against the simulated distribution obtained from a null model with 9999 (999 for modularity) resamplings.

3. Results

3.1. Prey Availability (Potential Trophic Niche)

During this study, 19 taxa of invertebrates were captured for a total of 650 individuals, of which 395 and 255 individuals were captured in the diurnal and nocturnal traps, respectively (Table 1).

Table 1. Environmental availability of invertebrates and number of invertebrates preyed by *Salamandra atra* in the study site.

Invertebrate Taxa	**Preyed Invertebrates**	**Environmental Availability of Invertebrates**		
	Stomach Contents	***Diurnal Traps (DT)***	***Nocturnal Traps (NT)***	***Pool captures (DT + NT)***
Arachnida	12	37	17	54
Chilopoda	17	5	3	8
Coleoptera	13	23	6	29
Coleoptera larvae	8	6	7	13
Collembola	3	74	101	175
Dermaptera	0	1	1	2
Diptera	3	66	12	78
Diptera larvae	38	0	1	1
Formicidae	0	93	61	154
Hemiptera	0	13	6	19
Isopoda	9	6	14	20
Lepidoptera	0	2	1	3
Lepidoptera larvae	6	1	1	2
Mecoptera larvae	0	0	5	5
Mollusca	19	2	17	19
Oligochaeta	6	1	1	2
Orthoptera	0	2	0	2
Rynchota	0	2	0	2
winged Hymenoptera	2	4	1	5

Diversity index (Table 2) did not differ significantly between diurnal and total prey availability (i.e., DT + NT) (diversity permutation test, $p = 0.91$) while the difference between nocturnal and total prey availability was highly significant (diversity permutation test, $p < 0.01$). The analysis of similarities (ANOSIM) between total and nocturnal prey availability also showed significant differences (R = 0.29; $p < 0.01$).

Table 2. Diversity index of the available prey.

Simpson Diversity Index	**1-D (95% C.I.)**	**Taxa**
Diurnal traps (DT)	0.82 (0.80–0.83)	17
Nocturnal traps (NT)	0.76 (0.72–0.80)	17
Pooled captures (i.e., DT + NT)	0.82 (0.80–0.83)	19

3.2. Sampling Predators

Fifty adult salamanders (i.e., with total length longer than 90 mm) were captured (26 females, 24 males) and stomach flushed with 41 positive, 4 individuals without prey in stomach, and 5 individuals with only indeterminate items (portions and fragments of prey unrecognizable, which were not attributable to an exact number of prey). By sorting of stomach contents, 176 invertebrates were obtained with a total of 139 analyzable items (n = 26 indeterminate, n = 11 parasite nematode) with an average of 2.78 ± 5.05 preys/stomach (mean ± s.d.; n = 45; range 0–34. Parasite nematodes were excluded) (Table 1).

3.3. Realized Trophic Niche

There was no overall difference in the diet composition between the sexes (ANOSIM, n = 41; global $R = -0.049$, $p = 0.882$). The analysis of the trophic niche, using the modification of Costello's graphical method [49,50], showed that *Salamandra atra* exhibited a generalized trophic strategy (Figure 1). Almost all prey categories are located in the left lower part of the graph, with both FO and $P_i < 0.50$, with only Diptera (fly) larvae located in the upper left quadrant (Figure 1). Fly larvae are the most abundant prey category in the diet, but they are eaten by a low number of individuals (FO = 0.097), thus suggesting a generalized trophic strategy at the population level.

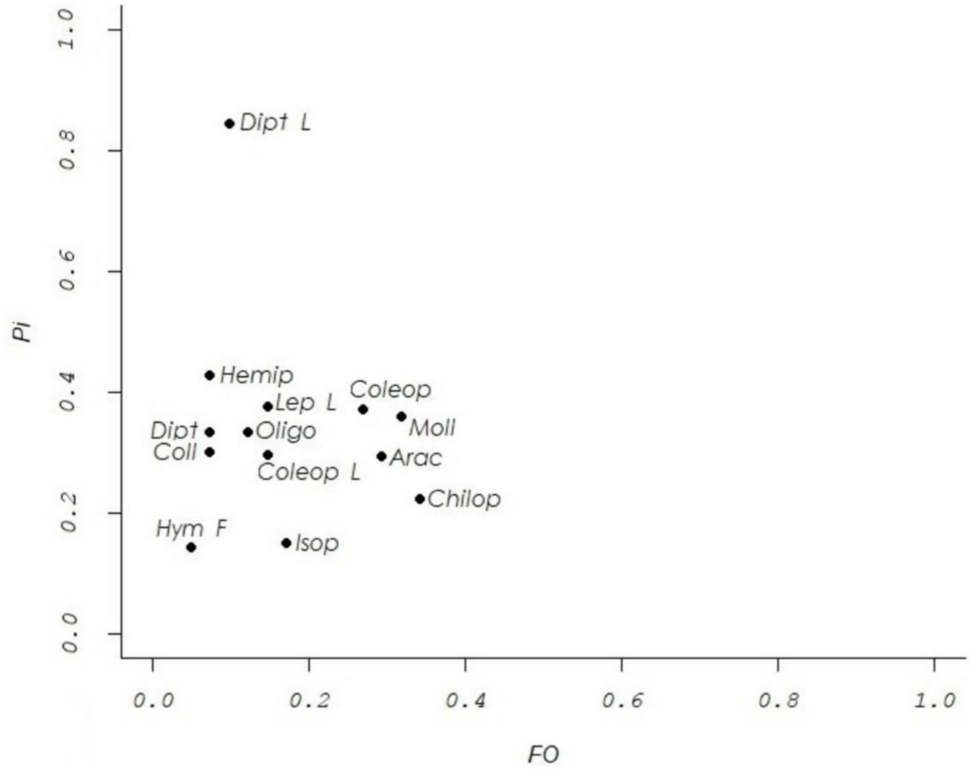

Figure 1. Modified Costello's plot [49,50], describing the trophic strategy of *Salamandra atra*. Legend: Arac = Aracnidae, Chilop = Chilopoda, Coleop = Coleoptera adults, Coleop_L = Coleoptera larvae, Coll = Collembola, Dipt = Diptera adults, Dipt_L = Diptera larvae, Hemip = Hemiptera, Hym_F = flying Hymenoptera, Isop = Isopoda, Lep_L = Lepidoptera larvae, Moll = Mollusca, Oligo = Oligochaeta.

The comparison of prey percentage in stomach of Alpine salamanders from our population and those of Fachbach et al. [30] is reported in Figure 2. Although some prey taxa were exclusive of a given population (e.g., Balttoidea, Dermaptera, Rhynchota), in the whole these three populations showed very similar diversity indices of the prey taxa (Simpson's index, 1-D, with 95% confidence limits, CL. Population 1: 1-D = 0.86, CL = 0.86–0.90; Population 2: 1-D = 0.85, CL = 0.83–0.90; Population 3: 1-D = 0.86, CL = 0.83–0.90).

3.4. Analysis of Trophic Strategy

Electivity index *E** was calculated using both nocturnal (Figure 3) and total (Figure 4) trophic availability. In both cases, *E** was negative for Hemiptera, Isopoda, Diptera, Aracnida, and Collembola, positive for Chilopoda, and proportional to environmental availability for Coleoptera larvae. Conversely, Mollusca resulted positively selected considering the total trophic availability, but they were randomly selected in the nocturnal availability. Worth noting is that E* provided completely opposite results for Coleoptera, which were negatively selected considering total availability but positively considering only nocturnal traps. Therefore, the two trophic availabilities (nocturnal and diurnal), when analyzed separately provided different or even an opposite pattern of prey's selectivity. These different results arised also considering the two main prey types, as shown by the realized trophic niche. Finally, salamanders operated a strong negative selection on the more representative categories in the trophic availability, which are Collembola, Diptera, and Formicidae.

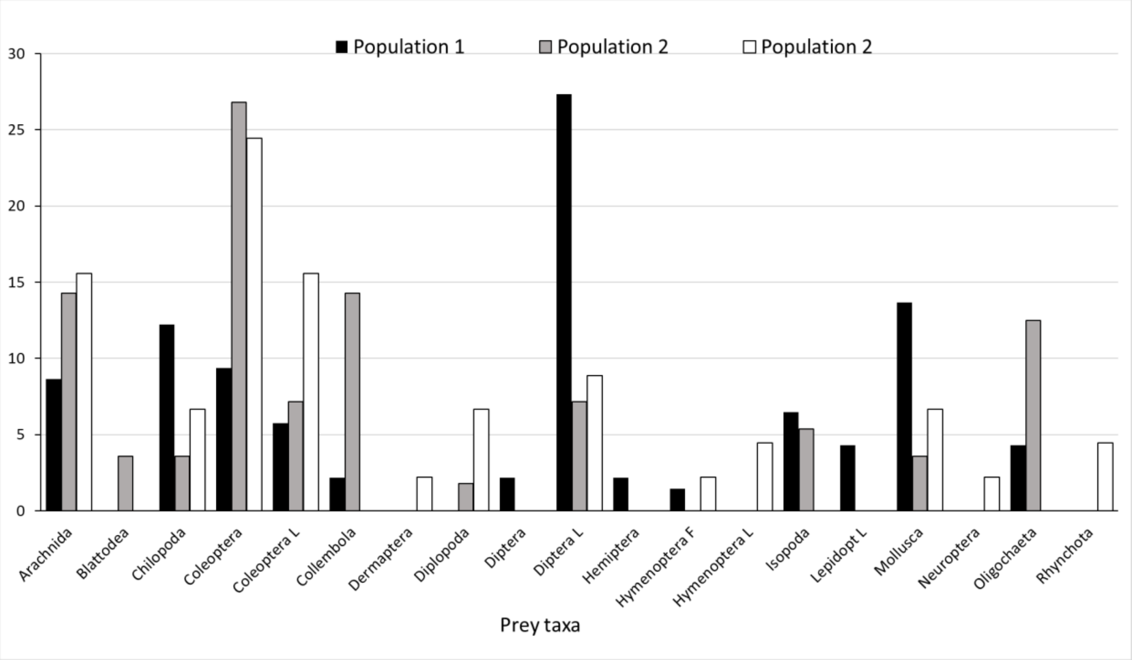

Figure 2. Comparison of prey percentage in stomach of Alpine salamanders from our population (Population 1, *n* = 41) and those of Fachbach et al. [30] (Population 2, from Gleinalmspeik, Germany, *n* = 15; Population 3, from Grimming, Germany, *n* = 26) [30]. The suffix L indicates the larval stage of the taxa; the suffix F (flying) indicates the winged taxa or the winged stage of the taxa.

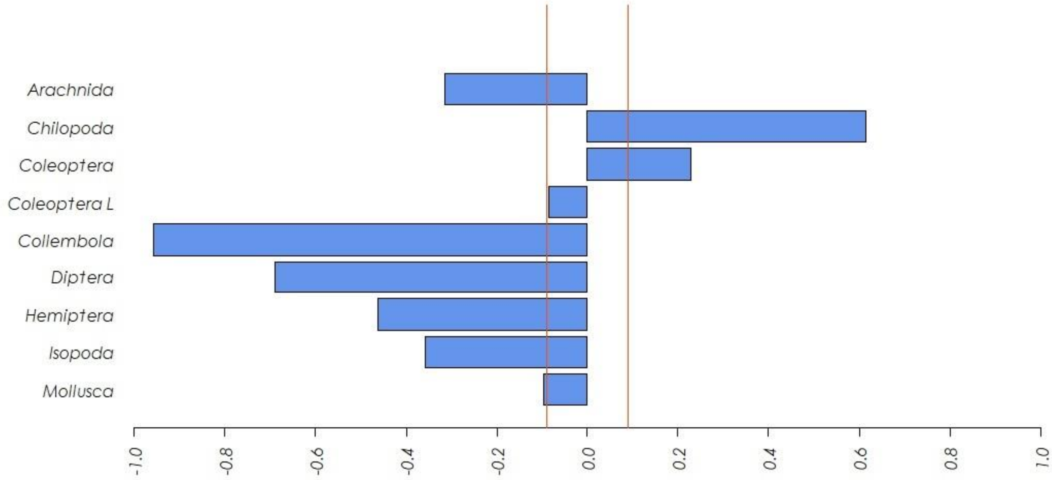

Figure 3. Relativized electivity index E* based on nocturnal trophic availability. Within the red vertical lines values are not statistically significant. The suffix L indicates the larval stage of the taxa.

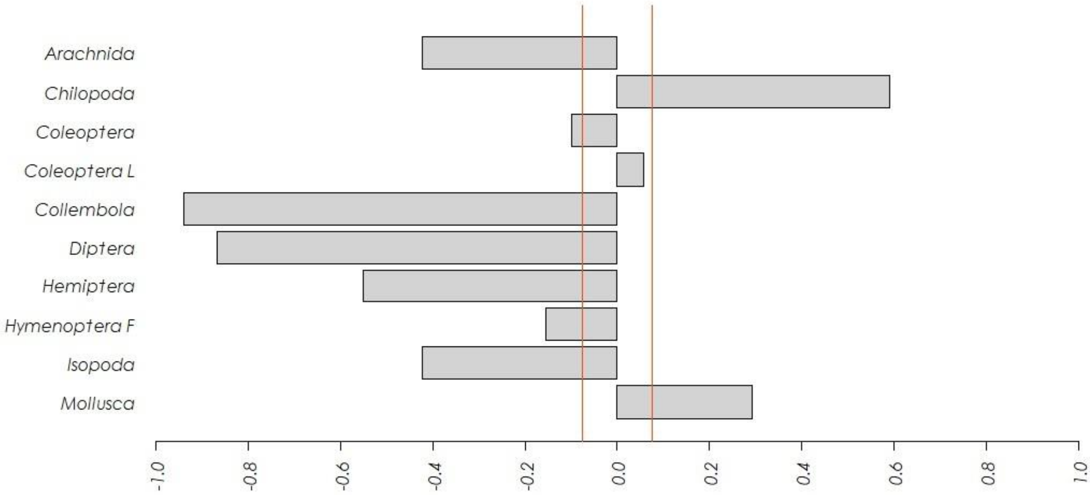

Figure 4. Relativized Electivity index E* based on total trophic availability. Within the red vertical lines values are not statistically significant. The suffix L indicates the larval stage of the taxa; the suffix F (flying) indicates the winged taxa or the winged stage of the taxa.

3.5. Analysis of Interindividual Diet Variation

The weighted bipartite network of individuals and resources is presented in Figure 5. The degree of diet variation $E = 0.87$ result was statistically highly significant ($p < 0.001$) and indicated a high variation among diets of individuals. The NODF = 22.4 metric indicated a moderate but significant ($p < 0.001$) nestedness in the network. At the same time, a high and significant modularity (Q = 0.58; $p < 0.001$) was present, and seven modules were identified (Figure 6).

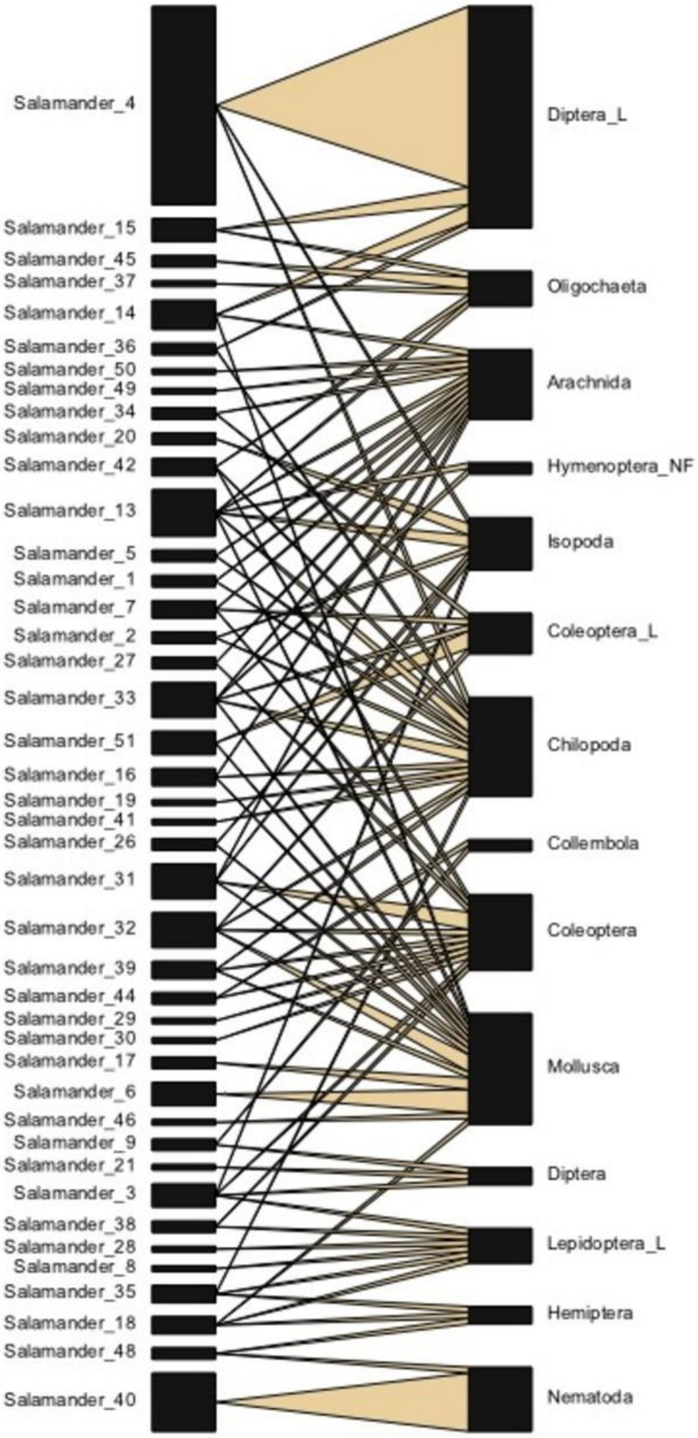

Figure 5. Bipartite network of salamanders and resources. Edge width represents frequency of use. The suffix L indicates the larval stage of the taxa; the suffix NF (not flying) indicates the not winged taxa or the not winged stage of a taxon.

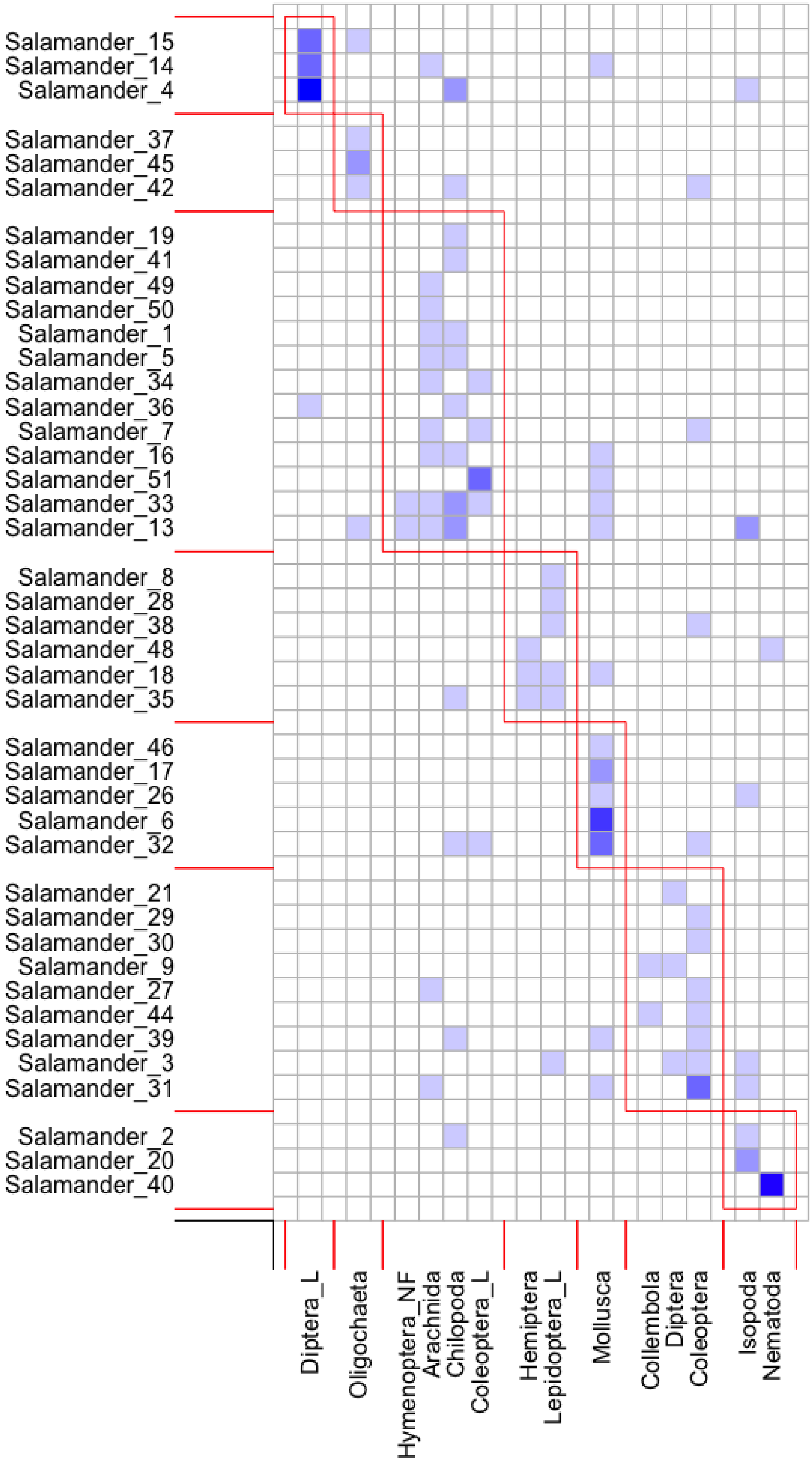

Figure 6. Adjacency matrix, with individual salamanders as rows and prey types as columns. Cell color represents frequency of use, red boxes indicate modules. The suffix L indicates the larval stage of the taxa; the suffix NF (not flying) indicates the not winged taxa or the not winged stage of a taxon.

4. Discussion

4.1. Prey Availability

Data about potential trophic niche highlighted rather low food availability, particularly in comparison with other studies about trophic strategy of other Italian salamanders carried out in similar periods with a similar number of pitfall traps [15,16,18]. These differences are not surprising due to the high-altitude alpine environment. Indeed, several studies confirm that conditions in mountains affect the species richness, composition, and density of different inver tebrates (e.g., [58–60]). Consequently, the low density of soil macroinvertebrates leads to a highly reduced amount of prey found in salamander's stomachs, in comparison with other species of terrestrial or semiterrestrial salamanders (e.g., ranging from 4.37 to 13.09 prey/stomach, [15]). The limited number of food items in the salamander stomachs may be due not only to the low prey availability, but also by the dimensional selectivity related to morphological characteristics of the predators and their predation strategy (see Section 4.2. and Section 4.3). ANOSIM and the diversity permutation test showed significant differences between total and nocturnal trophic availability, which strongly suggest that the actual daily activity pattern of the studied species must be taken into consideration because analyses that do not consider this factor can lead to misleading conclusions.

4.2. Realized Trophic Niche

There were no differences between males and females, according to previous studies [15,61,62]. The graphical Costello's method modified by Amundsen [48,49] identified a generalized trophic strategy in which Coloeptera and Mollusca represent the most valuable taxa of the diet (Figure 1). Diptera larvae is the only prey's category located in the upper-left quadrant of graph, indicating specialization. However, a low number of individuals preyed on this taxon ($n = 4$), and for only one of them, Diptera larvae represented 82% (31 of 38) of the diet. In this case, the high level of individual specialization, as exhibited by a single salamander, plays a marginal role in the overall picture of the population trophic strategy. The graphical plot showed that Alpine salamander avoided the use of small-sized taxa like Collembola, Diptera, and Formicidae, even if they are the dominant ones in the environment. The avoidance of small-sized taxa can be explained by considering the low number of preys/stomach (2.78) and the big size of this salamander. In fact, consumption of preys in amphibians is largely bound by morphological and physiological characteristics [63]. Furthermore, for Urodela, a positive correlation between dimension of the predator and prey has long been known [64]. Probably, predation strategy, not particularly refined and efficient to catch skillful prey, can also represent an important limit to usage of small-sized preys. Trophic strategy of *S. atra* pointed out by our study only partially agrees with observational information obtained in another Italian population [23], which exhibited a preference for Mollusca and Oligochaeta, followed by Orthoptera, Homoptera, Dermaptera, Isopoda, and Arachnida. Unfortunately, we could not perform an accurate comparison due to an absence of numerical data. Conversely, the study carried out by Fachbach et al. [30] on two German populations, although it did not report data for inferences on trophic strategy and selectivity, it provided numerical data on ingested preys. From the comparison of our data to those of the German populations (Figure 2), our population differed in the proportion of the prey taxa while the two German populations were more similar to each other (Figure 2). However, the overall diversity index of preyed taxa among the three populations was extremely similar. The most used categories in Fachbach and coauthors' study [30] were Coleoptera, Arachnida, and Coleoptera larvae, while few taxa seem to characterize just one population (e.g., Collembola in Population 2). In contrast, our study highlighted a preference for Diptera larvae, Mollusca, Chilopoda, and Coleoptera. Although the sampling methods to obtain information on preyed taxa were different (Fachbach and coauthors euthanized the salamanders), both methods collected preys directly by the stomach and may be considered comparable. Sample sizes were also comparable, although our salamanders belonged to the same population while the German ones were sampled from two different and distant populations. Consequently, differences among these populations in the ratio of preyed taxa may depend either on ecological variables in different environments (e.g., food availability) or on different behavioral traits at interpopulation level, or on both. The two German salamander populations [30] seem to occur in similar habitat (1200 m and 1600–1800 m a.s.l., calcareous geological substrate, open habitats mixed with coniferous woodland). Unfortunately, Fachbach et al. [30] reported only simple frequencies of preyed taxa for each population, but he did not report the number and type of prey per salamander and did not include trophic availability. Consequently, comparisons of electivity index or Amundsen method are inapplicable. However, the similar low average number of prey/salamander (2.78 from our study and 2.58 from Fachbach et al. [30]) suggest that the low number of ingested preys may be regarded as a characteristic trait of this species rather than that of a given population.

4.3. Trophic Selectivity

Definition of potential trophic niche is an indispensable tool to study trophic strategy and selectivity. Therefore, it is crucial to obtain data that are a good representation of the real trophic availability accessible to a given species. Dietary studies on other salamanders tried to achieve this target by using different types of traps that can collect samples from different habitats, like pitfall, sticky traps, and Berlese–Tullgren extractor [15,16]. Such methods are really effective to obtain data about the potential trophic niche, but should be used considering the activity period of the target species. Our results, for the first time, demonstrated that taking into account the temporal activity pattern of a predator may lead to a different interpretation of the trophic strategy. In literature, all studies about trophic strategy of salamanders perform arthropod sampling continuously for several days without considering the daily activity (and foraging) pattern of the studied species. However, we demonstrated that significant differences between total and nocturnal trophic availability could lead to different or even opposite interpretation of selectivity, which may also involve the most relevant taxa in the realized trophic niche. Electivity index, calculated with nocturnal trophic availability, shows a positive selection for Coleoptera and Chilopoda, highlighting the important role in summer’s trophic strategy of these taxa. Mollusca and Arachnida were also identified as relevant preys. The first was selected almost in relation to their abundance in the environment (they are just above the significance threshold, Figure 3), the second were exposed to a counter selection. Generally, relativized electivity index showed a negative selection for Collembola, Formicidae, and Diptera, which are, however, the most abundant in trophic availability. Therefore, selectivity results were essentially in agreement with tropic strategy defined by Amundsen’s method. As reported in Section 4.2, selection of prey type could be explained by dimension and hunting strategy of the Alpine salamander. Small number of preys per stomach could indicate a dimensional selectivity; for a large species such as *Salamandra atra,* foraging on few but large preys seems more worthwhile than eating many little ones, considering that a predator tends to maximize the energy intake consuming preys that give the best energy gain per time unit [65,66]. Given that selection seems to operate more on the prey’s size than on taxonomic categories, it will be useful to analyze trophic selectivity of Alpine salamander using volumetric classes that represent a possible measure of energy-intake [15], which can show a more detailed overview of trophic strategy of *Salamandra atra*.

4.4. Interindividual Diet Variation

The presence of interindividual diet variation in *Salamandra atra* is consistent with what was observed in other salamander species (e.g., [16,18]). Patterns of interindividual diet variation can be explained by optimal diet theory (ODT) [67,68], since individuals may have different traits affecting their ability to capture or handle different prey types and they will rank prey preferences accordingly. In this context, ODT theory accounts for three distinct patterns that may cause individual specialization [69]. Individuals may have different rank preferences and therefore specialize on different prey types. When competition is present, individuals should include new resources in their diet and increase overlap; we call it the distinct preferences model. Alternatively, under the competitive refuge model, individuals may share the first ranked prey and have different rankings for the less preferred ones. It implies that, under intraspecific competition, individuals will broaden their trophic niche, including different prey types according to their ranking preferences. The third model, the shared preferences model, assumes that individuals have identical preferences but differ in the willingness to include lower ranked prey in their diet. Consequently, concerning network analysis, the shared preferences model suggests the presence of significant nestedness [18] under intraspecific competition, but no modularity could be observed. Modularity in turn, should be present in case of the distinct preferences model, when resources are unlimited and competition is low, or by contrast, in the competitive refuge model when resources are limited and competition is high [53]. In our case study, the observed emergence of a significant modular network may be explained by both the competitive refuge model and the distinct preferences model. If the available resources are low, intraspecific competition is present and the preferred shared resource becomes scarce or less profitable, driving the individuals to the inclusion of different prey types, and to the emergence of several modules. The observed modules, however, could also arise from a distinct preferences model, where resources are unlimited and individuals, released by competition, specialize on their preferred prey items.

5. Conclusions

The diet of Salamandra atra, a fully terrestrial salamander, is investigated in depth for the first time, although during a short timeframe in the first half of August (i.e., about in the midseason of activity [23]). It showed a generalist trophic strategy; not all prey were consumed proportionally to their environmental availability. Considering diet variation at the individual level, the competitive refuge model and the distinct preferences model, within optimal diet theory, seem to equally fit our results. However, given the low trophic availability, the former is more supported. As a general rule, if the sampling period was short, it may not describe exhaustively the feeding habits of a given population. Although it is true that cross-sectional studies may overestimate individual specialization, in particular when prey distribution reflects a patchy environment [13,17], but our sampling was performed in a relatively small and homogenous area and this should reduce the bias. Furthermore, cross-sectional design represents the majority of data-type used in the analysis of interindividual diet variation (see reviews by [17,18]). Considering the lack of knowledge about trophic habits of Alpine salamander, the study of the trophic strategy is the first crucial step to understand the ecological role of this species. Further research will be necessary to investigate any possible variations in diet during the whole activity season and the functional relationship existing between predator and its prey, in terms of relative abundance and biomass. Finally, as a methodological contribution, we highlighted that considering the daily activity pattern of a species is a fundamental requirement for correct data collection and resulting interpretation. Our study was performed on a population of the nominal subspecies. Further investigations on the others, three subspecies with two of them extremely localized and Italian endemic [70,71], may elucidate any feeding differences among these taxa.

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References

1. Davic, R.D.; Welsh, H.H., Jr. On the ecological roles of salamanders. *Ann. Rev. Ecol. Evol. System.* **2004**, *35*, 405–434. [CrossRef]
2. Hairston, N.G. The local distribution and ecology of the plethodontid salamanders of the southern Appalachians. *Ecol. Monogr.* **1949**, *19*, 49–73. [CrossRef]
3. Petranka, J.W. *Salamanders of the United States and Canada*; Smithsonian Institution Press: Washington, DC, USA, 1998; pp. 1–587.
4. Costa, A.; Crovetto, F.; Salvidio, S. European plethodontid salamanders on the forest floor: Local abundance is related to fine–scale environmental factors. *Herpetol. Conserv. Biol.* **2016**, *11*, 344–349.
5. Romano, A.; Anderle, M.; Forti, A.; Partel, P.; Pedrini, P. Population density, sex ratio and body size in a population of *Salamadra atra atra* on the Dolomites. *Acta Herpetol.* **2018**, *13*, 195–199.
6. Burton, T.M.; Likens, G.E. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology* **1975**, *56*, 1068–1080. [CrossRef]
7. Best, M.L.; Welsh, H.H., Jr. The trophic role of a forest salamander: Impacts on invertebrates, leaf litter retention, and the humification process. *Ecosphere* **2014**, *5*, 1–19. [CrossRef]
8. Barton, K.A.; Zalewski, A. Winter severity limits red fox populations in Eurasia. *Glob. Ecol. Biogeogr.* **2007**, *16*, 281–289. [CrossRef]
9. Isbell, L.A. Sudden short–term increase in mortality of vervet monkeys (*Cercopithecus aethiops*) due to leopard predation in Amboseli National Park, Kenya. *Am. J. Primatol.* **1990**, *21*, 41–52. [CrossRef]
10. Berger, L.; Speare, R.; Thomas, A.; Hyatt, A. Mucocutaneous fungal disease in tadpoles of *Bufo marinus* in Australia. *J. Herpetol.* **2001**, *35*, 330–335. [CrossRef]
11. Chapman, C.A.; Wasserman, M.D.; Gillespie, T.R.; Speirs, M.L.; Lawes, M.J.; Saj, T.L.; Ziegler, T.E. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *Am. J. Phys Anthropol.* **2006**, *131*, 525–534. [CrossRef]
12. Hanya, G.; Chapman, C.A. Linking feeding ecology and population abundance: A review of food resource limitation on primates. *Ecol. Res.* **2013**, *28*, 183–190. [CrossRef]
13. Bolnick, D.I.; Yang, L.H.; Fordyce, J.A.; Davis, J.M.; Svanback, R. Measuring individual–level resource specialization. *Ecology* **2002**, *83*, 2936–2941. [CrossRef]
14. Wells, K.D. *The Ecology and Behavior of Amphibians*; Chicago University Press: Chicago, IL, USA, 2007.
15. Salvidio, S.; Romano, A.; Oneto, F.; Ottonello, D.; Michelon, R. Different season, different strategies: Feeding ecology of two syntopic forest dwelling salamanders. *Acta. Oecol.* **2012**, *43*, 42–50.
16. Costa, A.; Salvidio, S.; Posillico, M.; Matteucci, G.; De Cinti, B.; Romano, A. Generalisation within specialization: Inter–individual diet variation in the only specialized salamander in the world. *Sci. Rep.* **2015**, *5*, 13260. [CrossRef] [PubMed]
17. Bolnick, D.I.; Svanback, R.; Fordyce, J.A.; Yang, L.H.; Davis, J.M.; Hulsey, C.D.; Forister, M.L. The ecology of individuals: Incidence and implications of individual specialization. *Am. Nat.* **2003**, *161*, 1–28. [CrossRef]
18. Araújo, M.S.; Martins, E.G.; Cruz, L.D.; Fernandes, F.R.; Linhares, A.X.; Dos Reis, S.F.; Guimaraes, P.R., Jr. Nested diets: A novel pattern of individual-level resource use. *Oikos* **2010**, *119*, 81–88. [CrossRef]
19. Salvidio, S.; Oneto, F.; Ottonello, D.; Costa, A.; Romano, A. Trophic specialization at the individual level in a terrestrial generalist salamander. *Can. J. Zool.* **2015**, *93*, 79–83. [CrossRef]
20. Salvidio, S.; Costa, A.; Crovetto, F. Individual trophic specialisation in the Alpine newt increases with increasing resource diversity. *Ann. Zool. Fenn.* **2019**, *56*, 17–24. [CrossRef]
21. Rosa, G.; Costa, A.; Salvidio, S. Trophic strategies of two newt populations living in contrasting habitats on a Mediterranean island. *Ethol. Ecol. Evol.* **2020**, *32*, 96–106. [CrossRef]
22. Costa, A.; Baroni, D.; Romano, A.; Salvidio, S.; Lötters, S. Individual diet variation in *Salamandra salamandra* larvae in a Mediterranean stream (Amphibia: Caudata). *Salamandra* **2017**, *53*, 148–152.
23. Lanza, B.; Andreone, F.; Bologna, M.A.; Corti, C.; Razzetti, E. *Fauna d'Italia, vol. XLII, Amphibia*; Calderini Edition: Bologna, Italy, 2007; pp. 197–211.
24. Sillero, N.; Campos, J.; Bonardi, A.; Corti, C.; Creemers, R.; Crochet, P.A.; Isailovic, J.C.; Denoël, M.; Ficetola, G.F.; Gonçalves, J.; et al. Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphib. Reptil.* **2014**, *35*, 1–31. [CrossRef]

25. Bedriaga, J. von. *Die Lurchfauna Europa's II. Urdodela. Schwanzlurche*. Univ. typ. Moscow. Available online: http://www.europeana.eu/en/item/08711/item_23042?fbclid=IwAR368Ijnp_DRjaXfZmkOrOKIrDYic6VsgrfQ6EhAjySPxW9OcsBMZuga4dE (accessed on 16 May 2020).
26. Freytag, G.E. *Feuersalamander und Alpensalamander. Die Neue Brehm-Bücherei*; A. Ziemsen Verlag: Wittenberg Lutherstadt, Germany, 1955; p. 142.
27. Klewen, R.F. Untersuchungen zur Verbreitung, Öko-Ethologie und innerartlichen Gliederung von *Salamandra atra* (Laurenti 1768). Ph.D. Thesis, Universität Köln, Köln, Germany, 1986.
28. Klewen, R.F. *Die Landsalamander Europas 1: Die Gattungen Salamandra und Mertensiella*; A. Ziemsen Verlag: Wittenberg Lutherstadt, Germany, 1988.
29. Kuzmin, S.L. Feeding ecology of *Salamandra* and *Mertensiella*: A review of data and ontogenetic evolutionary trends. *Mertensiella* **1994**, *4*, 271–286.
30. Fachbach, G.; Kolossau, I.; Ortner, A.; Zur Ernährungsbiologie von Salamandra, s. salamandra und *Salamandra atra*. *Salamandra* **1975**, *11*, 136–144.
31. Scheller, H.V. Pitfall trapping as the basis for studying ground beetle predation in spring barley. *Tidsskr. Planteavl.* **1984**, *88*, 317–324.
32. Woodcock, B.A. Pitfall trapping in ecological studies. In *Insect Sampling in Forest Ecosystems*; Leather, S.R., Ed.; Blackwell Publishing Co.: New Jersey, NJ, USA, 2005.
33. Southwood, T.R.E.; Henderson, P.A. *Ecological Methods*, 3rd ed.; Wiley-Blackwell: Oxford, UK, 2000; p. 575.
34. Ausden, M. Invertebrates. In *Ecological Census Techniques: A Handbook*; Sutherland, W.J., Ed.; Cambridge University Press: Cambridge, UK, 1996.
35. Duellman, W.E.; Trueb, L. *Biology of Amphibians*; Johns Hopkins Univ. Press: Baltimore, MD, USA, 1994; p. 670.
36. Solé, M.; Rödder, D. Dietary assessments of adult amphibians. In *Amphibian Ecology and Conservation: A Handbook of Techniques*; Dodd, J., Ed.; Oxford University Press: Oxford, UK, 2010.
37. Fraser, D.F. Coexistence of salamanders in the genus Plethodon: A variation of the Santa Rosalia theme. *Ecology* **1976**, *55*, 238–251. [CrossRef]
38. Salvidio, S. Diet and food utilization in a rockface population of *Speleomantes ambrosii* (Amphibia, Caudata, Plethodontidae). *Vie et Milieu* **1992**, *42*, 35–39.
39. Schabetsberger, R. Gastric evacuation rates of adult and larval Alpine newts (*Triturus alpestris*) under laboratory and field conditions. *Freshw. Biol.* **1994**, *31*, 143–151. [CrossRef]
40. Caldwell, J.P. The evolution of myrmecophagy and its correlates in poison frogs (Family Dendrobatidae). *J. Zool.* **1996**, *240*, 75–101. [CrossRef]
41. Solé, M.; Beckmann, O.; Pelz, B.; Kwet, A.; Engels, W. Stomach–flushing for diet analysis in anurans: An improved protocol evaluated in a case study in Araucaria forests, southern Brazil. *Stud. Neotrop. Fauna Environ.* **2005**, *40*, 23–28. [CrossRef]
42. Clarke, K.R. Non–parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* **1993**, *18*, 117–143. [CrossRef]
43. Magurran, A.E. *Measuring Biological Diversity*; Blackwell Publishing: Oxford, UK, 2004; p. 256.
44. Wu, Z.-J.; Li, Y.; Wang, Y. A comparison of stomach flush and stomach dissection in diet analysis of four frog species. *Acta Zool. Sin.* **2007**, *53*, 364–372.
45. Hammer, O.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Paleontol. Electron.* **2001**, *4*, 1–9.
46. Vanderploeg, H.A.; Scavia, D. Calculation and use of selectivity coefficients of feeding: Zooplankton grazing. *Ecol. Mod.* **1979**, *7*, 135–149. [CrossRef]
47. Lechowicz, M.J. The sampling characteristics of electivity indices. *Oecologia* **1982**, *52*, 22–30. [CrossRef] [PubMed]
48. Ramos–Jiliberto, R.; Valdovinos, F.S.; Arias, J.; Alcaraz, C.; García–Berthou, E. A network–based approach to the analysis of ontogenetic diet shifts: An example with an endangered, small–sized fish. *Ecol. Compl.* **2011**, *8*, 123–129. [CrossRef]
49. Costello, M.J. Predator feeding strategy and prey importance: A new graphical analysis. *J. Fish Biol.* **1990**, *36*, 261–263. [CrossRef]
50. Amundsen, P.A.; Gabler, H.M.; Staldvik, F.J. A new approach to graphical analysis of feeding strategy from stomach contents data—Modification of the Costello (1990) method. *J. Fish Biol.* **1996**, *48*, 607–614.

51. Araújo, M.S.; Guimarães, P.R., Jr.; Svanbäck, R.; Pinheiro, A.; Guimarães, P.; Reis, S.F.D.; Bolnick, D.I. Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology* **2008**, *89*, 1981–1993. [CrossRef]
52. Pires, M.M.; Guimarães, P.R., Jr.; Araújo, M.S.; Giaretta, A.A.; Costa, J.C.L.; Dos Reis, S.F. The nested assembly of individual-resource networks. *J. Anim. Ecol.* **2011**, *80*, 896–903. [CrossRef]
53. Tinker, M.T.; Guimarães, P.R., Jr.; Novak, M.; Marquitt, F.M.D.; Bodkin, J.L.; Staedler, M.; Bentall, G.; Estes, J.A. Structure and mechanism of diet specialisation: Testing models of individual variation in resource use with sea otters. *Ecol. Lett.* **2012**, *15*, 475–483. [CrossRef]
54. Santamaría, S.; Enoksen, C.A.; Olesen, J.M.; Tavecchia, G.; Rotger, A.; Igual, J.M.; Traveset, A. Diet composition of the lizard *Podarcis lilfordi* (Lacertidae) on 2 small islands: An individual–resource network approach. *Curr. Zool.* **2020**, *66*, 39–49. [CrossRef]
55. Blüthgen, N.; Menzel, F.; Blüthgen, N. Measuring specialization in species interaction networks. *BMC Ecol.* **2006**, *6*, 9. [CrossRef] [PubMed]
56. Almeida–Neto, M.; Guimaraes, P.; Guimaraes, P.R., Jr.; Loyola, R.D.; Ulrich, W. A consistent metric for nestedness analysis in ecological systems: Reconciling concept and quantification. *Oikos* **2008**, *117*, 1227–1239. [CrossRef]
57. Beckett, S.J. Improved community detection in weighted bipartite networks. *R. Soc. Open Sci.* **2016**, *3*, 140536. [CrossRef] [PubMed]
58. Aubry, M.P.; Berggren, W.A.; van Couvering, J.; McGowran, B.; Pillans, B.; Hilgen, F.J. Quaternary: Status, rank, definition, survival. *Episodes* **2005**, *28*, 118–120. [CrossRef] [PubMed]
59. Sfenthourakis, S.; Anastasiou, I.; Strutenschi, T. The terrestrial isopod diversity of Mt. Panachaiko (Pelopon–nisos, Greece). *E. J. Soil Biol.* **2005**, *41*, 91–98. [CrossRef]
60. Lessard, S.J.; Rivas, D.A.; Stephenson, E.J.; Yaspelkis, B.B., III; Koch, L.G.; Britton, S.L.; Hawley, J.A. Exercise training reverses impaired skeletal muscle metabolism induced by artificial selection for low aerobic capacity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *300*, R175–R182. [CrossRef]
61. Lamb, T. The influence of sex and breeding condition on microhabitat selection and diet in the Pig Frog *Rana grylio*. *Am. Mid. Nat.* **1984**, *111*, 311–318. [CrossRef]
62. Romano, A.; Salvidio, S.; Palozzi, R.; Sbordoni, V. Diet of the newt, *Triturus carnifex* (Laurenti, 1768), in a flooded karstic sinkhole ("Pozzo del Merro", Central Italy). *J. Cave Karst Stud.* **2012**, *74*, 271–277. [CrossRef]
63. Duellman, W.E.; Trueb, L. *Biology of Amphibians*; Mc Graw-Hill Book Company: New York, NY, USA, 1986.
64. Whitaker, J.O., Jr.; Rubin, D.C. Food habits of Plethodon jordani metcalfi and Plethodon jordani shermani from North Carolina. *Herpetologica* **1971**, *27*, 81–86.
65. Krebs, J.R. Optimal foraging: Decision rules for predators. In *Behavioural Ecology: An Evolutionary Approach*; Krebs, J.R., Davies, N.B., Eds.; Backwell Scientific Publications: Oxford, UK, 1978; pp. 23–63.
66. Stephens, D.W. *Foraging Theory*; Princeton University Press: Princeton, NJ, USA, 1986.
67. Schoener, T.W. Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* **1971**, *2*, 369–404. [CrossRef]
68. Pulliam, H.R. On the theory of optimal diets. *Am. Nat.* **1974**, *108*, 59–75. [CrossRef]
69. Svanbäck, R.; Bolnick, D.I. Intraspecific competition affects the strength of individual specialization: An optimal diet theory method. *Evol. Ecol. Res.* **2005**, *7*, 993–1012.
70. Speybroeck, J.; Beukema, W.; Bok, B.; Van Der Voort, J. *Field Guide to the Amphibians and Reptiles of Britain and Europe*; Bloomsbury Natural History: London, UK, 2016.
71. Razpet, A.; Šunje, E.; Kalamujić, B.; Tulić, U.; Pojskić, N.; Stanković, D.; Krizmanić5, I.; Marić, S. Genetic differentiation and population dynamics of Alpine salamanders (*Salamandra atra*, Laurenti 1768) in Southeastern Alps and Dinarides. *Herpetol. J.* **2016**, *26*, 111–119.