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A network-based approach to the analysis of ontogenetic diet shifts: An example with an endangered, small-sized fish

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ABSTRACT

Many organisms exhibit ontogenetic shifts in their diet and habitat use, which often exert a large influence on the structure and expected dynamics of food webs and ecological communities. Nevertheless, reliable methods for detecting these niche shifts from consumption data are limited. In this study we present a new approach for the detection and analysis of ontogenetic diet shifts, based on complex network theory. As a case study, we apply these methods to the endangered, small fish *Aphanius iberus*. The stage-structured consumer population and its set of consumed prey are represented as an unweighted bipartite network. A statistical evaluation of the resulting network structure permits to uncover empirical patterns of ontogenetic diet shifts. We test for changes in niche breadth, as well as nestedness and diet modularity along ontogeny. These tests were carried out on the subnetworks describing consumption, positive electivity, and negative electivity on prey items. The statistical significance was established by means of null model analyses. Our analyses reveal a nested diet, along with a gradual decrease in diet breadth and a modular structure (i.e. abrupt changes) of elected preys along the ontogeny of *A. iberus*. The detection of network structure by means of the use of tools from complex network theory is shown to be a promising method for studying ontogenetic niche shifts.

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1. Introduction

Trophic relationships among coexisting species constitute a central theme in ecology, from the individual to the community level. Predator–prey relationships shape the structure of ecological communities, from population dynamics of the species involved (Chen and Cohen, 2001) to the robustness of the community to environmental perturbations (Dunne et al., 2002), and ultimately the persistence of species over time (Melián and Bascompte, 2002).

The architecture and magnitude of trophic interactions among species are, however, not static but highly variable for different causes (Bolker et al., 2003). One such cause is that the set of species a consumer feeds on can depend on consumer's age, size, or developmental stage (Werner and Gilliam, 1984). During the development and growth of organisms, their food requirements often change quantitatively and qualitatively because of the myriad of effects of predator size on metabolic costs, gape limitation, foraging ability and similar variables. In addition, development can be accompanied by shifts in habitat use (Gross et al., 1988), which result in changes of food availability and predation risk (Hjelm et al., 2000), which constrain the ability to exploit different prey types. As a consequence of the adaptation of consumers to changes in food availability, subjected to feeding constraints, many organisms exhibit either abrupt or gradual shifts in feeding habits along their ontogeny. Ontogenetic diet shifts (ODS) generate stage-structure, potentially leading the consumer population to behave as a set of distinctive trophic entities (Polis, 1984), each interacting with different prey assemblages (Werner and Gilliam, 1984). This ontogenetic niche partitioning, accompanied by heterogeneity in prey abundances, may exert a considerable influence on community dynamics (Polis et al., 1996; Takimoto, 2003).

There is an increasing trend in ecology toward the use of tools from complex systems theory, mainly derived from statistical mechanics (Albert and Barabási, 2002; Newman, 2003), for addressing a variety of natural phenomena. These include the analysis of food webs (Pascual and Dunne, 2006), mutualistic

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interactions (Bascompte and Jordano, 2007), and spatial dispersion (Fortuna et al., 2006). The complex network approach has provided a new and simple way for detecting properties of the structure of interactions among biological entities. In this paper, we show how the methods of complex systems theory can offer a new, simple, and robust way of revealing biologically meaningful patterns of ODS from field data. We studied ODS in the endangered fish *Aphanius iberus* by analyzing the network structure of a detailed quantitative record of consumed prey and their availability, previously published elsewhere (Alcaraz and García-Berthou, 2007; Alcaraz et al., 2008). This dataset is well suited for our objectives because of its detailed taxonomic precision, generally to the genus or species level (which is not frequent for fish dietary studies) and the small maximum size of the predator (which makes ODS less strong and apparent than in many other fishes). We analyzed: (i) ontogenetic shifts in diet breadth, (ii) nestedness in prey species composition, and (iii) modularity in prey species composition, for three kinds of diet matrices (consumption, electivity, and avoidance).

2. Methods

2.1. Dataset and adjacency matrices

The Spanish toothcarp (*A. iberus*) is an endangered cyprinodontid fish endemic to the Mediterranean coast of Spain. It is a small fish of total length <5 cm, sexually dimorphic, with longevity up to 2 years, euryhaline and eurythermal (Fernández-Delgado et al., 1988; García-Berthou and Moreno-Amich, 1992; Vargas and de Sostoa, 1997). This fish reproduces from April to October, laying up to 900 eggs in successive spawns, and reaches sexual maturity in a few months at a total length of <2 cm. The Spanish toothcarp is omnivorous, with a diet composed mainly of crustaceans such as harpacticoid copepods and amphipods, but also including plant debris and detritus (Alcaraz and García-Berthou, 2007).

In this paper, we analyze data previously reported elsewhere (Alcaraz and García-Berthou, 2007; Alcaraz et al., 2008) and we refer to those publications for full details on the field and laboratory procedures. Sampling of the toothcarp and its preys was carried out in Fra Ramon, a small coastal lagoon located at NE Spain, at 42°1′49″ N, 3°11′29″ E and 1.75 m below the sea level. The lagoon was sampled on the mornings of 21–22 November 2002. Prey organisms in the water column were sampled with a 1 m long transect of 50 μm mesh dip net (volume sampled = 0.038 m³). Benthic organisms were sampled with a 225 cm² benthic grab. Details on the post-sampling procedures can be found elsewhere (Alcaraz and García-Berthou, 2007; Alcaraz et al., 2008). Fish were sampled with a throw trap consisting of a 1 m high cylinder of 90 cm in diameter. From the samples taken at 38 sites, we analyzed the entire gut content of fishes and determined the relative density of each prey item in the environment (water column and benthos). In the laboratory, all fish individuals were measured and weighed to the nearest 0.1 mg, after being eviscerated. Prey organisms were identified with the highest possible taxonomic precision, usually to the genus or species level. The total number of fish and prey in the gut that were analyzed was 62 and 10,839, respectively, with prey belonging to 20 taxonomic groups.

Although gut contents give information about realized trophic interactions within food webs, the assessment of feeding preferences must consider the abundance of prey types in the environment. The use of food types in relation to their availability in the environment is often analyzed by means of electivity indices (see e.g. Alcaraz and García-Berthou, 2007; Mas-Martí et al., 2010). These indices allow detecting over- or under-representation of prey items in the diets compared to the resource availability. In this work, electivity on prey types by consumers was measured through the relativized electivity index e of Vanderploeg and Scavia (1979), an index strongly supported by comparative evaluations (Lechowicz, 1982):

$$e = \frac{w_i - n^{-1}}{w_i + n^{-1}}, \quad \text{with} \quad w_i = \frac{r_i}{p_i}\left(\sum_{k=1}^{n}\frac{r_k}{p_k}\right)^{-1},$$

where 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 (negative selection or avoidance). If prey were consumed according to their availability in the environment, then $e = 0$. Therefore we need to set a critical point above and below which observed values of e would be considered different from zero. We defined a threshold electivity value $u = 0.17$, which was the 5th percentile of the absolute values of the calculated e for each individual consumer on each prey type. Values of $|e| < 0.17$ were considered not significantly different from zero. Moderate variations in the value of u did not alter the outcome of our analyses.

The interaction system formed by the consumer's ontogenetic stages and their prey can be visualized as a bipartite network (Fig. 1), in which nodes represent consumers and prey. The links represent realized consumption interactions that can be classified into three types, i.e. whether a prey is being positively ($e > u$), negatively ($e < -u$) or neutrally ($-u < e < u$) selected by the consumer. Accordingly, the network is represented as a set of adjacency matrices (Fig. 1). The consumption matrix **C** contains information about the prey items recorded in the diet of each stage. In this matrix the element $c_{ij} = 1$ indicates that prey i is consumed by stage j, and $c_{ij} = 0$ otherwise. The electivity matrix **E** shows the prey items positively selected by each ontogenetic stage. In this matrix, elements $e_{ij} = 1$ indicate that prey item i displays a value of $e > u$ for stage j. Finally, avoidance matrix **A** contains elements
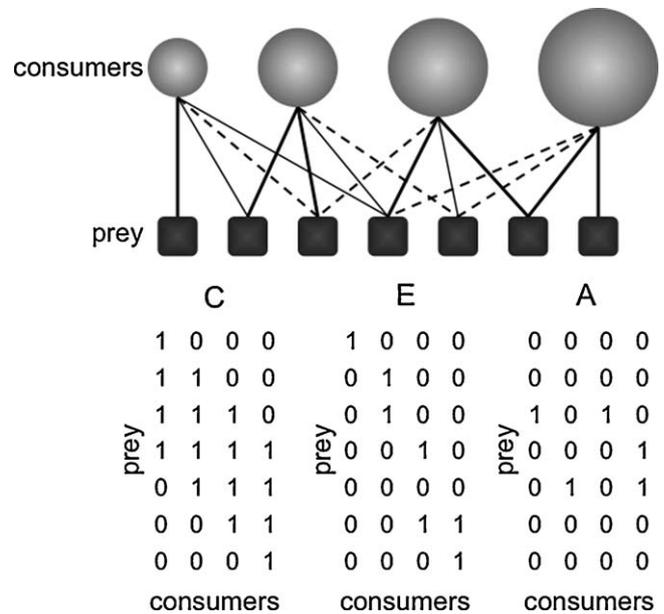

Fig. 1. Network (top) and matrix (bottom) representation of the interactions between a multi-stage consumer population and its prey resources, for the study of ontogenetic diet shifts. This figure does not represent real data. Spheres represent ontogenetic stages of the consumer species, squares represent prey items, and links represent realized prey–consumer interactions. Thick links indicate positive electivity of the prey, dashed links represent negative electivity (i.e. avoidance), and continuous thin links show null electivities. In the matrices, consumer stages and prey items are represented in columns and rows, respectively. In the consumption matrix **C**, ones indicate realized consumption interactions, and zeroes indicate unobserved consumption of item i by consumer stage j. Positive and negative electivities are represented in matrices **E** and **A**, respectively.

$a_{ij} = 1$, where item i displays a value of $e < -u$ for stage j. Consumers' ontogenetic stages were constructed on the basis of body mass categories. Using standard categorization procedures (Freedman and Diaconis, 1981), the recommended number of categories was found to be 10. Nevertheless, we made all the analyses for 8, 10, 12, and 14 stages in order to test the sensitivity of our methods to the chosen number of ontogenetic categories. If the size (mass) range of the individuals is very large, we recommend constructing the size classes using log-transformed mass. Using body length instead of mass should be expected to provide very similar results, given the usually high correlation between length and mass of animals.

Ontogenetic diet shift was analyzed by studying three structural properties of these matrices: ontogenetic shift in number of prey per stage (diet breadth), nestedness of prey types within successive stages, and modularity of diet along ontogeny (Fig. 2).

2.2. Diet breadth

The number of prey that each ontogenetic stage consumes, elects, or avoids is equivalent to the number of edges connected to the corresponding nodes (i.e. their degree) in the adjacency matrices **C**, **E**, and **A**, respectively. We decomposed diet breadth into three distinct measures: consumed diet breadth, elected diet breadth, and avoided diet breadth. We then computed the Spearman's rank correlation between the mean body mass of each ontogenetic stage and the degree of the nodes representing the stages. Significant positive and negative correlations were indicative of increasing and decreasing diet breadth along ontogeny, respectively.

2.3. Nestedness

Nestedness was measured through the NODF metric (Almeida-Neto et al., 2008), measuring the degree of nestedness only among columns (N_c), since we were interested in testing whether prey of some stages were subsets of the prey of other stages. This index ranges from 0 for perfectly nested networks to 100 for perfectly non-nested networks. A perfectly nested network implies that the prey types eaten by the more specialist stages, constitute proper subsets of the prey eaten by the more generalist stages. In order to evaluate the statistical significance of observed nestedness, we compared the N_c value of each matrix against the distribution of N_c obtained from 1000 matrices of equal size and similar fill,

generated from a null model which assumes that each prey-stage pair interacts with a probability proportional to the observed number of interactions of both nodes (Bascompte et al., 2003). Nestedness and null model analyses were measured using the freeware ANINHADO (Guimarães and Guimarães, 2006). Since we were interested in knowing whether prey types were nested within *successive* ontogenetic stages (for both natural and inverse order), we did not allow the software to pack the matrix.

2.4. Modularity

A network is said to be modular when its member nodes are organized into compartments (modules) within which interactions are more frequent than expected by chance. Conversely, interactions between modules are scarce. For our purposes, abrupt shifts in consumption habits, prey electivity or avoidance during the ontogeny of a consumer species must be reflected in a modular structure of the corresponding diet matrix. For detecting or rejecting this kind of network structure, we developed an algorithm based on the graph partitioning method of Newman (2006), and the bipartite extension of Barber (2007). The degree of modularity was measured through the index Q, defined as follows:

$$Q = \frac{1}{2m} Tr\, \mathbf{S}^{T} \mathbf{BS},$$

where m is the number of edges in the network, **S** is a $c \times n$ matrix that contains the belonging of each node $(1, \ldots, n)$ to each of the c modules; and **B** is defined with elements $b_{ij} = a_{ij} - p_{ij}$, where a_{ij} are the elements of the adjacency matrix (consumption, electivity or avoidance), and p_{ij} are the probabilities that each interaction exists by chance (see details in Newman, 2006; Barber, 2007). The optimization problem is to find a network partition that maximizes Q. For our purposes, we were interested in the optimal partition that preserves the natural sequence of consumers' ontogenetic stages. Therefore, we developed a modified version of the Barber's algorithm that measures Q in all the possible partitions of consumer stages with a number of modules c between 2 and 5, for testing 1–4 potential ontogenetic shifts within the life-cycle of the fish. The optimal adscription of prey types to each module was based on the eigenspectrum properties of **B**, as described in Barber (2007). For assessing the statistical significance of the specific modular structure obtained for each adjacency matrix, we compared the Q value of the optimally partitioned matrix against
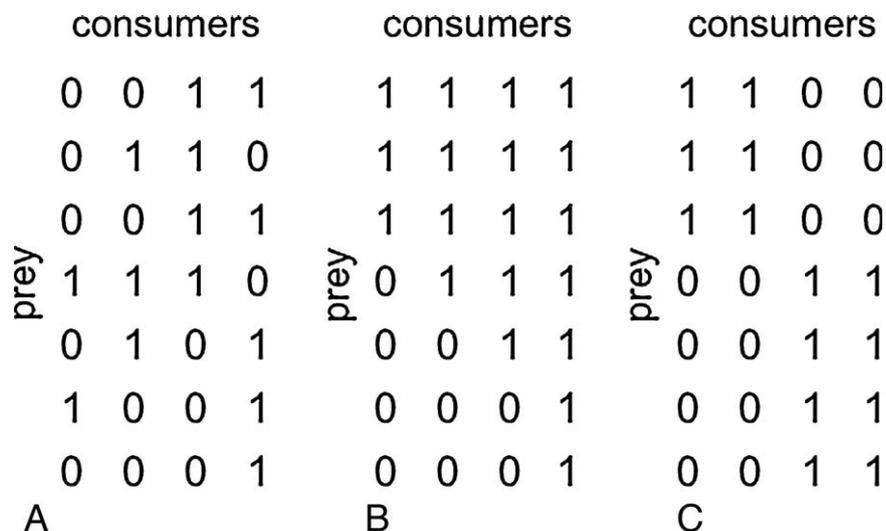
Fig. 2. Idealized prey–consumer matrices exhibiting different structures. System A exhibits an increase in diet breadth with age. System B exhibits a nested diet structure, where the diet of earlier stages consists of a subset of the prey items consumed by older stages. System C exhibits a modular diet structure, where there is no diet overlap between stages 1–2 and stages 3–4. Systems A and B show gradual shifts in diet structure along the ontogeny, while system C shows an abrupt ontogenetic shift.

Fig. 3. Consumption network of the 12-stage predator–prey system. Upper nodes represent the ontogenetic stages of the consumers (no diet record for stages 9–11), and lower nodes represent the consumed prey types.

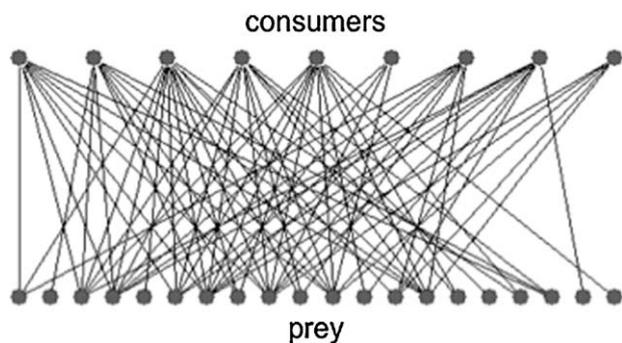a distribution of 200 Q values obtained from null matrices of equal size and similar fill (see methods of nestedness analyses). The null matrices also preserved the consumer stage partitioning obtained from the corresponding adjacency matrix, but the prey were randomly allocated to modules in each of the null matrices. Modularity was assumed to be significant when the Q value of the adjacency matrix fell above the 95% of the Q values of the null matrices.

3. Results

3.1. Diet breadth

A visual representation of the consumption network of the Spanish toothcarp is displayed in Fig. 3, for the case of the 12-stage configuration. Elected diet breadth, measured by the degree of the stages in the **E** matrix, decreases during the ontogeny of Spanish toothcarp (Table 1). In other words, as animals get older they show preference for less prey types, i.e. they become more specialists with respect to the food type that they select. These trends were less well supported for the **C**, and particularly, the **A** matrices, suggesting that ontogenetic shifts in diet breadth are only detectable at the level of positive preferences. Changes in the number of ontogenetic categories did not alter qualitatively the results obtained.

3.2. Nestedness

Our analyses showed that the diet of the Spanish toothcarp was significantly nested along the ontogeny (Table 2). Specifically, nestedness of diet habits appeared when the columns were sorted

Table 1
Rank correlation between the degree of ontogenetic stages of consumers and their body mass. Consumed diet breadth, elected diet breadth, and avoided diet breadth, are evaluated in the **C**, **E**, and **A** matrices, respectively. Significant values ($P < 0.05$) are marked in bold.

Matrix	Number of stages	r_S	P
C	8	−0.643	0.139
	10	−0.707	0.058
	12	−0.509	0.171
	14	−0.374	0.287
E	8	−0.849	**<0.05**
	10	−0.823	**<0.05**
	12	−0.882	**<0.01**
	14	−0.783	**<0.01**
A	8	−0.418	0.349
	10	−0.323	0.432
	12	−0.051	0.903
	14	−0.099	0.786

Table 2
Nestedness analysis of the diet matrices studied. Adjacency matrices **C**, **E**, and **A** contain information about consumed diet, elected diet, and avoided diet, respectively. The value of nestedness metrics and their associated P-values are shown for matrices whose stages were sorted in natural (increasing age) or inverse (decreasing age) order. Significant values ($P < 0.05$) are marked in bold.

Matrix	Number of stages	Natural		Inverse	
		Nestedness	P	Nestedness	P
C	8	61.43	**<0.05**	23.65	0.324
	10	60.24	**<0.01**	19.48	0.988
	12	50.35	**<0.05**	24.28	0.587
	14	47.6	**<0.05**	26.37	0.382
E	8	30.95	**<0.001**	0	0.265
	10	26.79	**<0.001**	1.79	0.455
	12	22.22	**<0.05**	1.39	0.291
	14	20	**<0.05**	3.33	0.574
A	8	38.29	0.101	26.98	0.096
	10	40.58	**<0.05**	26.21	0.273
	12	36.81	**<0.05**	30.26	0.105
	14	33.25	**<0.05**	27.89	0.113

according to the natural sequence of ontogenetic stages. This means that the consumed, elected and avoided prey items of older stages were a subset of those of earlier stages. The pattern is illustrated in Fig. 4A for the 12-stage classification. The results were independent of the number of ontogenetic categories used, except for the 8-stages avoidance matrix which resulted not significantly nested as compared with the null model. Note also that the strongest nestedness pattern was obtained for the electivity matrices, which were the only ones where a shift in diet breadth was detected by the correlation analyses (see Section 3.1).
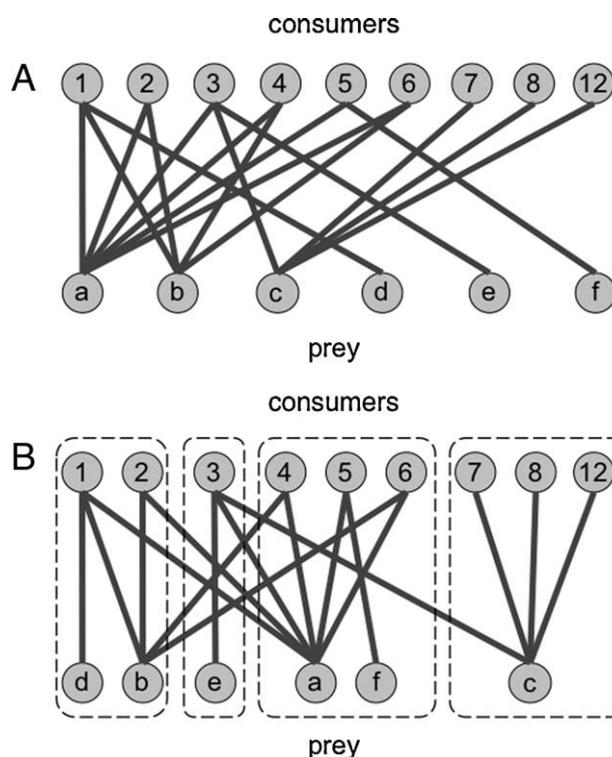

Fig. 4. Electivity network of the 12-stage predator–prey system, showing its nested (A) and modular (B) structures. Upper nodes represent the ontogenetic stages of the consumers (no diet record for stages 9–11), and lower nodes represent the elected prey types. Mass ranges of consumer's stages were: 1, <0.08 g; 2, 0.08–0.15 g; 3, 0.15–0.22 g; 4, 0.22–0.29 g; 5, 0.29–0.37 g; 6, 0.37–0.44 g; 7, 0.44–0.51 g; 8, 0.51–0.58 g; 12, 0.79–0.87 g. Elected prey were: a, *Mesochra lilljeborgi*; b, *Nereis diversicolor*; c, *Protracheroniscus occidentalis*; d, *Tisbe longicornis*; e, Acari; f, Hymenoptera.

Table 3
Modularity analysis of diet matrices for the Spanish toothcarp. Adjacency matrices **C**, **E**, and **A** contain information about consumed diet, elected diet, and avoided diet, respectively. Significance is assessed by contrasting the observed maximal modularity index of each matrix against the distribution of modularity values obtained from a null model that preserves the size, fill, and partition of consumer's stages into modules.

Matrix	Number of stages	Modularity P-value
C	8	0.116
	10	0.590
	12	0.629
	14	0.671
E	8	<**0.01**
	10	<**0.01**
	12	<**0.01**
	14	<**0.01**
A	8	0.112
	10	0.114
	12	0.488
	14	0.302

3.3. Modularity

Our modularity results showed that the electivity matrix of the Spanish toothcarp was significantly modular (Table 3). Ontogenetic stages were organized into four (for matrices with 8–12 stages) or five (for 14 stages) modules. Each module included a set of successive ontogenetic stages of the consumer and a set of prey that were strongly preferred by those stages and weekly preferred by stages of other modules. For the 12-stage classification (see Fig. 4B), the shifts in diet preferences occurred at consumer body masses of 0.15, 0.22, and 0.44 g. For a 10-stage classification, these values changed to 0.18, 0.27, and 0.44 g, respectively. Similar to the former results, these trends were qualitatively insensitive to moderate variations in the chosen number of stages. Consumption and avoidance matrices did not reveal any ontogenetic shift in diet, based on this analysis.

In Fig. 4B, the structure of the 12-stage electivity network is shown. Note that stages 9–11 were eliminated from the analysis since no fish of that size range were captured. The first module contained predator stages 1 and 2, and the prey *Nereis diversicolor* (Polichaeta) and *Tisbe longicornis* (Copepoda); the second module

contained predator stage 3 and unidentified Acari; the third module contained stages 4–6, the copepod *Mesochra lilljeborgi* and unidentified Hymenopteran; and the fourth module contained stages 7, 8, and 12, and the isopod prey *Protracheroniscus occidentalis*. The body length of elected prey belonging to different modules differed significantly (Kruskal–Wallis test, $P < 0.001$) and indeed they differed among any pair of modules (multiple comparisons by ranks, $P < 0.001$). Remarkably, the largest elected prey item (isopods) was the only prey of the module that included the largest predator stages.

In Table 4 we present the matrix of electivity data for the 12-stage configuration, in order to show that the module identification process is far from being trivial. The network approach presented here permits to accurately find the modular organization of the target matrix.

4. Discussion

In this study we have introduced an alternative approach for the study of ontogenetic niche shifts based on network theory. By representing a stage-structured consumer population and its set of consumed prey as a bipartite network, it becomes possible to test several structural patterns within the network. Among the structural measures, niche breadth, nestedness, and modularity, are of particular relevance for the study of ODS. Moreover, we have shown that considering the availability of prey in the environment for the construction of adjacency matrices based on electivity provides further insight about consumers' feeding decisions. Employing these methods for the analysis of a real dataset in order to illustrate the applicability of our approach, revealed a shift in diet breadth and a modular network structure when considering the matrix of actively elected prey. In addition, the consumed, as well as the elected and avoided prey items resulted to be significantly nested among consumers' stages. These results indicate that the fish predator decreased the richness of their consumed, elected and avoided prey types along its ontogeny, although the decrease in number of elected prey showed to be stronger. Most important, our modularity analysis revealed that different subsets of ontogenetic stages selected different prey items. Although providing a biological explanation to the observed trends is beyond the scope of this work, we hypothesize that gape limitation could be a factor responsible of the shift from module 3

Table 4
Electivity values of the consumer stages on each prey type. A value of −1 indicates that a given prey was not consumed by organisms of that stage. The values of positive electivity above the threshold value 0.17 are shown in bold, and were used for constructing the binary electivity matrix that resulted to be significantly modular.

Prey	Stage								
	1	2	3	4	5	6	7	8	12
1	−0.903	−1.000	−0.992	−1.000	−1.000	−1.000	−0.998	−1.000	−1.000
2	−1.000	−0.933	−1.000	−1.000	−1.000	−1.000	−1.000	−1.000	−1.000
3	0.068	−0.123	−0.714	0.020	−0.706	−1.000	−1.000	−0.888	−1.000
4	**0.202**	**0.346**	−0.389	**0.655**	−0.048	**0.334**	−0.194	−0.229	−1.000
5	−1.000	−1.000	**0.663**	−1.000	−1.000	−1.000	−1.000	−1.000	−1.000
6	−0.918	−1.000	−0.986	−0.990	−0.998	−1.000	−0.998	−0.999	−1.000
7	**0.495**	**0.655**	**0.205**	**0.542**	**0.405**	**0.308**	−0.916	−0.710	−0.845
8	−1.000	−0.740	−0.979	−1.000	−0.936	−1.000	−1.000	−1.000	−1.000
9	**0.236**	−0.764	−0.619	−0.188	−0.231	−0.822	−0.968	−0.977	−0.999
10	−1.000	−1.000	−0.987	−0.710	−1.000	−1.000	−0.915	−1.000	−1.000
11	−0.839	−0.867	−0.952	−0.967	−0.971	−0.982	−1.000	−0.999	−1.000
12	−1.000	−1.000	−1.000	−1.000	−0.055	−1.000	−1.000	−0.528	−1.000
13	−1.000	−1.000	**0.678**	−1.000	−1.000	−1.000	**0.756**	**0.773**	**0.662**
14	−0.688	−0.965	−0.912	−0.678	−0.924	−1.000	−0.954	−1.000	−0.994
15	−1.000	−1.000	−1.000	−0.901	−0.756	−1.000	−1.000	−1.000	−1.000
16	−1.000	−1.000	−1.000	−0.926	−1.000	−1.000	−1.000	−1.000	−1.000
17	−1.000	−1.000	−0.431	−1.000	0.103	−1.000	−1.000	−1.000	−1.000
18	0.167	−0.022	−1.000	−0.222	−1.000	−1.000	−1.000	−1.000	−1.000
19	−1.000	−1.000	−1.000	−1.000	−1.000	−1.000	−1.000	−0.944	−1.000
20	−1.000	−1.000	−1.000	−1.000	**0.696**	−1.000	−1.000	−1.000	−1.000

to module 4, since body lengths of prey fed by the oldest stages were markedly larger.

Note that in this study we considered that diet shifts comprise changes not only of what actually is being preyed on, but also changes in the prey that are being elected or avoided, in relation to resource availability. This particular definition of diet shift allows detecting changes in food preferences, independent of passive ingestion of abundant prey types. As a result, different patterns of ODS could be found for a single species inhabiting different environments. Although this could challenge a concept of ODS as a species-invariant trait, recent developments show that ODS patterns are not expected to be fixed within species, but varying as a function of food requirements as well as food availability (Takimoto, 2003). Careful comparison of ODS among different habitats could provide new insights about the causes and consequences of feeding behavior.

In previous studies, it has been suggested that certain measures of network structure could be informative for the characterization of ODS. For example, Woodward and Hildrew (2002) reported that diet breadth increased with predator size, and conjectured that the dietary composition of the aquatic predators studied exhibited a nested structure during ontogeny. Likewise, Werner and Gilliam (1984) suggested that certain types of ODS occur when "the niche of all smaller size-classes is included in that of all larger classes." (p. 395), i.e. a nested pattern. Nevertheless, to our knowledge, diet nestedness during ontogeny had not been statistically tested previously. On the other hand, abrupt changes in diet habits during ontogeny have been recognized since long ago, both in metamorphic and non-metamorphic species (Werner and Gilliam, 1984). This kind of ODS has been customarily assessed through evaluating the degree of diet overlap among predator's ontogenetic stages (Woodward and Hildrew, 2002; Anneville et al., 2007; Graham et al., 2007). Although hierarchical clustering can be used for the classification of ontogenetic stages based on diet similarity (e.g. Rezsu and Specziár, 2006), it becomes problematic to choose which clusters or modules to extract from the hierarchy. It is also not easy to know how reliable the obtained clustering is, compared to other models. Therefore, when the aim is at identifying discrete sets of related entities, a partitioning method should be preferred, in association with proper null model analysis.

The network approach presented here offers a simple method for detecting ontogenetic diet shifts, and combined with null model analyses provides a robust means of detecting gradual and abrupt shifts in diet composition or preferences. Our main contribution rests on adapting topological analyses of networks to the study of ODS. Particularly, we presented a method for testing modularity without altering the order of the ontogenetic stages of the consumer. Furthermore, we show the utility of building binary electivity matrices based on quantitative diet records, for the search of trends in diet preferences along the ontogeny. This approach is flexible enough to be adapted for a wide range of specific questions, and prone to be used for a systematic comparison of diet shifts, both within and among species. For example, it would be possible to test whether the size or age of consumers explain observed niche shifts, or find the phenotypic traits of prey that are best candidates as drivers for observed consumer's niche shifts. This would be accomplished by using different network configurations, e.g. defining the consumers' stages by means of size or age classes, and classifying the prey types by species, body size, or any other phenotypic trait.

However, the performance of any quantitative method will be a function of the quality of data. Regarding the detection of ODS for a particular species, individual consumers of all ages or sizes should be sampled in different sites to ensure the independence of the feeding preferences of each ontogenetic stage. Moreover, a careful sampling design should be carried out in order to deal with spatial and temporal heterogeneity of most natural systems. Probably, stages will not be homogenously distributed over space and time, and thus they will be exposed to different prey assemblages. In these cases, heterogeneous prey availability may obscure conclusions about ontogenetic shifts if sampling effort is relatively reduced or not well planned. On the other hand, high-quality field information will allow using quantitative indices of network structure currently under development (e.g. Galeano et al., 2009), instead of indices as the ones used here based on binary information. Nevertheless, we chose using binary indices because they are well established in the literature and are less sensitive to sampling error. However, the fast advance in network theory portends the availability of new quantitative tools to be applied on high-resolution data. In the same vein, detailed data would allow networks to be constructed using individual consumers instead of stages, an approach that deserves further methodological development.

Ontogenetic differences in diet preferences can easily exceed interspecific differences (Polis, 1984; Woodward and Hildrew, 2002). ODS can thus exert a large influence on community structure and functioning (Polis et al., 1996) and a reliable detection of ODS patterns can help to improve our understanding of food webs.

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