

## Biochemical fingerprints in zooplankton

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### SUMMARY

The use of the role of the species in the habitat (the niche), rather than systematics, for studying the factors that determine which and how many species live in a specific habitat (community assembly), is an approach that has been limited by obvious difficulties in the characterization of the niche. This study shows that is possible to use biochemical fingerprints as indicators of both "requirement niche" and "impact niche" in zooplankton species. Protein content is a good indicator of the nutritive state of the animals and, also, it is a good tool for elucidating food-competitive capacities among species. Reproductive success is positively related with the organic content of the egg (the sum of proteins, carbohydrates, and lipids). Finally, the amino acid composition of the species is a good indicator of the trophic niche and the adaptations of the species to abiotic factors.

**Key words:** Proteins, carbohydrates, lipids, amino acids, zooplankton, copepods, cladocerans, rotifers, niche, community assembly.

### RESUMEN

*La utilización del papel de las especies en el hábitat (el nicho), en lugar de la sistemática, para estudiar los factores que determinan cuantas y cuales especies viven en un hábitat (ensamblaje de la comunidad), se ha visto limitado por dificultades obvias a la hora de caracterizar el nicho. Este estudio muestra que es posible usar huellas bioquímicas como indicadores del "nicho de requisitos" y el "nicho de impacto" en especies del zooplancton. El contenido en proteínas es un buen indicador del estado nutritivo de los animales y también es una herramienta útil para dilucidar entre las capacidades competitivas de las especies por el alimento. El éxito reproductivo está relacionado positivamente con el contenido orgánico del huevo (la suma de proteínas, carbohidratos y lípidos). Finalmente, la composición de aminoácidos de las especies es un buen indicador del nicho trófico y de las adaptaciones de las especies a los factores abióticos.*

**Palabras clave:** proteínas, carbohidratos, lípidos, aminoácidos, zooplancton, copépodos, cladóceros, rotíferos, nicho, ensamblaje de comunidades.

### INTRODUCTION

One of the main aims of Ecology is to determine the processes that determine which and how many species live in a specific habitat (community assembly). The identification of the main factors structuring the assemblage of the zooplankton communities usually relies on studies based on the co-occurrence (or lack of it) of species, and changes in the abundance of the species according to changes in the abiotic factors.

A different approach for studying community assembly is to compare the niches of the species. For instance, if the main factor structuring a community were a depletable factor in the environ-

ment, hence potentially limiting, then the co-existence of the species would be the result of a reduced overlap in resource use and, therefore, the niche of the species would be different. Co-occurring species would have a similar niche, if the assemblage of the community were mainly governed by environmental factors that limit populations (e.g., temperature, salinity, etc).

The ecological approach based on niche comparison has been limited by obvious difficulties in the characterization of the niche of the species. A correct description of the niche requires characterising the two distinct niche components (Leibold, 1995): 1) the requirement niche, describing the response of the species to the

environment and, 2) the impact niche, describing the effect of the species on the habitat.

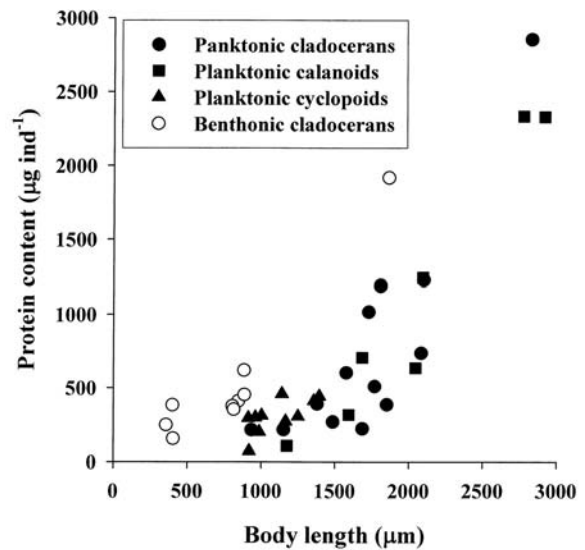
In this review I show that it is possible to describe both the requirement niche and the impact niche of zooplankton species, by using some biochemical fingerprints. These fingerprints are indicators of the optimal growth and reproduction of zooplankton species for the different ecological factors. Therefore, they are indicators of the adaptations of the species to the abiotic factors (requirement niche), and also of the differential use of the niche (impact niche).

### NUTRITIONAL CONDITION

Food availability is one of the most important factors affecting the population dynamics of zooplankton species (Threlkeld, 1976; Tessier & Goulden, 1982; Gliwicz 1985; Guisande & Toja, 1988; Duncan, 1989; Müller-Navarra & Lampert 1996). The amount of food affects intraspecific variations in population abundance because it controls the reproductive output, but it may also play a significant role in determining the co-occurrence of zooplankton species, because of the exclusion of those with lower competitive abilities or the co-existence of others as a result of a reduced overlap in food resource use.

Field studies have shown that there is a log positive relationship between protein content of the individuals and food abundance (Serrano & Guisande, 1989; Guisande *et al.*, 1991b). Therefore, protein content of the animals is a good indicator of the nutritive state in zooplankton, i.e., if the species is food limited or is at the optimum nutritive state in the habitat.

Moreover, the protein content-length regressions allow for determining which species are favoured when food concentration is low (Guisande *et al.*, 1991b) and hence, it is a good tool for elucidating food competitive abilities among species. Figure 1 is an example that protein content may be a good tool for comparing competitive abilities among zooplankton species. The protein content was higher in the benthic than in the planktonic species collected from Pyrenean lakes (Fig. 1). Therefore, in



**Figure 1.** Relationship between body length and protein content, considering only 15 amino acids (see Guisande *et al.*, 2003), of different species of cladocerans, diaptomids and cyclopoids collected from Pyrenean mountain lakes. *Relación entre la longitud del cuerpo y el contenido en proteínas, considerando sólo 15 amino ácidos (ver Guisande *et al.*, 2003), de diferentes especies de cladóceros, diaptómidos y ciclópodos recolectados en lagos de montaña del Pirineo.*

these oligotrophic lakes, it seems that those species that can feed on periphyton or detritus, which are mainly found on the water body bottom, may have a competitive advantage in comparison with planktonic species.

Protein content also allows for the discrimination between the behaviour of individuals or sub-populations (Guisande *et al.*, 1991a). Zooplankton species perform diel vertical migrations as a trade-off between reduced mortality and reduced output due to lower food availability in their refuge. Length-body protein regression in cladocerans, as a measure of nutritional state, allowed for the discrimination between migrating and non-migrating individuals of *Daphnia hyalina-galeata* (Guisande *et al.*, 1991a). The daphnids that remain in the surface and, hence, do not migrate, have a higher protein content than those animals that do migrate. Therefore, in addition to discriminate between migrating and non-migrating animals, the protein content, as an indicator of nutritional condition, also allowed for the demonstration that migrating animals gain no metabolic advantage over non-migrating ones.

## REPRODUCTIVE SUCCESS

In addition to changes in breeding intensity, much of the seasonal change in population densities of zooplankton species can also be ascribed to variability in reproductive success.

Zooplankton species are able to modify their reproductive strategy according to changes in temperature, food level, intra-specific competition and/or risk of predation, in order to maximize offspring fitness (see for review Guisande & Gliwicz, 1992; Guisande 1993). The change in reproductive strategy is usually associated to a trade-off between offspring size and number (see Guisande & Mazuelos, 1991; Guisande *et al.*, 1996), which represents a compromise between neonate fitness and maximum possible clutch size (Tessier & Consolatti, 1989). Since egg size varies directly with protein, carbohydrate, and lipid content of the eggs (Guisande & Gliwicz, 1992; Guisande, 1993; Galindo *et al.*, 1993; Guisande & Harris, 1995), and offspring survival depends on the biochemical composition of the egg (Guisande & Gliwicz, 1992; Guisande *et al.*, 1993; Guisande & Harris, 1995; Guisande *et al.*, 1996; Guisande *et al.*, 1998; Riveiro *et al.*, 2000; 2004), the organic content of the egg is a good indicator of reproductive success in zooplankton species.

Reproductive success also depends on food quality. A diet that is optimally balanced is an important factor affecting zooplankton reproductive success (see for review Guisande *et al.*, 1999). If the biochemical composition of the food is too different from the biochemical composition of the zooplankton species, the lag time between food ingested and it being converted into the production of eggs is probably too short to produce eggs with an optimal proportion of biomolecules (amino acids, fatty acids, etc.) and elements (N, P, etc.). Therefore, food quality may be an important factor affecting the population dynamics of zooplankton species, but the problem is the difficulty of quantifying food quality. A higher reproductive success is observed as the amino acid composition of copepod species becomes closer to the amino acid composition

of food (Guisande *et al.*, 1999). Therefore, the comparison between the amino acid composition of zooplankton species and their food is a good indicator of food quality.

## AMINO ACID COMPOSITION AS AN INDICATOR OF DIFFERENTIAL USE OF NICHE AND THE ADAPTATION TO THE ABIOTIC ENVIRONMENT

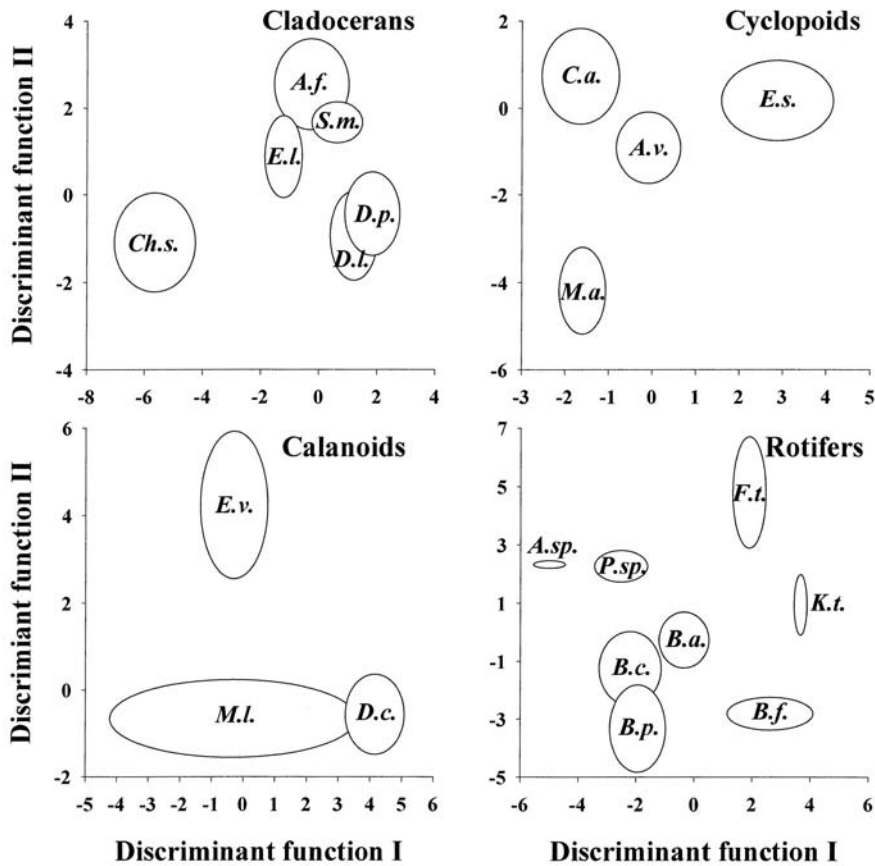
*Amino acid composition is species-specific.* A biochemical fingerprint, which informs about the differential use of the niche of the species and the adaptation to the environment, must be species-specific. This has been demonstrated in zooplankton species (Guisande *et al.*, 2002; 2003; Riveiro *et al.*, 2003; Boëchat & Adrian, 2005).

A discriminant analysis performed on the amino acid composition of cladocerans, cyclopoids and calanoids from Pyrenean lakes with altitudes ranging between 1,875 and 2,990 m (Guisande *et al.*, 2003), and rotifers from peridunal ponds of Doñana National Park revealed that the percentage of species correctly classified was 86.3 %, 94.1 %, 100 % and 94.2 % respectively (Fig. 2). Therefore, despite the intraspecific variation, it is possible to identify each species according to its amino acid composition.

## Trophic niche

Food resource partitioning may play a significant role in determining the co-occurrence of zooplankton species. However, evidence of exploitative competition usually relies on the co-existence (or lack of it) of species assigned to functional groups or guilds based solely on systematics. Few studies have directly examined the types of food consumed by zooplankton or the way in which food is gathered, because there are obvious difficulties in performing field observations of food use.

The amino acid composition of zooplankton species can be used to study food resource partitioning in zooplankton communities because it is a good indicator of both the “requirement niche” and the “impact niche” (Leibold, 1995).



**Figure 2.** Plots of the mean  $\pm$  SD of first two discriminant function scores for the amino acids of the species. Species abbreviation: *Daphnia longispina* (D.l.), *Daphnia pulex* (D.p.), *Alona affinis* (A.f.), *Chydorus sphaericus* (Ch.s.), *Scapholeberis mucronata* (S.m.), *Eurycercus lamellatus* (E.l.), *Cyclops abyssorum* (C.a.), *Macrocyclus albidus* (M.a.), *Acanthocyclops vernalis* (A.v.), *Eucyclops serrulatus* (E.s.), *Eudiaptomus vulgaris* (E.v.), *Diaptomus cyaneus* (D.c.), *Mixodiaptomus laciniatus* (M.l.), *Poyarthra* sp. (P.sp.), *K. tropica* (K.t.), *Filinia terminalis* (F.t.), *B. plicatilis* (B.p.), *B. falcatus* (B.f.), *B. calyciflorus* (B.c.), *B. angularis* (B.a.) and *Asplanchna* sp. (A.sp.). Representación de la media  $\pm$  DE de las dos primeras funciones discriminantes de los amino ácidos de las especies. Abreviación de las especies: *Daphnia longispina* (D.l.), *Daphnia pulex* (D.p.), *Alona affinis* (A.f.), *Chydorus sphaericus* (Ch.s.), *Scapholeberis mucronata* (S.m.), *Eurycercus lamellatus* (E.l.), *Cyclops abyssorum* (C.a.), *Macrocyclus albidus* (M.a.), *Acanthocyclops vernalis* (A.v.), *Eucyclops serrulatus* (E.s.), *Eudiaptomus vulgaris* (E.v.), *Diaptomus cyaneus* (D.c.), *Mixodiaptomus laciniatus* (M.l.), *Poyarthra* sp. (P.sp.), *K. tropica* (K.t.), *Filinia terminalis* (F.t.), *B. plicatilis* (B.p.), *B. falcatus* (B.f.), *B. calyciflorus* (B.c.), *B. angularis* (B.a.) y *Asplanchna* sp. (A.sp.).

Guisande *et al.*, (1999; 2000; 2002) showed that the amino acid composition of some marine copepods is species-specific and remains relatively constant despite distinct nutritional supplies, and there are important differences in the amino acid composition among zooplankton and phytoplankton species (Guisande *et al.*, 1999). Moreover, the more similar the amino acid composition of the food to the amino acid composition of the species, the greater the reproductive success (Guisande *et al.*, 1999).

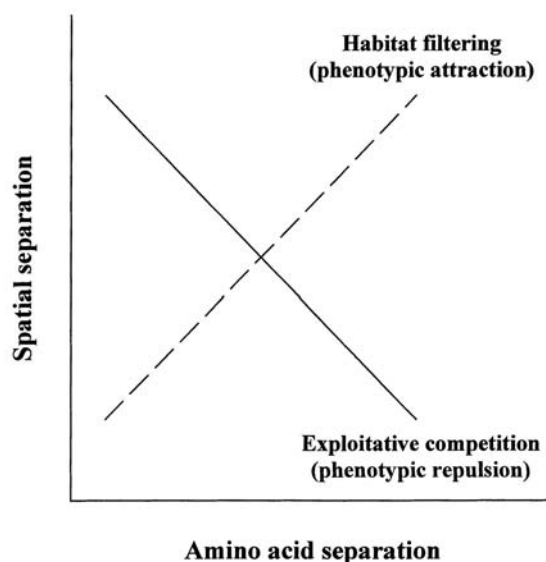
Therefore, amino acid composition is a good indicator of the “requirement niche” for optimal growth and reproduction. On the other hand, although it has been shown that copepods can assimilate some amino acids selectively (Cowie & Hedges, 1996), the main differences in amino acid composition among zooplankton species are mainly due to differences in diet (Guisande *et al.*, 2002). Therefore, zooplankton amino acid composition is also a good indicator of the “impact niche” of each species.

Mountain lakes are an appropriate setting in which to study the role of food resource partitioning in zooplankton, mainly because these lakes are oligotrophic to ultra-oligotrophic, thus food limitation may have played a significant role in evolutionary history. Guisande *et al.*, (2003) analysed the amino acid composition of 6 cladocera and 7 copepod species from 29 Pyrenean mountain lakes. A discriminant analysis showed that each species could be distinguished according to its amino acid composition. A negative relationship between amino acid differentiation and co-occurrence among the cladocera and cyclopoid copepod was observed. As the differences in the amino acid composition among zooplankton species indicate distinct food sources (Guisande *et al.*, 2002; McClelland & Montoya, 2002), the relationship found indicates that trophic-niche differentiation plays a key role in determining the assemblage of these zooplankton communities. Therefore, by driving co-evolutionary histories either at present or in the past, exploitative competition played a significant factor in structuring the cladocera and cyclopoid communities in these oligotrophic lakes.

### Abiotic niche

In the previous section it was shown that amino acid composition of zooplankton species is a good indicator of the trophic niche of the species. Protein expression can also be directly related to stress in that proteins are used by zooplankton species to adapt to changing habitat conditions (Kimmel & Bradley, 2001). Therefore, amino acid composition may also provide a natural tag for the adaptation of each species to its habitat, in other words, information about the abiotic niche of the species. The identification of common adaptations of co-occurring species may allow identifying the main abiotic factors in structuring community assemblages.

In a study in ponds of Doñana National Park (SW, Spain), a negative relationship between amino acid separation and spatial overlap among rotifer species was observed, indicating that those species with a similar amino acid composition have a higher spatial overlap.



**Figure 3.** Theoretical relationship between amino acid composition separation and spatial overlap among species under a situation of phenotypic repulsion (solid line) and phenotypic attraction (dashed line). *Relación teórica entre la separación de la composición de amino ácidos y el solapamiento espacial entre especies bajo una situación de repulsión fenotípica (línea continua) y atracción fenotípica (línea discontinua).*

Therefore, there is an opposite pattern in the rotifer community of the ponds in contrast to the one observed in cladocera and copepods of Pyrenean lakes (Guisande *et al.*, 2003). Those species with a similar amino acid composition and hence, with a similar trophic niche, spatially co-occur, indicating that trophic-niche differentiation was not the main factor in structuring rotifer assemblages in the ponds.

The high similarity in the amino acid composition among co-occurring species could be interpreted as evidence for a predominant role in habitat filtering, indicating the relevant role of abiotic factors on the assemblages of the rotifer community in the ponds. The positive relationship between amino acid separation and species separation according to their weighted mean for the concentration of silicates indicates that salinity was the main abiotic factor in structuring the assembly of rotifer communities in the ponds.

Therefore, amino acid composition of zooplankton species, in addition to being species-specific and a good indicator of the trophic niche of the species (Guisande *et al.*, 2002; 2003; McClelland & Montoya, 2002; Boëchat

& Adrian, 2005), is also a good indicator of the adaptation of the species to the abiotic conditions of the habitat. Therefore, amino acid composition is a good indicator of the ecological niche of zooplankton species and therefore, a good tool to elucidate the way zooplankton communities assemble from a regional pool of species. The relationship between amino acid separation and spatial separation among zooplankton species will be negative when exploitative competition (phenotypic repulsion), either at present or in the past by driving co-evolutionary histories, is the most significant factor in structuring the zooplankton community (Fig. 3). The relationship will be positive when there is a predominant role for habitat filtering (phenotypic attraction).

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#### BIBLIOGRAPHY

- BOËCHAT, I. G. & R. ADRIAN. 2005. Biochemical composition of algivorous freshwater ciliates: You are not what you eat. *FEMS Microbiol. Ecol.*, 53: 393-400.
- COWIE G. L. & J. I. HEDGES. 1996. Digestion and alteration of the biochemical constituents of a diatom (*Thalassiosira weissflogii*), ingested by an herbivorous zooplankton (*Calanus pacificus*). *Limnol. Oceanogr.*, 41: 581-594.
- DUNCAN A. 1989. Food limitation and body size in the life cycles of planktonic rotifers and cladocerans. *Hydrobiologia*, 186/187: 11-28.
- GALINDO, M. D., C. GUISANDE & J. TOJA. 1993. Reproductive investment of several rotifer species. *Hydrobiologia*, 255/256: 317-324.
- GLIWICZ, Z. M. & C. GUISANDE. 1992. Family planning in *Daphnia*: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia*, 91: 463-467.
- GLIWICZ, Z. M. 1985. Predation or food limitation: an ultimate reason for extinction of planktonic cladocera species. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, 39: 419-430.
- GUISANDE, C. & J. TOJA. 1988. The dynamics of various species of the genus *Brachionus* (ROTATORIA) in the Guadalquivir river. *Archiv. Hydrobiol.*, 112: 579-595.
- GUISANDE, C. & L. SERRANO. 1989. Analysis of protein, carbohydrate and lipid in rotifers. *Hydrobiologia*, 186/187: 339-346.
- GUISANDE, C. & N. MAZUELOS. 1991. Reproductive pattern of *Brachionus calyciflorus* Pallas at different food concentrations. *J. Plankton Res.*, 13: 279-286.
- GUISANDE, C., A. DUNCAN. & W. LAMPERT. 1991a. Trade-offs in *Daphnia* vertical migration strategies. *Oecologia*, 87: 357-359.
- GUISANDE, C., J. TOJA & N. MAZUELOS. 1991b. The effect of food on protein content in rotifer and cladoceran species: a field correlational study. *Freshwat. Biol.*, 26: 433-438.
- GUISANDE, C. & Z. M. GLIWICZ. 1992. Egg size and clutch size in two *Daphnia* species grown at different food levels. *J. Plankton Res.*, 14: 997-1007.
- GUISANDE, C. 1993. Reproductive strategy as population density varies in *Daphnia magna* (CLADOCERA). *Freshwat. Biol.*, 29: 463-467.
- GUISANDE, C., M. D. GALINDO, F. M. GALAN & F. OLIVEROS. 1993. The cost of reproduction in the rotifer *Brachionus calyciflorus*. *Int. Revue ges. Hydrobiol.*, 78: 493-499.
- GUISANDE, C. & R. HARRIS. 1995. Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. *Limnol. Oceanogr.*, 40: 476-482.
- GUISANDE, C., J. SÁNCHEZ, I. MANEIRO & A. MIRANDA. 1996. Trade-off between offspring number and offspring size in the marine copepod *Euterpina acutifrons* DANA at different food concentrations. *Mar. Ecol. Progr. Ser.*, 143: 37-44.
- GUISANDE, C., I. RIVEIRO, A. SOLÁ & L. VALDÉS. 1998. Effect of biotic and abiotic factors on the biochemical composition of wild eggs and larvae of several fish species. *Mar. Ecol. Progr. Ser.*, 163: 53-61.
- GUISANDE, C., I. MANEIRO & I. RIVEIRO. 1999. Homeostasis in the essential amino acid composi-

- tion of the marine copepod *Euterpina acutifrons*. *Limnol. Oceanogr.*, 44: 691-696.
- GUISANDE, C., I. RIVEIRO & I. MANEIRO. 2000. Comparison between the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality on copepod reproduction. *Mar. Ecol. Progr. Ser.*, 202: 135-142.
- GUISANDE, C., I. MANEIRO, I. RIVEIRO, A. BARRERO & Y. PAZOS. 2002. Estimation of copepod trophic-niche in the field using amino acids and marker pigments. *Mar. Ecol. Progr. Ser.*, 239: 147-156.
- GUISANDE, C., F. BARTUMEUS, M. VENTURA & J. CATALAN. 2003. Role of food partitioning in structuring the zooplankton community in mountain lakes. *Oecologia*, 136: 627-634.
- KIMMEL, D. G. & B. P. BRADLEY. 2001. Specific protein responses in the calanoid copepod *Eurytemora affinis* (Poppe, 1880) to salinity and temperature variation. *J. Exp. Mar. Biol. Ecol.*, 266: 135-149.
- LEIBOLD, M. A. 1995. The niche concept revisited: Mechanistic models and community context. *Ecology*, 76: 1371-1382.
- MCCLELLAND, J. W. & J. P. MONTOYA. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, 83: 2173-2180.
- MÜLLER-NAVARRA D. & W. LAMPERT. 1996. Seasonal patterns of food limitation in *Daphnia galeata*: separating food quantity and food quality effects. *J. Plankton Res.*, 18: 1137-1157.
- RIVEIRO, I., C. GUISANDE, M. LLOVES, I. MANEIRO & J. M. CABANAS. 2000. Importance of parental effect on larval survival in *Sardina pilchardus*. *Mar. Ecol. Progr. Ser.*, 205: 249-258.
- RIVEIRO I, C. GUISANDE, C. FRANCO, A. LAGO DE LANZÓS, I. MANEIRO I & A. R. VERGARA. 2003. Egg and larval amino acid composition as indicators of niche resource partitioning in pelagic fish species. *Mar. Ecol. Progr. Ser.*, 260: 255-262.
- RIVEIRO, I., C. GUISANDE, I. MANEIRO & A. R. VERGARA. 2004. Parental effects in the European sardine (*Sardine pilchardus*). *Mar. Ecol. Progr. Ser.*, 274: 225-234.
- THRELKELD S. T. 1976. Starvation and the size structure of zooplankton communities. *Freshwat. Biol.*, 6: 489-496.
- TESSIER, A. J. & N. L. CONSOLATTI, 1989. Variation in offspring size in *Daphnia* and consequences for individual fitness. *Oikos*, 56: 269-276.
- TESSIER, A. J. & C. E. GOULDEN. 1982. Estimating food limitation in cladocera populations. *Limnol. Oceanogr.*, 27: 707-717.