

Stable carbon and nitrogen isotope quantitative structural assessment of dominant species from the Vaccarès Lagoon trophic web (Camargue Biosphere Reserve, France)

Ana Persic, Hélène Roche*, François Ramade

University of Paris-Sud XI, CNRS UMR 8079, ESE Ecology Systematic and Evolution, Bldg 362, F91405 Orsay Cedex, France

Received 31 July 2003; accepted 5 January 2004

Abstract

The Vaccarès Lagoon (Camargue Biosphere Reserve), a complex brackish ecosystem, is the natural habitat for numerous freshwater, marine and euryhaline species forming a particularly intricate food web. The main objectives of this study were to describe its trophic relations and investigate factors influencing its structure and dynamics. The combined stable C and N isotope method was used to establish a quantitative assessment of the trophic status of Vaccarès organisms. Although the levels of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ showed large intraspecific and interspecific variations, the isotopic signatures of the species assessed revealed a general trend of ^{15}N enrichment with trophic level. Distribution of the biota into four trophic compartments—depositivore, zooplanktivore, predator, and top-predator—was corroborated by the general analysis of the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Analysis of seasonal and annual isotopic variations showed that natural environmental changes (increase in food abundance, climatic and salinity variations) as well as individual metabolic capacities would affect the relative stability of an organism's trophic position. No significant correlation was found between isotopic variation and size and growth in fishes except for the eel population where the positive correlation between these two parameters confirmed intra-population trophic variations. Finally, separately examined species exhibited significant, although incoherent, correlation between their isotopic signature and lipid content. This study confirms the efficiency of the isotopic approach in trophic studies and highlights the need for further investigations of anthropic environmental modifications occurring in this protected area and frequently disturbing its food web.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: wetland; stable isotopes; trophic relations; protected area; Camargue

1. Introduction

The French National Nature Reserve of Camargue (NNRC) ranks as the largest strict Nature Reserve (IUCN—UN Category n° 1) of the protected coastal wetlands in Western Europe. Spreading over about 14 000 ha, the NNRC, which is located in the central part of a landscape protected area, the Regional Natural Park of Camargue, is representative of a threatened type of ecosystem and as such was designated in 1977 by UNESCO as an 'International Biosphere' Reserve.

The NNRC currently preserves a complex mosaic of both terrestrial and aquatic habitats enabling extensive

biodiversity (Fig. 1). The Vaccarès Lagoon (6400 ha) stands as the largest among the numerous shallow lagoons that occur in the lowest central and southern parts of the NNRC. It is located between two arms of the Rhone River and the coastline. Erection of dikes, high input of fresh water and low water exchange with Mediterranean Sea has generated a positive southward gradient of salinity within the lagoon. In such littoral wetlands the hydrological balance depends on both seasonal variations and freshwater input from canals (Ramade, 1998, 2002; Gelin et al., 2001; Pampoulie et al., 2001). The sheer number, diversity, and complexity of abiotic and biotic factors (Rosecchi et al., 1995; Bardin and Pont, 2002; Roche et al., 2003) render this ecosystem unique and the description of its dynamic food web has proven particularly challenging. Since the food web relationships play a major structuring role in

* Corresponding author.

E-mail address: helene.roche@ibaic.u-psud.fr (H. Roche).

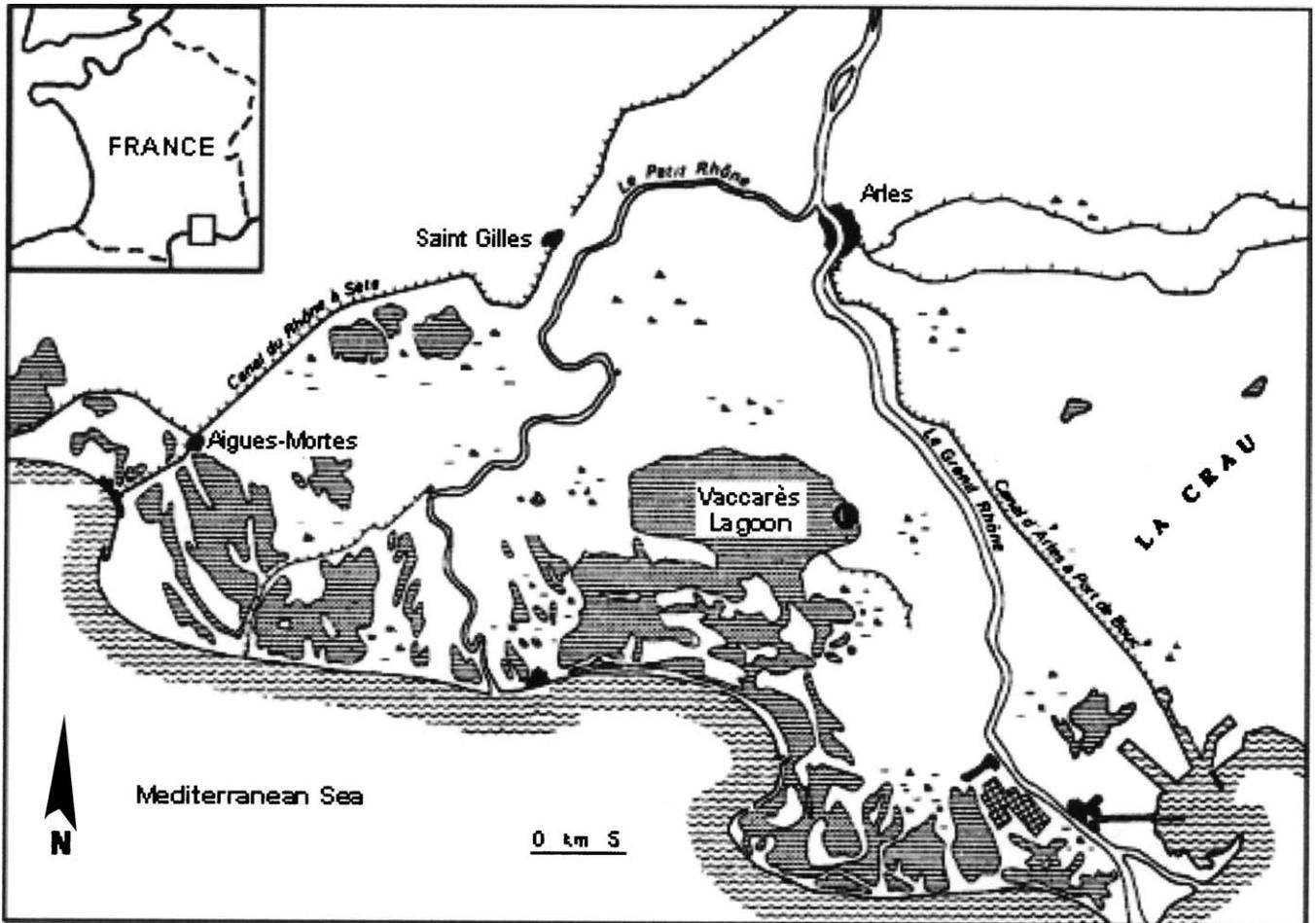


Fig. 1. The Camargue Biosphere Reserve. The sampling site La Capelière (C) is located in the East of the Vaccarès Lagoon.

aquatic ecosystems, determination of the trophic position of an organism highlights numerous aspects of its biology as well as a wide range of ecological patterns and processes. The identification of trophic levels is however confounded by a high level of food web complexity and spatial or temporal variations at a number of scales. Traditional approaches to food web descriptions include gut or stomach content analysis, together with field and laboratory observations (Richter et al., 1999; Beaudoin et al., 2002). These studies highlight what animals feed on with a high degree of taxonomic precision. However, they are labor intensive, subject to errors and delineating what was actually digested and assimilated by consumers remains speculative. The stable isotope method, based on the selective metabolic partition which leads to a preferential waste of lighter isotopes during respiration and excretion (De Niro and Epstein, 1978; Rau et al., 1983), seems to overcome some of these difficulties. This integrative approach distinguishes assimilated rather than ingested food reflecting the complexity of food webs over longer periods (Guiguer et al., 2002; Renones et al., 2002). It has been shown that

naturally occurring stable isotopes of nitrogen and carbon display a stepwise enrichment between prey and consumer tissue. In particular, the heavier nitrogen isotope becomes progressively enriched from prey to predator (3–5‰). The values of $\delta^{15}\text{N}$ therefore provide a continuous variable which is able to quantify relative trophic positioning of biota (Van der Zanden et al., 1997). Stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$; $\delta^{13}\text{C}$ values) also show trophic enrichment (1‰) but are principally used to elucidate sources of primary production in aquatic ecosystems and to trace carbon flow from primary producers to tertiary consumers (Gu et al., 1996; Vizzini et al., 2002).

This paper presents the first attempt to make an accurate description and representation of the Vaccarès Lagoon food web using the combined stable C and N isotope method. The objectives of this work were to characterize trophic relationships in dominant species within the lagoon, to describe their changes in time and to examine factors influencing the trophic status of individual organisms whose feeding history is poorly understood.

2. Materials and methods

2.1. Biological material and sample collection

Species were selected in accordance with the annual NNRC biodiversity report, an internal publication of the French National Nature Conservancy Society (SNPN). Sixteen different aquatic species were screened according to their estimated trophic level in the Vaccarès Lagoon food web (Crivelli, 1981; Bardin and Pont, 2002). Common and scientific names and the biometry of the investigated fish species are given in Table 1.

For the present study, the lowest trophic level was the zooplankton level, which is mainly composed of phytoplanktivorous copepods. Species in the lower trophic level studied were the cockle *Cerastoderma glaucum*, a bivalve, and crustacean species: Mysidaceae *Mysis* sp.; *Gammarus* sp., gammarid shrimps and *Palaemonetes varians* and *Crangon crangon* pink and brown shrimps, respectively. According to data in the literature, based mainly on classical gut content analysis *C. glaucum* is a filter-feeding species that feeds on phytoplankton and on particulate organic matter deposits. The mysids and the gammarid shrimps are mainly zooplanktivorous, feeding on copepods and detritus. At higher trophic levels three 'groups' of fishes were considered; the first consisted of organisms that feed mainly on smaller crustaceans and zooplankton (*Atherina boyeri*, *Gobius niger*, *Pomatoschistus* sp., *Gasterosteus aculeatus*, *Syngnathus acus*), the second was represented by the predator fishes feeding on smaller fishes (*Anguilla anguilla*, *Lepomis gibbosus*, *Stizostedion lucioperca*). *Abramis* sp. and Mugilidae sp. comprised the third 'group', i.e. preferentially freshwater fishes. Juveniles of mullets and breams generally feed on periphyton and detritus but may also prey on macroinvertebrates such as insect larvae. The sampling site, an area called La Capelière, is located in the eastern part of the lagoon, approximately 300 m to the north of the mouth of the canal which drains rice field irrigation waters (Fig. 1). Biological samples were collected in March, April, May, June and September 2001 and in March, April and May 2002.

Fishes were sampled with fixed fishing nets, bivalves were collected with metallic-mesh strainers and crustaceans were trawled in the sampling sites. When collected, all the samples were stored frozen for transport to the laboratory at the Paris-Sud University. The stable isotope analyses were performed on the whole body mass of invertebrates and smaller fishes, whereas samples of dorsal muscle of predator fishes were taken and subsequently analyzed. The same sample, which was pooled for the invertebrates and processed individually for the fishes, was used for C and N measurements.

2.2. Stable isotope analysis

After lipid extraction and separation (chloroform/methanol treatment), the lipid-free residues were filtered and the obtained tissue samples were freeze-dried and powdered. The 1 mg powder sub-samples were packed into 3.3 × 5 mm tin capsules for stable isotope measurements performed with a continuous flow isotope ratio mass spectrometer (VG Optima; Model NA-1500, Carlo Erba). The C and N stable isotopes were analyzed on two separate sub-samples. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The R standard values were based on PeeDee Belemnite (PDB) for ^{13}C and atmospheric N_2 for ^{15}N . Replicate measurements of internal laboratory standards indicate measurement errors of 0.2‰ and 0.3‰ for carbon and nitrogen isotope measurements, respectively.

2.3. Data analysis

Statistical analyses were achieved with the Statview program. When the normality and the variance homogeneity of the data were demonstrated, statistical differences were checked using the parametric Student's

Table 1
Biometry of the analyzed fish species (p: pools; (n) = number of measures)

| | Common name | n | Size (cm) | Weight (g) |
|-----------------------------------|--------------------------|-------|-----------|------------|
| <i>Atherina boyeri</i> | Sand smelt | 41 | 4.0–10.0 | 0.6–8.0 |
| <i>Gobius niger</i> | Black goby | 4 p. | 6.0–6.3 | 1.77–1.88 |
| <i>Pomatoschistus</i> sp. | Sand goby | 16 | 2.2–5.6 | 0.24–2.36 |
| <i>Gasterosteus aculeatus</i> | Three-spined stickleback | 24 | 2.0–5.3 | 0.09–1.84 |
| <i>Syngnathus acus</i> | Pipe-fish | 10 p. | 5.5–11.6 | 0.20–0.77 |
| <i>Anguilla anguilla</i> juvenile | European eel | 19 | 20.0–33.0 | 10.4–89.6 |
| <i>Anguilla anguilla</i> yellow | European eel | 14 | 39.0–57.6 | 107–387 |
| <i>Lepomis gibbosus</i> | Common sunfish | 4 | 10.5–12.4 | 26.4–44.7 |
| <i>Stizostedion lucioperca</i> | Pike-perch | 4 | 27.0–27.9 | 152–170 |
| <i>Abramis</i> sp. | Common bream | 4 | 9.0–17.6 | 7.8–52.5 |
| Mugilidae sp. | Mullet | 6 | 2.2–4.1 | 0.12–0.65 |

t-test. Correlations were calculated with Pearson's coefficient.

3. Results

3.1. Stable isotope composition

Sixteen species (1 pooled group of copepods; 5 invertebrates; 10 fishes) of the Vaccarès community were analyzed for stable nitrogen and carbon isotopes. This non-exhaustive list was established, notably for fishes, as a function of the capture success by authorized fishermen in this protected area and therefore corresponds to the current (2001–2002) most common species encountered in the Vaccarès Lagoon.

The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ levels exhibited intraspecific and large interspecific variations (Fig. 2). The mean values of $\delta^{15}\text{N}$ ranged from $5.65 \pm 0.09\text{‰}$ in copepods to $13.2 \pm 1.0\text{‰}$ in top-predator sunfish revealing a general enrich-

ment with increasing trophic position. The mean values of $\delta^{13}\text{C}$ showed different, irregular patterns, ranging from $-14.4 \pm 0.1\text{‰}$ in brown shrimp to $-26.4 \pm 0.04\text{‰}$ in pike-perch. The particularly low zooplankton ^{13}C signature ($-26.9 \pm 1.9\text{‰}$) can be explained by a non-coherent chemical treatment prior to isotope analysis (non-defatted). De Niro and Epstein (1978) showed that lipids present more negative $\delta^{13}\text{C}$ values compared to other tissues, so higher lipid content in samples would result in lower $\delta^{13}\text{C}$ in samples. Despite wide variability, the $\delta^{15}\text{N}$ profiles reflected to a large extent the theoretical trophic emplacement of the analyzed species. The mullet was an exception due to its specific situation in the lagoon. This preferentially herbivorous fish, which is a periphyton consumer (i.e. a primary consumer) also feeding on macroinvertebrates and insect larvae, is present in the Vaccarès only at the juvenile stages. Consequently its $\delta^{15}\text{N}$ signature ($10.9 \pm 0.1\text{‰}$) did not differ from those of the secondary consumers. With the

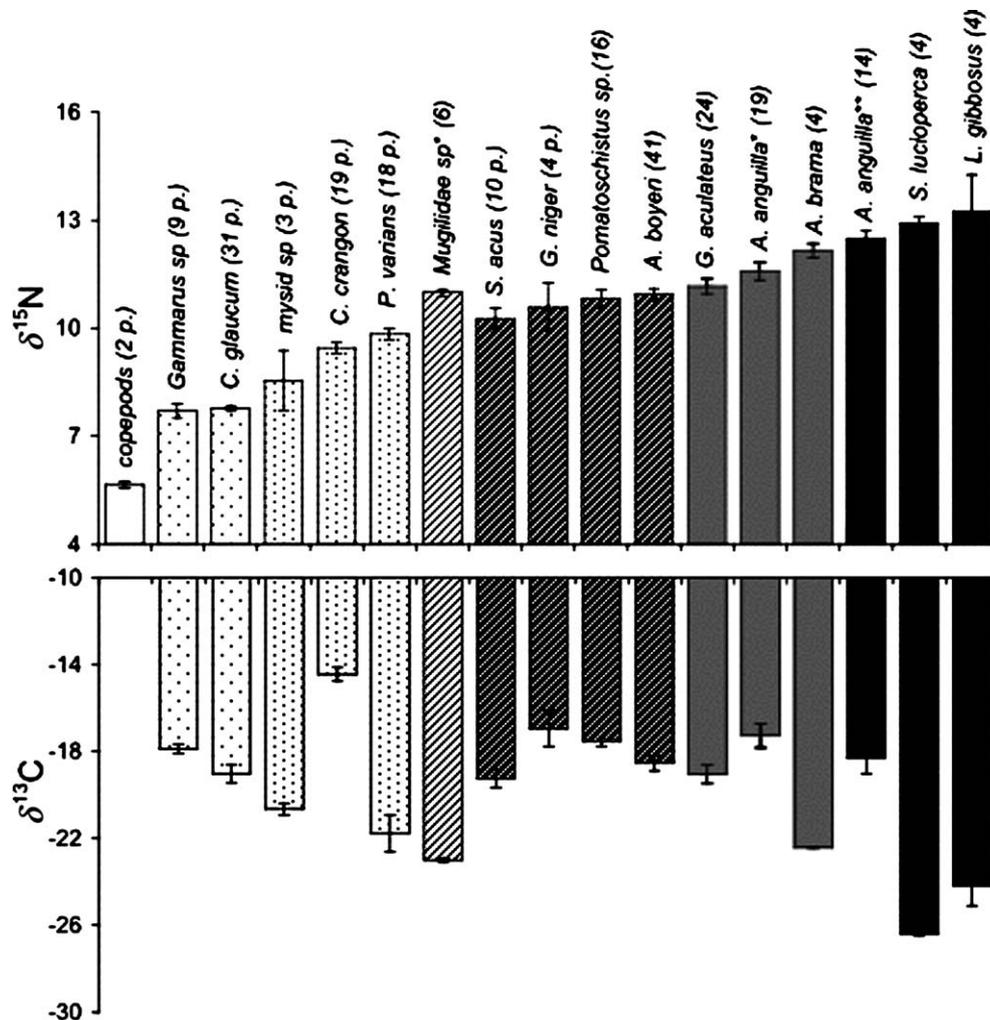


Fig. 2. Mean stable isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in species collected in the Vaccarès Lagoon (Camargue Biosphere Reserve, France). *: Juvenile; **: yellow; p: pools; (n) = number of individuals. The bars represent standard errors; □, primary consumer phytoplanktivorous; ▤, primary consumer depositivorous; ▥, secondary consumer zooplanktivorous; ▦, primary consumer herbivorous/omnivorous fish; ▧, secondary consumer benthivorous; ▨, secondary consumer predator; ▩, secondary consumer top-predator.

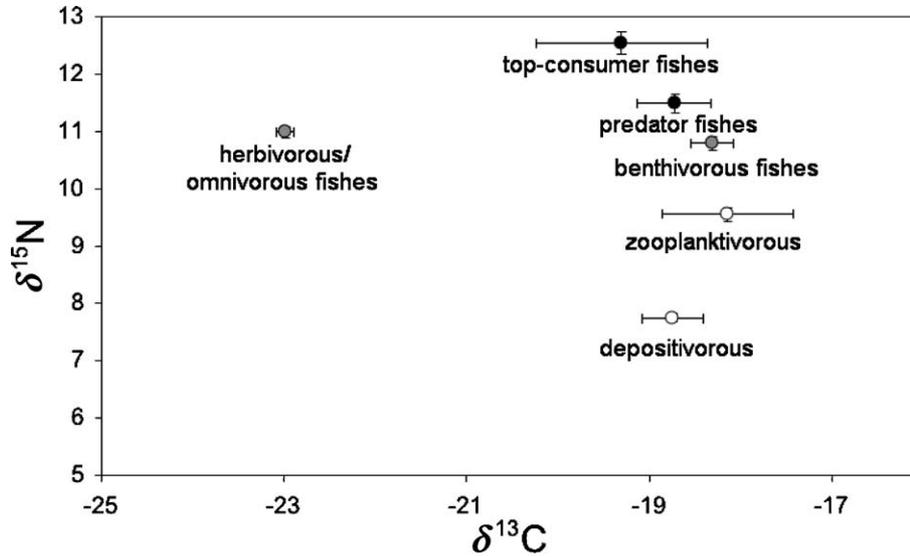


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of some trophic categories in the Vaccarès Lagoon.

exception of copepods, which were not processed using the chloroform/methanol procedure, and the juvenile mullet, for the above-mentioned reasons, the $\delta^{13}\text{C}$ profile corresponded more or less properly to feeding behavior of the species (Fig. 3).

No correlation was found between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($r^2 = -0.09$, Pearson p -value = 0.16, $n = 229$) when data from the entire studied community were pooled together, indicating a non-linear food web ($r^2 = 0.009$). Fig. 4 displays the occurrence of two distinct groups, pelagic versus benthic species, which differ by their pooled mean values of stable isotopes, and also illustrates the presence of approximately three trophic ranks or compartments.

3.2. Isotopic enrichment and trophic levels

Mean isotopic enrichment values were calculated for known consumer–prey couples that were established on the basis of stomach content analysis and other data in the literature. The $\delta^{15}\text{N}$ values of consumers exhibited enrichment with increasing food web position but the increase of the $\delta^{13}\text{C}$ value was less significant. Consistent with former studies of Hobson and Welch (1992), we noticed that $\delta^{15}\text{N}$ enrichment among successive levels was included between 2.8‰ and 3.8‰ with an average of $3.20 \pm 0.14\%$. Conversely, as previously suggested, the $\delta^{13}\text{C}$ did not fit into such classification, because protein, carbohydrate, and lipid components, even in

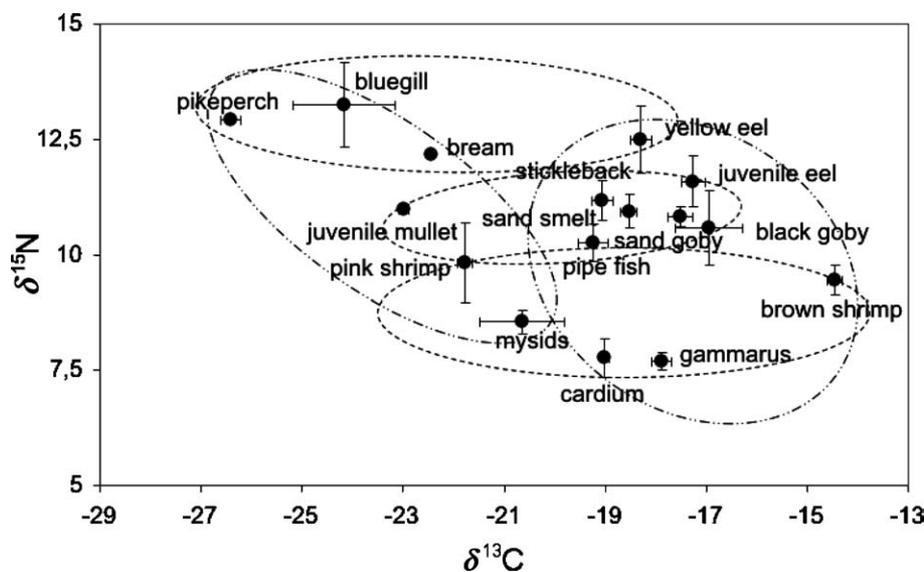


Fig. 4. Trophic relationships between the studied organisms of the Vaccarès Lagoon (Camargue Biosphere Reserve, France), drawn on the basis of stomach contents, literature descriptions, and professional observations and confirmed by ^{15}N analysis (arrow with filled lines), determined from 3.2‰ ^{15}N enrichment (arrow with dotted lines) and 3.2‰ ^{15}N enrichment without possible feeding relations (irregular dotted lines).

previously defatted samples, impinge on the rates of ^{13}C enrichment (Wada et al., 1987). The study was focused on nitrogen isotopes and the trophic level of each of the organisms was estimated on the basis of $\delta^{15}\text{N}$ enrichment using the formula:

Trophic position

$$= \lambda + (\delta^{15}\text{N organism} - \delta^{15}\text{N base of the food web})/3.2$$

where $\lambda = 2$ is the trophic position of copepods used to estimate the $\delta^{15}\text{N}$ base of the food web, $\delta^{15}\text{N}$ organism is measured directly and 3.2 is the calculated average enrichment in $\delta^{15}\text{N}$ per trophic level in the Vaccarès Lagoon food web. The results were quite similar after taking into account the estimation of the isotopic enrichment from depositivores.

Consequently the trophic levels were ranked from 2 in zooplankton to 4.37 in the piscivorous sunfish (Table 2). Ultimately, a scheme describing the feeding relationships of the analyzed dominant components of Vaccarès Lagoon trophic web (Fig. 5) was achieved by integrating the theoretical data with the results from the investigations described above.

3.3. Influence of annual cycle on the isotopic composition of food web components

Seasonal variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of the Vaccarès biota were evaluated. In particular the time course was compared of the isotopic profile of two dominant species which have a large population in the lagoon over the entire annual cycle: *Atherina boyeri* ($n = 23$) and *Cerastoderma glaucum* ($n = 17$ pools). Their isotopic composition was monitored from the end of the winter (March) through spring (April, May, June) to the end of the summer (September) 2001.

The results show a general trend towards a slight enrichment of the $\delta^{15}\text{N}$ values for both species, from the end of winter to summer (Fig. 6a) with a significant increase of $\delta^{15}\text{N}$ signatures in June followed by a slight decrease in late summer. Seasonal changes of $\delta^{13}\text{C}$ profiles followed a completely different and rather irregular pattern (Fig. 6b). However, the more or less variable changes in the $\delta^{13}\text{C}$ signatures of the two investigated species were similar throughout the seasons,

though a rather brisk distortion was observed for the September samples, decreasing for the sand smelt and increasing significantly for the cockle.

The annual spring variations were assessed by comparing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained in spring 2001 and 2002 for primary (*Cerastoderma glaucum*, *Gammarus salinus*) and secondary (*Atherina boyeri*, juvenile *Anguilla anguilla*) consumers which served as representatives of two different, successive trophic levels (Fig. 7). Some of the considered species showed a tendency towards a slight decline of $\delta^{15}\text{N}$, significant in the bivalve and the smelt (DF = 10, t -value = 2.5, $p = 0.03$; DF = 22, t -value = 2.13, $p = 0.04$, respectively) and conversely a slight growth of $\delta^{13}\text{C}$, which was significant in juvenile eels and cockles (DF = 10, $t = -4.40$, $p = 0.007$; DF = 8, t -value = -2.70 , $p = 0.03$, respectively). The observed variations, never exceeding 2‰ nitrogen enrichment, did not suggest any change in the trophic structure of the Vaccarès Lagoon, but rather confirmed the influence of natural environmental variations on isotope composition of its community.

3.4. Influence of size and growth on the isotopic composition of food web components

The correlations between isotopic composition and weight, length and relative condition index, calculated as a function of the Vaccarès population in 7 fish species were also evaluated (Table 3). A positive significant correlation between size and $\delta^{13}\text{C}$ values was found in 3 fishes: three-spine stickleback, juvenile mullet and sand smelt. Although these results might suggest that intra-population habitat and trophic differences may be responsible for the observed variation in body size, no relationship was found regarding the $\delta^{15}\text{N}$ signatures with the exception of the European eel whose $\delta^{15}\text{N}$ values increased with increasing size.

4. Discussion

The outstanding biodiversity of the Vaccarès Lagoon is related to environmental factors. The structure of its trophic web depends on the balance between tolerance

Table 2

Trophic levels calculated from an enrichment level of ^{15}N equal to 3.2 in food-web components of the Vaccarès Lagoon (Camargue Biosphere Reserve, France)

| | Trophic level | | Trophic level | | Trophic level |
|--------------|---------------|-----------------|---------------|------------|---------------|
| Zooplankton | 2.00 ± 0.03 | Pipefish | 3.44 ± 0.09 | Carp bream | 4.03 ± 0.21 |
| Gammarids | 2.64 ± 0.06 | Black goby | 3.54 ± 0.21 | Yellow eel | 4.14 ± 0.07 |
| Cockle | 2.66 ± 0.03 | Sand goby | 3.62 ± 0.08 | Pike-perch | 4.27 ± 0.06 |
| Mysids | 2.91 ± 0.26 | Sand smelt | 3.65 ± 0.05 | Sunfish | 4.37 ± 0.32 |
| Brown shrimp | 3.19 ± 0.05 | Juvenile mullet | 3.67 ± 0.03 | | |
| Pink shrimp | 3.31 ± 0.05 | Stickleback | 3.72 ± 0.07 | | |
| | | Juvenile eel | 3.85 ± 0.08 | | |

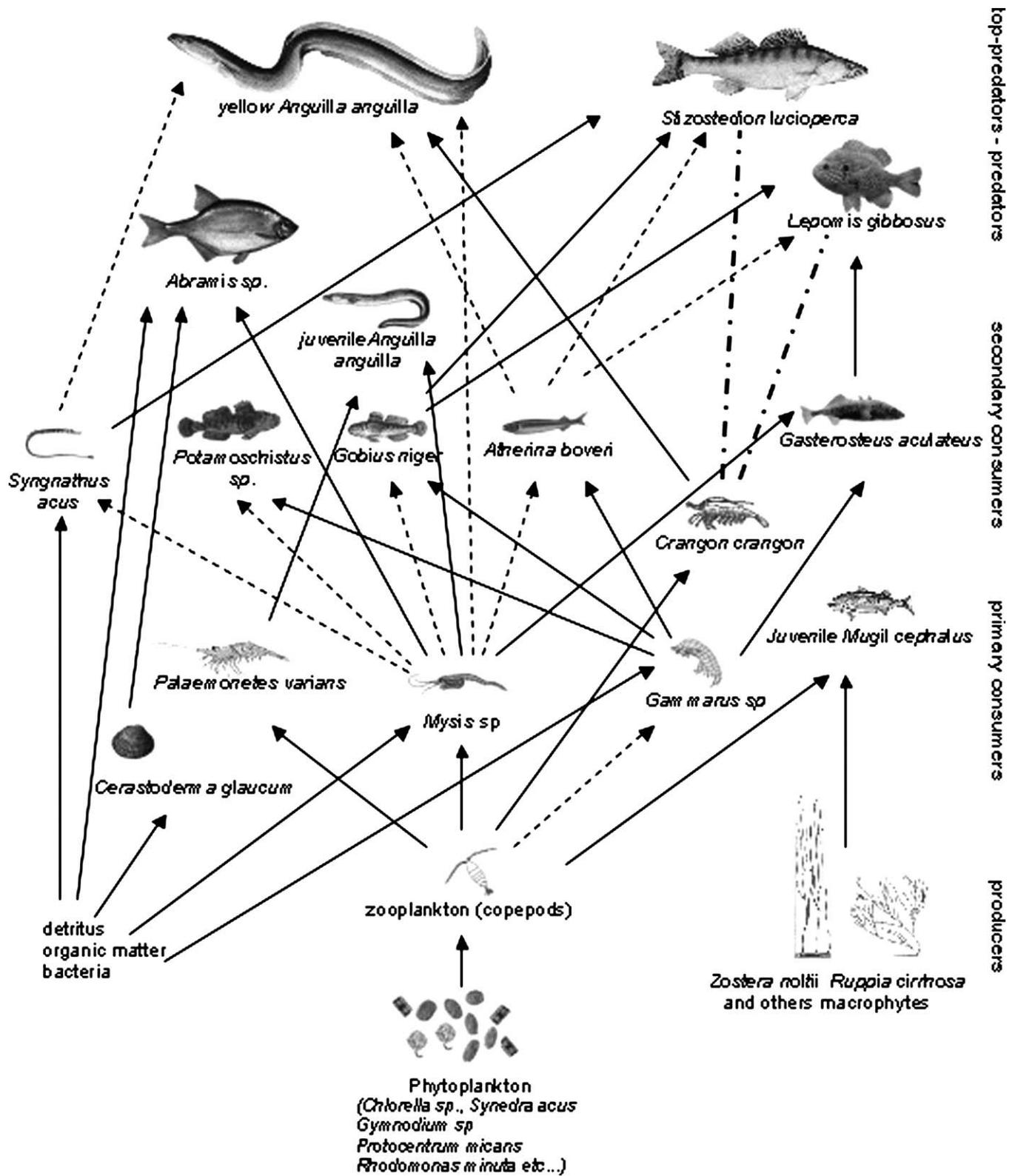


Fig. 5. Stable-carbon and stable-nitrogen isotope values of components of the Vaccarès food web (Camargue Biosphere Reserve, France).

and impacts of their variations (Ramade, 1998, 2002). A large body of work in studies listed by Bardin and Pont (2002) has been devoted to the influence of environmental factors, diel and tidal cycles, tidal currents or

wind-induced current patterns, water turbidity, and hydrographic regime prevailing on the numbers of species, notably young fishes, entering the lagoon. The number of fish entering the lagoon is largely influenced

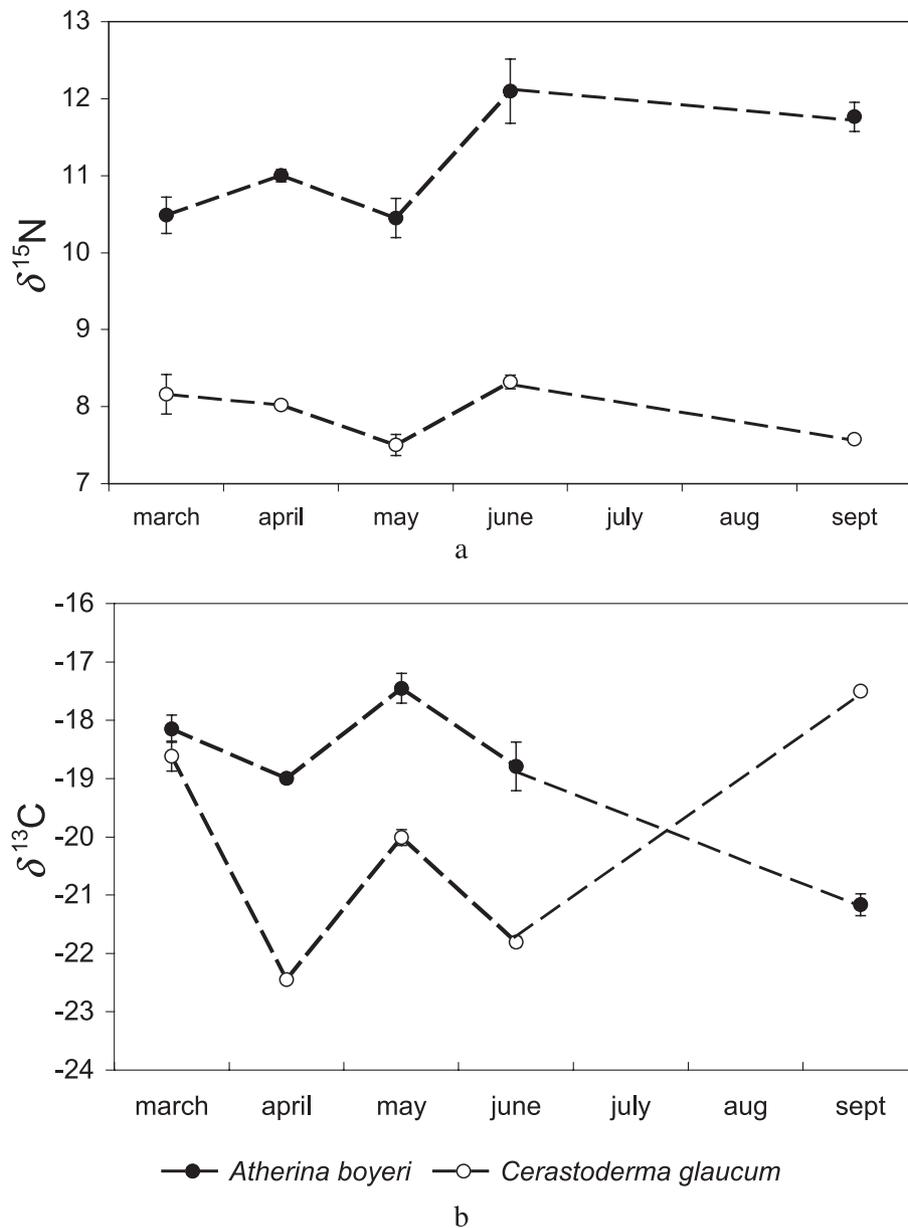


Fig. 6. Seasonal variations of (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ in the cockle (*C. glaucum*) and the sand smelt (*A. boyeri*) from the Vaccarès Lagoon during the year 2001.

by actively inflowing currents defined as selective tidal stream transport. In previous work we have also demonstrated the effects of anthropic factors on fish species in Camargue (Roche et al., 2002a,b, 2003). The present investigation involving the Vaccarès trophic web, addresses another ecological topic: consumer–prey relations between organisms living in this community.

The isotopic signatures of species assessed in the present work revealed a general increase of ^{15}N enrichment with trophic level. The lowest values were found for copepods (5.6‰) and the highest for the three ‘superpredator’ fishes ($\approx 13\text{‰}$) (*Stizostedion lucioperca*, *Anguilla anguilla* and *Lepomis gibbosus*). Conversely, this type of pattern was not seen for $\delta^{13}\text{C}$. ^{13}C reflects

carbon flow from organic matter to consumers (Gu et al., 1996) and consequently can vary intensely with food web elements (Hobson et al., 2002). The interspecific variations of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were associated with a large dispersion of individual data suggesting both intraspecific and intra-population disparities. Isotopic signatures are known to vary greatly within taxonomic groups. Hobson and Welch (1992), who studied an arctic marine ecosystem and Vizzini and Mazzola (2002), who studied a Mediterranean coastal area, have largely explained these differences by the type of habitat and feeding habits. Indeed these authors found a correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the pelagic species and a lower trophic enrichment of ^{13}C in the benthic species.

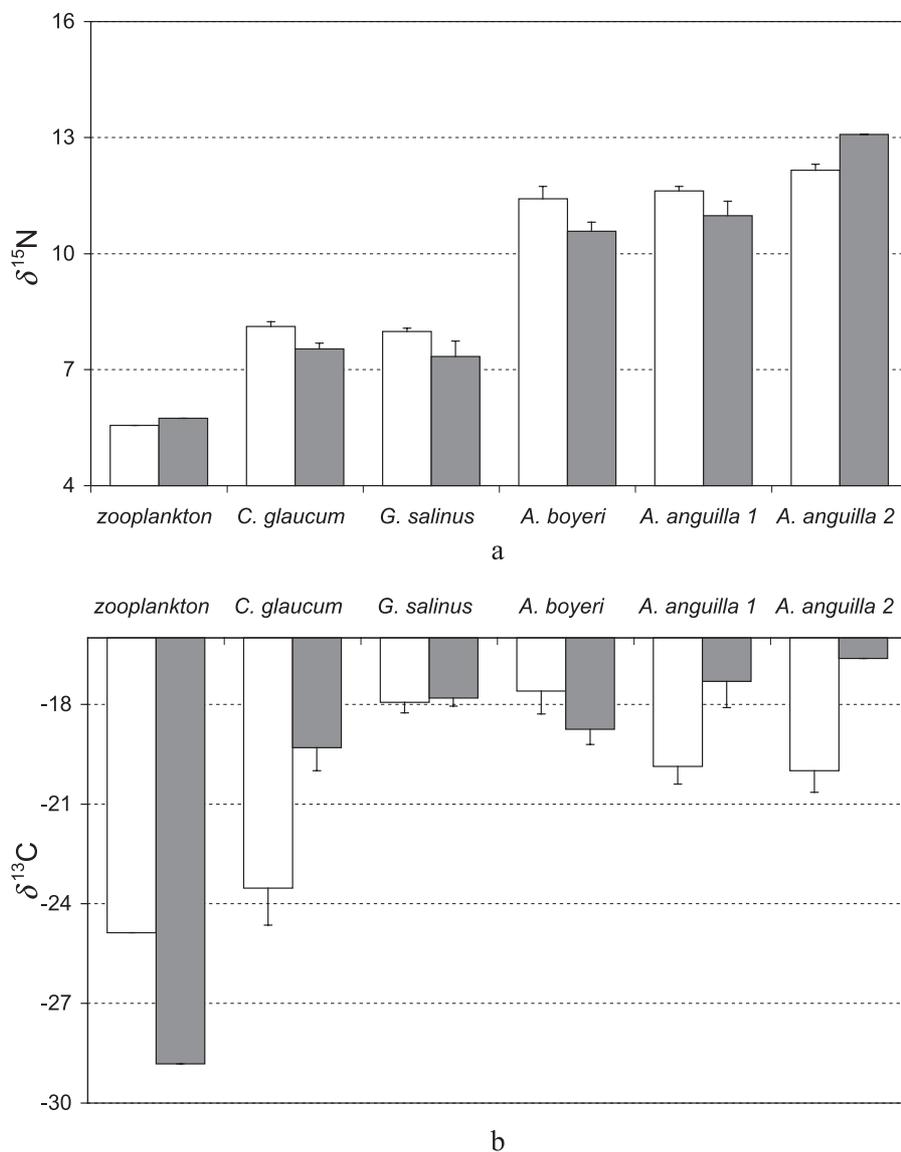


Fig. 7. Annual variations of (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ in depositivores: the cockle (*Cerastoderma glaucum*) and the gammarid (*Gammarus salinus*) and in small consumer fishes: the sand smelt (*Atherina boyeri*) and the juvenile eel (*Anguilla anguilla* 1) and in the yellow eel (*Anguilla anguilla* 2) from the Vaccarès Lagoon. The bars represent standard errors; □, spring 2001; ■, spring 2002.

In the Vaccarès Lagoon, it was not possible to demonstrate any correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, whether within the total community of the ecosystem or in the pelagic group. Hecky and Hesslein (1995) showed that the use of stable C isotope ratios increases N isotope trophic position evidence and provides a means of discrimination between benthic and pelagic sources of production. Benthic species become more enriched in ^{13}C than pelagic species. In the present work the lack of concordance with proposed models (Hobson et al., 2002) is undoubtedly due to the isotopic variability linked to the dietary activities of the majority of the studied species. In such brackish ecosystems, the trophic web structure depends on environmental and seasonal factors; consequently many opportunistic feeders are periodically present. Nevertheless analysis of the relation-

ship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provided a good distinction between the trophic compartments: depositivores; zooplanktivores (planktonic or nektonic microphagous predators); medium size macrophagous predators; and large top-predators.

The use of $\delta^{13}\text{C}$ was limited in the determination of trophic positions, and $\delta^{15}\text{N}$ enrichment was used to calculate the trophic levels (TL). Indeed, known feeding modes and habitat preferences were associated with individual ^{15}N values, demonstrating a regular enrichment of 3.2‰ from one level to another. The $\delta^{15}\text{N}$ value in copepod pools was about 5.5‰ suggesting that in most cases, herbivorous zooplankton had been collected. Level 2 was consequently attributed, empirically, to the zooplankton group. This enabled calculation of the TL of each consumer sample by taking into account

Table 3

Significant correlations between stable isotopes values and body parameters (weight, size and condition index) in fishes from the Vaccarès Lagoon (Pearson coefficient, p -value < 0.05)

| | $\delta^{15}\text{N}$ vs | | | $\delta^{13}\text{C}$ vs | | |
|---------------------|--------------------------|----------------------|--------------------------|--------------------------|-----------------------|--------------------------|
| | Weight | Length | Relative condition index | Weight | Length | Relative condition index |
| Pipefish (10) | | | | | | |
| Stickleback (20) | | | -0.515 $p = 0.019$ | 0.555 $p = 0.009$ | 0.606 $p = 0.004$ | |
| Juvenile mullet (6) | | | | 0.876 $p = 0.019$ | 0.979 $p < 0.0001$ | |
| Sand goby (16) | | | | | | |
| Black goby (4) | | | | | | |
| Sand smelt (41) | | | | 0.608 $p < 0.0001$ | 0.641 $p < 0.0001$ | |
| Eel (35) | 0.41 $p = 0.026$ | 0.485 $p = 0.007$ | | | | |

the constancy of $\delta^{15}\text{N}$ enrichment among trophic levels of the aquatic food web, an approach previously employed by Hobson et al. (2002). TL was less than 3 for depositivorous species and ranged from 3 to 4 for the group of consumers composed of shellfishes plus non-piscivorous fishes. For top-predator fishes, TL was greater than 4. The trophic web structure drawn up from this isotope evidence validated the assumed feeding habits and revealed other previously unknown feeding relationships. The 'feeding map' presented in Fig. 4 highlights the central position of mysids as often described in brackish waters of Western Europe (Sondergaard et al., 2000; Vizzini and Mazzola, 2002). Mysids feed on diverse particulate organic matter, phytoplankton and smaller zooplankton and are consumed by other carnivorous zooplanktons, fishes and birds (Mulkins et al., 2002). Mysids are constantly present in lagoons of Camargue despite salinity fluctuations, but the abundance of the population exhibits strong seasonal variations with an 'explosion' in the summer months. Like Mysids, the Gammarids stand as a prevalent prey of small-sized fish species, sand smelt, various gobies species and three-spined stickleback. Their isotopic signature confirms their function as essential elements of the trophic web. The population of the shrimp *Crangon crangon* exhibits large seasonal variations. Gelin et al. (2000, 2001) considered that the brown shrimp population is annual in the Vaccarès Lagoon. Indeed juveniles enter the lagoon in summer where they reach sexual maturity before returning to the sea. The cockle *Cerastoderma glaucum*, which assimilates organic matter, microphytobenthos and deposits (and will be considered as the sentinel organism in our current ecotoxicological studies) has only one potential predator, the carp bream (TL 4). The carp bream is a freshwater fish that occurs occasionally in the Vaccarès Lagoon. Small-sized species such as pelagic *Atherina boyeri* and benthic Gobiidae sp. dominate the ichthyofauna. For the benthic population, the recent increase in salinity since

1998 has led to predominance of the common goby (Pampoulie et al., 2001). The sedentary behavior of these species (Bouchereau et al., 1991; Rosecchi et al., 1995) is questioned by the recent work of Bardin and Pont (2002) who demonstrated that large numbers of *A. boyeri* and *Potamoschistus microps* enter the Camargue Lagoons during spring. Nevertheless these small-sized fishes occupied trophic levels ranging from 3.4 to 3.8, together with the pipefish, the juveniles of eels and mullet, and the three-spined stickleback. The mullet occupies a particular position in the Vaccarès trophic web because of its omnivorous habits (periphyton and microinvertebrates). The top consumers exhibited a TL above 4. The pike-perch and the sunfish occupied the highest trophic position, 4.3 and 4.4, respectively, and the yellow eel (TL 4.1) considered traditionally as omnivorous (invertebrate consumer), was here occasionally piscivorous due to a predictable relationship between the yellow eel and the sand smelt.

The seasonal variations in $\delta^{15}\text{N}$ values of two different components of the Vaccarès trophic web—the sand smelt and the cockle—were characterized by an accentuated increase in June. More likely than a change in trophic level, this seasonal pattern probably reflects the effects of other associated natural variations (increase in food abundance, climatic and salinity variations). Spring and summer in the Camargue Biosphere Reserve are characterized by the highest annual temperatures leading to increased evaporation and salinity, and therefore the highest salinity induces the additional fractionation, implicated in adaptative mechanisms of euryhaline species, favoring the heavier isotope of nitrogen and leading to an increase in $\delta^{15}\text{N}$ values. Moreover as suggested by Dufour and Gerdeaux (2001) seasonal variations in $\delta^{15}\text{N}$ that occur at the primary producer level may be reflected in $\delta^{15}\text{N}$ values at higher trophic levels.

The seasonal $\delta^{13}\text{C}$ profiles of the sand smelt and the cockle were quite different, probably reflecting changes

in carbon allocation during respiratory metabolism (or other metabolic processes) as well as the composition of phytoplankton species as suggested by Fisk et al. (2001). The most pronounced interspecies distortion occurred in September: decreased $\delta^{13}\text{C}$ for fish and increased $\delta^{13}\text{C}$ for the bivalve. This might be explained by the fact that *Atherina boyeri* and *Cerastoderma glaucum*, although both part of the same food web, do not depend on the same primary producers since they inhabit different ecological niches (the cockle living buried in sand and the sand smelt being a fish which crosses the water column and feeds on both benthic and pelagic zones (Focant et al., 1999) participating in two trophic chains).

The relative trophic positions of the components of the Vaccarès aquatic community exhibit a surprising annual stability. Since the populations of some species are strictly annual (i.e. *Crangon crangon* Gelin et al., 2001; *Gasterosteus aculeatus* Poizat et al., 2002), we assessed the inter-annual variations (Spring 2001 vs Spring 2002) of stable isotopes in organisms from 'multi-generation' populations: two detritivores (*Cerastoderma glaucum* and *Gammarus salinus*) and two secondary consumer fishes (*Atherina boyeri* and juvenile *Anguilla anguilla*). $\delta^{15}\text{N}$ values were lower in 2002 compared with 2001 in all the tested organisms. In the same way, the $\delta^{13}\text{C}$ variations, when significant, showed a decrease in organisms caught in 2002. This may suggest that these inter-annual variations reflect the capacity of such species to adapt to 'the local environmental resources and their trophic plasticity' as demonstrated by Vizzini and Mazzola (2002) for *A. boyeri*.

The stability of a linear food chain depends on energetic demand and the predator–prey body size ratio (Jonsson and Ebenman, 1998). The potential correlations between stable isotope contents and fish body size were therefore investigated. The absence of a systematic isotopic variation with fish size and growth can be attributed to weak predator size–prey size relationships as proposed by Fry et al. (1999). Nevertheless the positive relationship between $\delta^{15}\text{N}$ and body size in the eel population confirmed the intra-population trophic variations. As described above, the trophic position of the juvenile *Anguilla anguilla* (TL 3.8) was always lower than 4 whereas the opportunely generalist feeding yellow eel (TL 4) almost reached the trophic level of the top-predator and thus may be considered as a top-consumer. This is not an unexpected finding since variable species morphology is not uncommon in the top-predator community; eels for instance are typically serpentiform. In a quite different ecosystem, the lakes of the Boreal Plain of Canada, Beaudoin et al. (2002) observed that all the top predators occupy similar trophic positions despite large differences in body size and trophic morphology. According to the observation of Van der Zanden et al. (1998) in smallmouth bass, the positive relationship between $\delta^{13}\text{C}$ and body size in the sand smelt

population indicated a change in feeding habits from benthic to pelagic preys as a function of age.

5. Conclusion

Stable isotope analysis, coupled with literature data, provide an excellent tool in determining and quantifying trophic relations between aquatic organisms even in particularly dynamic systems such as the Vaccarès Lagoon. In this study a constant 3.2‰ $\delta^{15}\text{N}$ enrichment between two successive trophic levels has been shown. Since a brackish ecosystem requires specific adaptations, the isotopic approach offers a sensitive indicator of natural or inadvertent environmental changes. Therefore, despite the hydrological seasonal balance, which leads to variations of feeding strategies and species composition, the Mysids and Gammarids exhibit a constant dominant position in communities of brackish waters of Camargue. They may be considered as the major prey of secondary consumer fishes. Conversely the pike-perch and the sunfish occupy the highest trophic positions, above the European yellow eel, a common euryhaline fish in Camargue lagoons.

Acknowledgements

We thank the staff of the National Nature Reserve of Camargue, especially François Lescuyer, for his technical field assistance during biota sampling and Eric Coulet for biological information and valuable advices. We are also grateful to Caroline Lelarge for stable isotopes measurements.

References

- Bardin, O., Pont, D., 2002. Environmental factors controlling the spring immigration of two estuarine fishes *Atherina boyeri* and *Pomatoschistus* spp. into a Mediterranean lagoon. *Journal of Fish Biology* 61, 560–578.
- Beaudoin, C.P., Prepas, E.E., Tonn, W.M., Wassenaar, L.I., Kotak, B.G., 2002. A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. *Freshwater Biology* 46, 465–477.
- Bouchereau, J.L., Quignard, J.P., Joyeux, J.C., Tomsini, J.A., 1991. Stratégies et tactiques de reproduction de *Pomatoschistus microps* (Kroyer, 1838) et de *Pomatoschistus minutus* (Pallas, 1770) (Pisces, Gobiidae) dans le golfe du lion (France). Nids, déterminismes de la sédentarité et de la migration. *Cybius* 15, 315–346.
- Crivelli, A.J., 1981. Les peuplements de poissons de la Camargue. *Revue d'Ecologie La terre et la vie* 35, 617–671.
- De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42, 495–506.
- Dufour, E., Gerdeaux, D., 2001. Apports des isotopes stables ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$, $^{36}\text{S}/^{34}\text{S}$, $^{87}\text{Sr}/^{86}\text{Sr}$) aux études écologiques sur les poissons. *Cybius* 25, 369–382.

- Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environmental Science and Technology* 35, 732–738.
- Focant, B., Rosecchi, E., Crivelli, A.J., 1999. Attempt of biochemical characterization of sand smelt *Atherina boyeri* Risso, 1810 (Pisces *Atherinidae*) populations from the Camargue (Rhône delta, France). *Comparative Biochemistry and Physiology B* 122, 261–267.
- Fry, B., Mumford, P.L., Tam, F., Fox, D.D., Warren, G.L., Havens, K.E., Steinman, A.D., 1999. Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 590–600.
- Gelin, A., Crivelli, A.J., Rosecchi, E., Kerambrun, P., 2001. Can salinity changes affect reproductive success in the brown shrimp *Crangon crangon*? *Journal of Crustacean Biology* 2, 905–911.
- Gelin, A., Crivelli, A.J., Rosecchi, E., Kerambrun, P., 2000. Is the brown shrimp *Crangon crangon* (L.) population of the Vaccarès lagoon (Camargue, France, Rhone delta) an annual population. *Comptes-rendus de l'Académie des Sciences III Life Sciences* 323, 741–748.
- Gu, B., Schelske, C.L., Hoyer, M.V., 1996. Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. *Journal of Fish Biology* 49, 1233–1243.
- Guiguer, K.R.R.A., Reist, J.D., Power, M., Babaluk, J.A., 2002. Using stable isotopes to confirm the trophic ecology of Arctic charr morphotypes from Lake Hazen, Nunavut, Canada. *Journal of Fish Biology* 60, 348–362.
- Hecky, R.E., Hesslein, R.H., 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society* 14, 631–653.
- Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Marine Ecology Progress Series* 84, 9–18.
- Hobson, K.A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M., 2002. A stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep-Sea Research II* 49, 5131–5150.
- Jonsson, T., Ebenman, B., 1998. Effects of predator–prey body size ratios on the stability of food chains. *Journal of Theoretical Biology* 193, 407–417.
- Mulkins, L.M., Jelinska, D.E., Karagatzides, J.D., Carra, A., 2002. Carbon isotope composition of mysids at a terrestrial–marine ecotone, Clayoquot Sound, British Columbia, Canada. *Estuarine, Coastal and Shelf Science* 54, 669–675.
- Pampoulie, C., Chauvelon, P., Rosecchi, E., Bouchereau, J.L., Crivelli, A.J., 2001. Environmental factors influencing the gobiid assemblage of a Mediterranean Lagoon: empirical evidence from a long-term study. *Hydrobiologia* 445, 175–181.
- Poizat, G., Rosecchi, E., Crivelli, A.J., 2002. Life-history variation within a three-spined stickleback population in the Camargue. *Journal of Fish Biology* 60, 1296–1307.
- Ramade, F., 1998. *Dictionnaire Encyclopédique des Sciences de l'Eau*. Ediscience International, Paris, 800 pp.
- Ramade, F., 2002. *Dictionnaire encyclopédique de l'écologie et des sciences de l'environnement*. Dunod Sciences ed, Paris, 1085 pp.
- Rau, G.H., Mearns, A.J., Young, D.R., Olson, R.J., Schafer, H.A., Kaplan, I.R., 1983. Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64, 1314–1318.
- Roche, H., Buet, A., Ramade, F., 2002a. Relationship between persistent organic chemicals residues and biochemical constituents in fishes from a protected area, the French National Nature Reserve of Camargue. *Comparative Biochemistry and Physiology C* 133, 393–410.
- Roche, H., Buet, A., Ramade, F., 2002b. Accumulation of lipophilic micro-contaminants and biochemical responses in eels from the Biosphere Reserve of Camargue. *Ecotoxicology* 11, 9–18.
- Roche, H., Buet, A., Tidou, A., Ramade, F., 2003. Contamination du peuplement de poissons d'un Étang de la Réserve Naturelle Nationale de Camargue, le Vaccarès, par des polluants organiques persistants. *Revue d'Ecologie La terre et la vie* 58, 77–102.
- Renones, O., Polunin, N.V.C., Goni, R., 2002. Size related dietary shifts of *Epinephelus marginatus* in a western Mediterranean littoral ecosystem: an isotope and stomach content analysis. *Journal of Fish Biology* 61, 122–137.
- Richter, H., Focken, U., Becker, K., 1999. A review of the fish feeding model MAXIMS. *Ecological Modelling* 120, 47–64.
- Rosecchi, E., Crivelli, A.J., Balvay, G., 1995. Sand smelt (*Atherina boyeri*) migration within the water system of the Camargue, southern France. *Hydrobiologia* 300–301, 289–298.
- Sondergaard, M., Jeppesen, E., Aaser, H.F., 2000. *Neomysis integer* in a shallow hypertrophic brackish lake: distribution and predation by three-spined stickleback (*Gasterosteus aculeatus*). *Hydrobiologia* 428, 151–159.
- Van der Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing the trophic position of littoral fish estimated using stable nitrogen isotopes ($\delta^{15}\text{N}$) and diergol data. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 1142–1158.
- Van der Zanden, M.J., Hulshof, M., Ridgway, M.S., Rasmussen, J.B., 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. *Transactions of the American Fisheries Society* 127, 729–739.
- Vizzini, S., Sara, G., Michener, R.H., Mazzola, A., 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecologica* 23, 277–285.
- Vizzini, S., Mazzola, A., 2002. Stable carbon and nitrogen ratios in the sand smelt from a Mediterranean coastal area: feeding habits and effect of season and size. *Journal of Fish Biology* 60, 1498–1510.
- Wada, E., Terazaki, M., Kabaya, Y., Nemoto, T., 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. *Deep-Sea Research* 34, 829–841.