Food web of a SW Atlantic shallow coastal lagoon: spatial environmental variability does not impose substantial changes in the trophic structure

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ABSTRACT: We performed a detailed analysis of the food web structure of Laguna de Rocha, a temporally open coastal lagoon in the Southwest Atlantic, characterised by spatial gradients in salinity, nutrient levels and trophic status. Carbon and nitrogen stable isotopes of main producers, invertebrate and vertebrate consumers, and stomach contents of ichthyofauna were analysed seasonally at freshwater (north) and marine-influenced (south) sites to determine whether environmental differences induced changes in food web structure. Contribution of primary and secondary food sources and trophic linkages were assessed with the multisource-partitioning model IsoSource and an index that quantifies the contribution of each organism as food for the ecosystem. Isotopic analyses were performed for 10 primary organic matter sources (OM), 13 invertebrates and 9 fish species. Suspended and sediment OM constituted the most important primary sources, while direct consumption of macrophytes and macroalgae was marginal. The small invertebrates *Neomysis americana*, *Nephtys fluviatilis*, *Pseudodiaptomus richardii* and an amphipod were the most important intermediate consumers at both sites (except *P. richardii*, which was present only in the north). Top predators were the fish species *Micropogonias furnieri*, *Paralichthys orbygnianus* and *Hoplias malabaricus*, and the maximum estimated trophic level (between 3.4 and 4.8) varied seasonally, but not between sites. Fish stomach content analyses largely confirmed results from the IsoSource mixing model. Overall results indicated that, despite environmental differences between sites, the structure of the biological assemblages and general trophic patterns were similar at both sites.

KEY WORDS: Food web structure · Coastal lagoon · Estuaries · Benthic–pelagic coupling · Isotopic mixing models

INTRODUCTION

Coastal lagoons are complex ecosystems ranking among the most productive on Earth (Harrison & Parsons 2000). They occupy ca. 10% of the sea–land interface worldwide and provide feeding, reproduction and nursery areas for marine and terrestrial biota,

fisheries, aesthetic values and tourism. Complexity and productivity result from their shallowness, inputs of new nutrients from land, and the coexistence of diverse communities of primary producers (Day et al. 1989).

Important issues related to the functioning of coastal lagoons include characterisation of main primary

organic matter (OM) sources, energy pathways supporting consumers, and the processes modulating their spatial and temporal variability (Chanton & Lewis 2002, Froneman 2004). Most studies in shallow estuaries have focused on identifying OM sources that sustain specific consumer groups. Traditional views of macrophyte detritus as a fuel for secondary production (Odum & Heald 1975) have received only partial support (Deegan & Garrit 1997, Kwak & Zedler 1997, Chanton & Lewis 2002, Kibirige et al. 2002, Perissinotto et al. 2003, Vizzini & Mazzola 2003, Bouillon et al. 2004). Microphytobenthic algae, phytoplankton, macrophytes and detritus can all be important sources, and relative contributions differ between systems according to environmental characteristics (Kanaya et al. 2007).

Less effort has been devoted to identifying the most significant secondary-level food sources (i.e. heterotrophic OM sources for secondary and higher consumers). The structure of the assemblage of intermediate consumers that link basal OM sources and higher predators can be very relevant for food web processes and ecosystem functioning. For instance, disappearance of specific intermediate consumers may result in a simplification of the food web that compromises the efficiency of energy transfers ('energetic bottlenecks', sensu Heath & Roff 1996, Sherwood et al. 2002, Iles & Rasmussen 2005). The assemblage of consumers can be modulated by food availability and environmental conditions, i.e. salinity regime (Attrill 2002).

Estuaries are characterised by spatial gradients in salinity and associated environmental variables (e.g. nutrients, seston load), and in the structure of biological communities. It could be expected that primary OM sources and the general trophic structure of estuaries change along these environmental gradients (Kanaya et al. 2007). In coastal lagoons such variability has been suggested to affect the taxonomy and production of the plankton (Froneman 2002, Perissinotto et al. 2003) and the network structure and energy flows (Froneman 2004, Scharler & Baird 2005). Natural or human-induced alterations in sea-level, in freshwater inflow, or in the lagoon–ocean connection directly affect hydrographic gradients and may also impact ecological functions. Understanding the correlation between environmental variability and trophic processes in coastal lagoons can contribute to improve the management and conservation of these ecosystems.

We performed a detailed study of the food web of Laguna de Rocha (LR), a relatively pristine lagoon estuary in the southern Atlantic. We sampled 2 sites representative of freshwater and marine-influenced areas to evaluate the hypothesis that main primary food sources and intermediate consumers differ at contrasting sites of the main environmental gradient. Prior

studies showed that besides salinity, selected sites differ in trophic status (nutrient levels and biomass of primary producers), grain size and organic content in sediments, seston load, and in the diversity and abundance of submerged and emergent vegetation (Conde et al. 1999, 2000, 2002). Selected sites are ca. 12 km apart and represent the extremes of the environmental gradient at LR, but are potentially open to the exchange of highly mobile organisms (e.g. fish); however, physico-chemical characteristics (particularly salinity) restrict such mobility, and some fish species occur only at either the marine-influenced or limnetic end. Analyses included dual stable isotopic composition (δ^{13}C and δ^{15}N) of main components of the biological community and complementary diet analyses of fish fauna. Trophic interactions were explored using the multi-source mixing model approach IsoSource (Phillips & Gregg 2003). The relative trophic significance of every group as a food source was quantified using an index of trophic importance based on the outputs of the mixing models.

MATERIALS AND METHODS

Study site. Laguna de Rocha is a shallow brackish lagoon (mean depth = 0.6 m; area = 72 km^2) located on the Atlantic coast of South America (34° 38' S, 54° 17' W; Fig. 1). It is part of a protected area (MaB-UNESCO) and communicates with the sea in the southernmost region through an inlet that opens naturally several times per year at irregular intervals (Conde et al. 2000). Marshes of *Schoenoplectus californicus* and areas covered by *Potamogeton* sp. are widespread close to the freshwater end of the lagoon in the north area (N), which is dominated by fine-grained sediments with an organic content of 2 to 4% (Conde & Sommaruga 1999). Sandy sediments containing <1% of OM dominate the south (S). The lagoon is a nursery and feeding ground for highly valued crustaceans and fish (pink shrimp *Farfantepenaeus paulensis*, blue crab *Callinectes sapidus*, white croaker *Micropogonias furnieri* and flatfish *Paralichthys orbygnianus*), and also for resident (e.g. black-necked swan *Cygnus melancoryphus*, coscoroba swan *Coscoroba coscoroba*) and migratory birds (common tern *Sterna hirundo*, Chilean flamingo *Phoenicopterus chilensis*). The Rocha River is the main tributary (mean flow = 13.4 m^3 s^{-1}), which receives treated industrial and domestic sewage from a city of ~25 000 inhabitants 12 km upstream, although there is still no evidence of impact on the water quality of the lagoon (Arocena et al. 1996).

Hydrographic sampling and analysis. Two sampling sites representative of typical freshwater and marine-influenced areas (Stations N and S, respectively; Fig. 1)
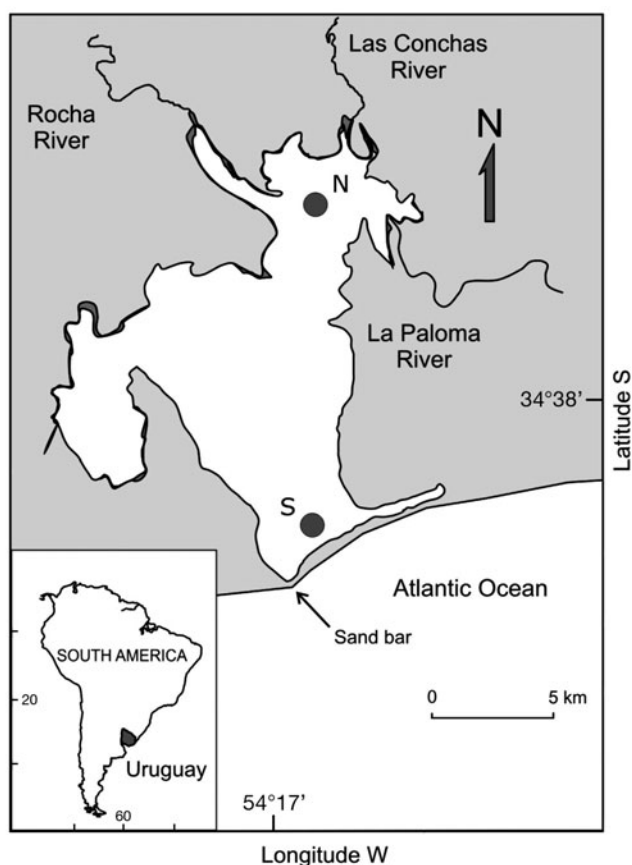
Fig. 1. Location of Laguna de Rocha on the southeastern coast of South America. North (N) and south (S) sampling sites are indicated

were visited once per season in May (autumn), August (winter) and October (spring) in 2004, and in February (summer) in 2005, to estimate the stable isotopic composition of dominant groups of producers and consumers. Physical environmental variables measured on each occasion included water depth (m), temperature (°C) and dissolved oxygen (mg l^{-1}; Horiba OM-14), pH and conductivity (mS cm^{-1}; Horiba D-24). Salinity values were derived from conductivity and temperature measurements using the UNESCO international equation of state for seawater.

Suspended particulate organic matter (POM) was determined by filtering 0.5 to 1 l water through precombusted GF/F Whatman filters as loss of weight of dried filters after ignition at 450°C for 15 min (APHA 1995). Concentrations of nitrate, soluble reactive phosphorus (SRP) and reactive silicate were measured spectrophotometrically (APHA 1995). Chlorophyll *a* was determined spectrophotometrically by filtering 0.1 to 0.5 l onto GF/F Whatman filters, and after 24 h of dark and cold (ca. 0°C) extraction in 90% acetone. In both analyses a Beckman DU 650 spectrophotometer was used.

Biological sampling. Biological samples were collected at N and S to estimate abundance of different groups and for isotopic analyses (see next subsection). Phytoplankton was collected in plastic bottles and preserved with acid Lugol solution; phytoplankton >3 µm was identified under an inverted microscope with phase contrast at 1000×, using sedimentation chambers of 5 to 10 ml (Hasle 1978), and abundance was expressed as cells per millilitre. Macroalgae and seagrasses were collected with an Ekman benthic grab (0.052 m^2 × 20 cm vertical penetration) and picked by careful examination of whole samples; samples were then dried and weighed, and biomass was expressed as g dry weight m^{-2}.

Zooplankton was collected by towing a 35 cm mouth diameter plankton net fitted with 200 µm mesh and a flowmeter to estimate the volume of filtered water. Mobile epifauna (mysid *Neomysis americana*, freshwater shrimps *Palaemonetes argentinus*, crabs *Cyrtograpsus angulatus* and amphipods) were collected with a sledge trawl designed for coastal lagoons (rectangular mouth, 85 cm width, 70 cm height) fitted with 500 µm plankton mesh and a flowmeter; the trawl sampled most of the water column and effectively collected larger zooplankton like adult copepods and mysids. In all cases samples were preserved in 5% buffered formaldehyde. Zooplankton and epifauna were sorted, identified and quantified under a binocular microscope, and abundance was expressed as ind. m^{-3}. Benthic infauna was collected with an Ekman benthic grab (0.052 m^2), sorted with a 500 µm sieve, and identified and counted under a binocular microscope, and abundances were expressed as ind. m^{-2}.

Stable isotope analyses. Stable isotope analyses were performed for macrophytes, periphyton, POM (phytoplankton and other seston), and for the top millimetre of the sediment layer (sediment organic matter, SOM, representing microphytobenthos plus the organic fraction in sediments), as well as for the most abundant species of consumers in the zooplankton, macrobenthos and nekton (fish). Biological samples were collected as described above, with the exception of fish, which were collected using gillnets by local fishermen.

In order to avoid interference of non-assimilated gut contents on the isotopic signature (Levin & Michener 2002), small invertebrates were transferred alive to a nearby field laboratory in large, thermally insulated buckets (ca. 20 l), picked under low magnification binocular microscope using forceps (wide-bore pipettes for zooplankton) and kept alive in 0.7 µm filtered lagoon water for 1 to 24 h according to the size of organisms. For crabs, the muscle of one walking leg from each specimen was withdrawn. For fish, only muscle of the dorsal region was sampled, as its isotopic

composition is less variable than that of other body parts (Pinnegar & Polunin 1999). Manipulation of samples was done with methanol-rinsed forceps or pipettes.

To meet mass requirement for isotopic analyses (1.2 mg for animal tissue, 2 mg for plants and 40 mg for sediments), animals of small individual size were pooled in one sample (e.g. for copepods, ca. 250 ind. sample^{-1}); also, macrophytes and macroalgae represented taxonomically homogeneous composite samples. When possible, 3 replicates were collected for each group. All samples were rinsed in Milli-Q water and kept frozen at –20°C in pre-combusted glass flasks or filters until further processing. Periphyton was obtained by carefully scraping leaves of the emergent hydrophyte *Schoenoplectus californicus.* SOM was collected by carefully scrapping the upper millimetre of a sediment sample taken manually with an acrylic corer. POM was collected by pre-sieving up to 2 l of surface water through 63 μm mesh to remove zooplankton and large detrital particles, and then filtered onto a pre-combusted (500°C for 4 h) Whatman GF/F glass microfibre filter (0.7 μm pore size).

All samples were oven dried until constant weight at 60°C. Subsamples were packed into 5 × 9 mm tin capsules and acidified by adding 1% $PtCl_2$ solution before analysis to remove carbonates (Levin & Michener 2002). Stable isotope ratios of carbon and nitrogen were measured at the University of California, Davis, USA, by continuous flow isotope ratio mass spectrometry (20-20 mass spectrometer, PDZEuropa) after sample combustion to CO_2 and N_2 at 1000°C in an on-line elemental analyser (PDZEuropa ANCA-GSL). Gases were separated on a Carbosieve G column (Supelco) before introduction to the isotope ratio mass spectrometer (IRMS) via an open split interface. Sample isotope ratios were compared to those of standard gases injected directly into the IRMS before and after the sample peaks, and provisional $\delta^{15}N$ (air) and $\delta^{13}C$ (PDB) values were calculated. Provisional isotope values were adjusted to bring the mean values of working standard samples distributed at intervals in each analytical run to the correct values of the working standards. Working standards are a mixture of ammonium sulphate and sucrose with $\delta^{15}N$ versus air 1.33‰ and $\delta^{13}C$ versus PDB –23.83‰, and periodically calibrated against international isotope standards. Isotopic values were expressed in parts per thousand (‰), as deviations from standards following the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Trophic level (TL) of each organism was estimated on the basis of $\delta^{15}N$ enrichment, according to Persic et al. (2004):

$$TL = \lambda + (\delta^{15}N \text{ organism} - \delta^{15}N \text{ base of the food web}) / 3.4$$

where $\delta^{15}N$ organism is measured and 3.4 is an average $\delta^{15}N$ enrichment per TL (Post 2002). $\delta^{15}N$ base of the food web is $\delta^{15}N$ of organisms that met 2 criteria (hereafter referred to as the reference organism): (1) they are as close as possible to true herbivorous and (2) they are sessile or have very limited mobility, so they provide a good isotopic baseline for estimating the trophic position of higher trophic level consumers, while capturing spatial and temporal variation at the base of food webs (Post 2002). In our case these were the benthic filter feeder clam *Erodona mactroides* (for the seston POM source) and the snail *Heleobia australis* (for the SOM source). λ is the TL (= 2) of reference organisms.

Fish diets. The stomach contents of the same fish used for stable isotopic analyses were determined as complementary information and to allow a qualitative comparison of both approaches. For each specimen, the stomach was extracted and preserved in 5% buffered formaldehyde until analysis. Stomachs were dissected, and the contents were sorted under a binocular microscope, identified to the lowest possible taxonomic level, and the individual weight of each item was recorded (fresh wet weight, mg).

Data analysis. The contribution of the food sources to every consumer at Laguna de Rocha was assessed with the source-partitioning model IsoSource, outlined in Phillips & Gregg (2003), to statistically constrain the relative proportions of various sources to consumers. IsoSource evaluates all possible contributions of each potential source (from 0 to 100%) in user-defined increments (1 or 4%) to identify source combinations that sum to the known isotopic signature of the mixture within a prescribed small tolerance (±0.1‰). This procedure creates a frequency distribution and estimates contribution within a defined range of every potential source (1 to 99 percentiles as recommended; Phillips & Gregg 2003). Isotopic signature was adjusted considering an isotopic fractionation (enrichment) of 0.4‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ (Post 2002). Potential food sources to consumers were assigned based on literature results using stable isotopes and traditional methods (e.g. gut content analyses) for the same or similar species, and on information of fish gut contents obtained during the present study.

Given the nature of the outputs from IsoSource, when large numbers of primary sources, consumers and potential food items are available for every consumer, interpretation of results and exploration of trophic patterns, i.e. sources of OM common to different consumers, can turn cumbersome. As a data-reduction procedure we used IsoSource outputs to

define an index that quantifies the contribution as food for the ecosystem (CFE) of every organism, e.g. the extent to which every component is used by the consumers within the system. The relative importance of organism j as food within the ecosystem was defined as:

$$CFE_j = PC \times Cavg_j$$

where PC is the number of potential consumers, defined as those for which the 99th percentile in the frequency distribution of organism j contribution to consumer diet was >5%. $Cavg_j$ is the average contribution of source j to all consumers potentially feeding on it, which was estimated as the average of the mean contribution of that source for all potential consumers:

$$Cavg_j = \frac{\Sigma C_{ij}}{n}$$

where C_{ij} is the mean contribution of source j to the ith consumer, and n is the number of consumers on that source.

Isotopic signals of selected groups (e.g. those present at both sites and in all or most sampling occasions) were compared between sites and seasons separately by Mann-Whitney (M-W) and Kruskal-Wallis (K-W) tests, respectively. Differences between sites in the TL of selected organisms and in maximum TL were assessed by a M-W test. For between-site comparisons seasonal data were pooled when no significant differences existed according to the K-W test. Comparison of maximum TL considered results of that species with the highest estimated TL at each site and season, as well as that species with the second highest TL if standard deviations of the estimated TL of those 2 species overlapped.

RESULTS

Environmental characteristics

The connection between lagoon and ocean was interrupted during most of the study period. Water depth was similar between N and S, with a minimum of 0.4 m in winter and a maximum of 1.2 m during summer. Temperature was also similar between N and S and showed a typical seasonal variation (from 14.1 ± 0.2°C in winter to 23.5 ± 0.2°C in summer; Table 1). Conductivity difference between sites was largest in winter (higher at S) and progressively converged to similar values in summer. Oxygen levels indicated near saturation at both sites and for all samplings (from 8.7 ± 0.1 to 12.4 ± 0.7 mg l^{-1}). Dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) and reactive silicate were higher at N and maximum during winter (except silicate) (Table 1). Chlorophyll a at N ranged from 7.3 (spring) to 11.0 µg l^{-1} (autumn) and at S between 4.4 (autumn) and 12.4 µg l^{-1} (spring) (Table 1).

Assemblages of primary producers and consumers

Primary producers

Phytoplankton was consistently dominated by nanoplankton size classes. Composition and abundance of taxa varied between sites and seasons, although some groups were abundant at both sites in several periods. The most representative taxa at N were the diatoms *Melosira moniliformis* and *Paralia sulcata*, and the euglenoid *Eutreptiella* cf. *gymnastica*. The most representative taxa at S were also the diatoms *M. moniliformis*,

Table 1. Environmental variables recorded at Laguna de Rocha during the study period. N: northern site; S: southern site; Z: depth; T: temperature; DO: dissolved oxygen; K_d: light extinction coefficient; OM: suspended organic matter; SRP: soluble reactive phosphorus; SiO$_2$: reactive silicate; NO$_3$: nitrate average. Values ±1 SD are indicated

Period/ Area	Z (m)	T (°C)	DO (mg l^{-1})	Conductivity (mS cm^{-1})	Salinity	K_d (m^{-1})	Seston (mg l^{-1})	OM (%)	SRP (µg l^{-1})	SiO$_2$ (µg l^{-1})	NO$_3$ (µg l^{-1})	Chl a (µg l^{-1})
Autumn												
N	1.2 ± 0.0	14.2 ± 0.2	12.4 ± 0.7	8 ± 0.9	5.7 ± 0.7	5.5 ± 0.3	69.2 ± 21.5	24.8 ± 2.9	20.3 ± 2.0	3156.4 ± 212.0	10.7 ± 1.8	11.0 ± 0.4
S	1.2 ± 0.1	14.6 ± 0.3	12.3 ± 0.5	13.8 ± 0.3	10.2 ± 0.2	1.1 ± 0.2	21.7 ± 2.5	34.2 ± 12.8	13.2 ± 2	2508.8 ± 66.9	–	4.4 ± 1.4
Winter												
N	0.4 ± 0.1	14.6 ± 0.3	10.3 ± 0.7	1.3 ± 0.8	0.8 ± 0.5	2 ± 0.7	40.2 ± 22.8	52.9 ± 21.6	35.0 ± 9.7	4971.7 ± 361.0	45.7 ± 10.1	7.5 ± 1.5
S	0.3 ± 0.1	14.1 ± 0.3	10.8 ± 0.1	21.6 ± 1.6	16.8 ± 1.4	3.9 ± 0.2	64.4 ± 12.4	38.1 ± 38.1	9.2 ± 9.1	664.7 ± 267.2	7.3 ± 2.1	10.5 ± 7.2
Spring												
N	1.0 ± 0.0	16.7 ± 0.2	–	4.4 ± 0.5	2.9 ± 0.3	3.9 ± 0.2	33.6 ± 5.7	55.1 ± 7.6	10.0 ± 0.5	2648.1 ± 311	6.8 ± 1.5	7.3 ± 2.2
S	0.9 ± 0.1	19.5 ± 0.1	–	11.4 ± 0.4	7.3 ± 0.25	2.6 ± 0.9	30.0 ± 13.9	70.5 ± 33.9	6.9 ± 4.0	1945.0 ± 222.8	5.7 ± 0.7	12.4 ± 5.4
Summer												
N	0.8 ± 0.0	23.5 ± 0.2	10.3 ± 0.5	9.8 ± 0.01	5.7 ± 0.01	2.3 ± 0.3	78.4 ± 18.9	32.8 ± 10.3	22.4 ± 2.4	2114.8 ± 179.4	11.2 ± 3.6	7.4 ± 4.9
S	0.8 ± 0.1	22.5 ± 0.2	8.7 ± 0.1	9.8 ± 0.02	5.7 ± 0.03	1.7 ± 1.8	29.6 ± 16.9	54.6 ± 13.1	21.4 ± 0.6	2093.3 ± 207.8	24.0 ± 5.4	5.5 ± 3.6

P. sulcata, *Plagioselmis nannoplanctica* and *Cyclotella* cf. *menghiniana*. Seasonally, N exhibited changes in dominant groups, from diatoms in summer to euglenoids in winter, while at S diatoms dominated throughout the year. Macrophyte composition varied between sites and seasons (Table 2). *Ruppia maritima* and *Zannichellia palustris* were common to both sites. Macrophyte biomass (in g dry weight [DW]) was higher at N and dominated by *Myriophyllum quitense* at N (19.4 ± 15.5 g DW m^{-2}; mean ± 1 SD) and by *R. maritima* at S (4.2 ± 3.7 g DW m^{-2}). Seasonally, *M. quitense* dominated in August at N (33.1 g DW m^{-2}), and *R. maritima* dominated in February at S (9.6 g DW m^{-2}) (Table 2).

Consumers

The zooplankton assemblage varied little between sites (Table 2), and dominant taxa were common to both. Seasonal changes were moderate, except for at S in autumn, when an unidentified ctenophore occurred in large numbers and copepods were absent. N was dominated by the copepod *Pseudodiaptomus richardii* (annual average 13.9 ± 21.2 ind. m^{-3}), while *Acartia tonsa* dominated at S (annual average 129.3 ± 217.5 ind. m^{-3}). The mysid *Neomysis americana* was abundant at both sites (annual average 24.5 ± 28.5 ind. m^{-3}).

Benthic community composition also varied little between sites (Table 2). The gastropod *Heleobia australis* (annual average 6570 ± 6386 ind. m^{-2}), the bivalve *Erodona mactroides* (annual average 1637 ± 2906 ind. m^{-2}) and the polychaete *Nephtys fluviatilis* (annual average 264 ± 127 ind. m^{-2}) were dominant at both sites throughout the year (Table 2). The crabs *Callinectes sapidus* and *Cyrtograpsus angulatus* occurred only at S, but no data on their abundances are available.

Fish were represented by 9 taxa (Table 2). The white croaker *Micropogonias furnieri*, the mullet *Mugil* sp.,

Table 2. Quantitative and presence/absence data for trophic groups in Laguna de Rocha. Biomass is indicated for sources (g DW m^{-2}), and abundance is indicated for planktonic (ind. m^{-3}) and benthic (ind. m^{-2}) invertebrates. Values ±1 SD are indicated. •: species present, abundance data not available; –: species absent; N: northern site; S: southern site; n.i.: not identified

Taxon	Acronym	Autumn		Winter		Spring		Summer	
		N	S	N	S	N	S	N	S
PRODUCERS									
Enteromorpha sp.	Ent	–	•	–	•	–	•	–	•
Eleocharis sp.	Ele	15.9 ± 8.2	–	4.2 ± 2.2	–	0.6 ± 0.2	–	2.6 ± 1.2	–
Myriophyllum quitense	Myr	22.7 ± 1.0	–	33.1 ± 7.0	–	–	–	2.5 ± 2.5	–
Periphyton	Per	•	–	•	–	•	–	•	–
Potamogeton sp.	Pot	–	–	0.4 ± 0.1	–	1.3 ± 0.8	–	3.8 ± 4.6	–
Ruppia maritima	Rup	1.2 ± 1.1	3.5 ± 1.7	0.1 ± 0.1	1.3 ± 0.8	0.7 ± 0.5	2.3 ± 1.3	5.6 ± 5.2	9.6 ± 4.7
Schoenoplectus californicus	Sch	•	–	•	–	•	–	•	–
Zannichellia palustris	Zan	–	1.2 ± 0.2	–	0.4 ± 0.2	–	0.1 ± 0.0	3.4 ± 2.7	1.1 ± 0.5
CONSUMERS									
Invertebrates									
Acartia tonsa	Aca	0.4	–	0.11	196.2	14.5	545.2	19	–
Amphipoda	Amp	132 ± 57	57 ± 38	–	32 ± 39	6 ± 11	19 ± 19	–	19 ± 19
Callinectes sapidus	Cal	–	•	–	•	–	•	–	•
Cyrtograpsus angulatus	Cyr	–	•	–	•	–	•	–	•
Ctenophora	Cte	–	•	–	–	–	–	–	–
Erodona mactroides	Ero	57 ± 65	189 ± 33	–	832 ± 182	82 ± 142	536 ± 97	2993 ± 1479	8399 ± 10685
Heleobia australis	Hel	2577 ± 1321	14121 ± 3815	76 ± 0	14209 ± 295	416 ± 344	7196 ± 3462	926 ± 597	8399 ± 10685
Isopoda	Iso	164 ± 48	744 ± 265	57 ± 0	1084 ± 236	–	126 ± 11	95 ± 105	529 ± 82
Neomysis americana	Neo	5	0.5	0.4	132	7.9	69	3.3	•
Nephtys fluviatilis	Nep	139 ± 66	183 ± 95	170 ± 53	189 ± 87	246 ± 173	277 ± 134	498 ± 137	410 ± 299
Heteromastus similis	Sim	63 ± 76	139 ± 61	28 ± 40	504 ± 139	69 ± 55	101 ± 58	239 ± 176	989 ± 1223
Palaemonetes argentinus	Pal	0.02	0.01	0.5	–	0.01	0.04	1.7	–
Pseudodiaptomus richardii	Pse	45.3	–	1.76	0.4	0.1	–	8.6	•
Fish									
Brevoortia aurea	Bre	–	•	–	•	–	–	–	–
Cyphocharax voga	Cyp	–	–	–	–	–	•	–	–
Engraulidae n.i.	Eng	•	–	–	–	–	–	–	–
Hoplias malabaricus	Hop	•	–	–	–	•	–	•	–
Leporinus sp.	Lep	•	–	–	–	–	–	–	–
Micropogonias furnieri	Mic	•	•	•	•	–	•	•	–
Mugil sp.	Mug	•	–	–	•	–	•	–	–
Odontesthes sp.	Odo	•	–	–	•	–	•	–	–
Paralichthys orbygnianus	Par	•	–	•	•	–	•	•	•

the silverside *Odontesthes* sp. and the flatfish *Para-lichthys orbignyanus* occurred at both sites. *Hoplias malabaricus* and a non-identified freshwater engraulid occurred only at N, while the menhaden *Brevoortia aurea* occurred only at S.

Stable isotope composition

A total of 32 groups were analysed for stable carbon and nitrogen isotopic composition, including 10 primary sources of OM, 13 invertebrate taxa and 9 fish species (see Appendix 1 in the Electronic Supplementary Material available at http://www.int-res.com/articles/suppl/m362p069_app.pdf).

Primary producers

$\delta^{13}C$ values for producers varied from $-25.3 \pm 0.5‰$ in *Schoenoplectus californicus* to $-13.1 \pm 0.4‰$ in *Zannichellia palustris*. $\delta^{15}N$ ranged from $2.1 \pm 2.1‰$ in *S. californicus* to $6.5 \pm 1.9‰$ in *Eleocharis* sp. (Fig. 2). Spatial variation (Δ) of $\delta^{13}C$ was <3‰, and significant differences occurred in autumn (POM and SOM), spring and summer (POM) (Appendix 1, Table 3), while differences in $\delta^{15}N$ between sites were restricted to spring (SOM). Seasonal $\Delta \delta^{13}C$ was >3‰ for most producers, and significant differences existed for periphyton and POM (N). These groups were more ^{13}C-enriched in spring and summer compared to autumn and winter (Appendix 1, Table 4). Seasonal differences
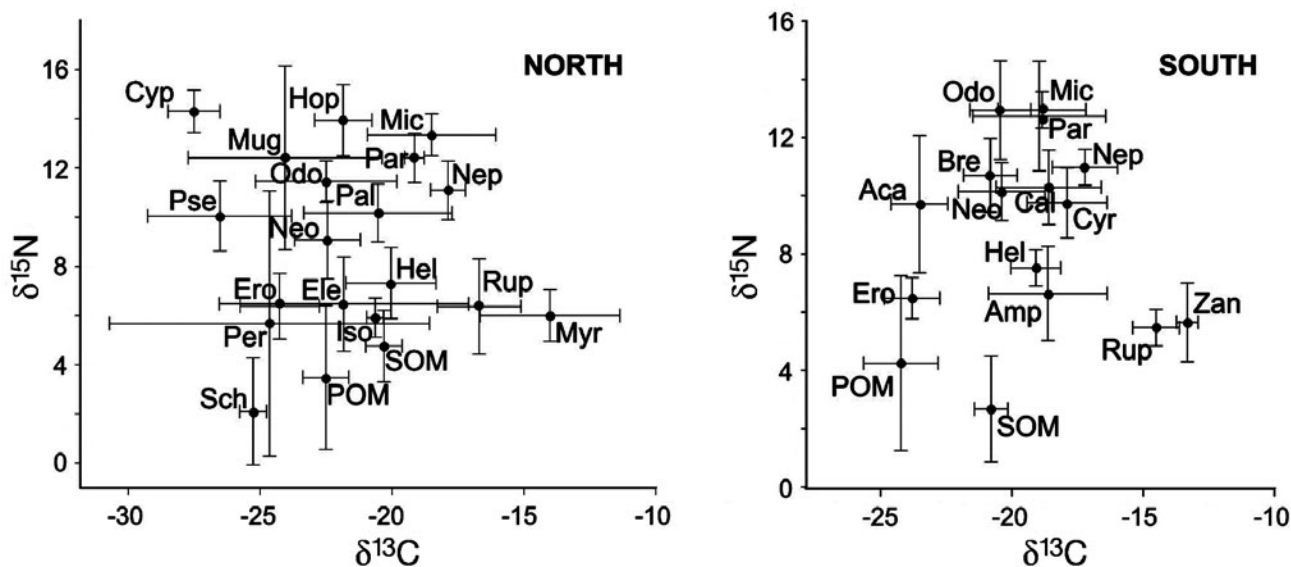

Fig. 2. Annual average stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the main biological components of Laguna de Rocha ecosystem in the north and south areas. Error bars indicate ±1 SD. POM: particulate organic matter; SOM: sediment organic matter; for full taxonomic names and acronyms, see Table 2

Table 3. Comparison of $\delta^{13}C$ and $\delta^{15}N$ isotopic signals of selected organisms from the north versus south sites of Laguna de Rocha at different seasons. Numbers shown are probability values resulting from a Mann-Whitney test and corresponding sample size (in brackets). *Significant differences with a probability level <5%. –: cases for which analyses were not performed due to absence of the species at N or S or without replicates; POM: particulate organic matter; SOM: sediment organic matter; for full taxonomic names, see Table 2

Group	Autumn		Winter		Spring		Summer	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
SOM	0.049 (6)*	0.513 (6)	0.513 (6)	0.127 (6)	0.513 (6)	0.049 (6)*	0.127 (6)	0.275 (6)
POM	0.049 (6)*	0.513 (6)	0.275 (6)	0.827 (6)	0.049 (6)*	0.827 (6)	0.049 (6)*	0.513 (6)
E. mactroides	0.999 (7)	0.034(7)*	0.275 (6)	0.049 (6)*	0.049 (6)*	0.513 (6)	0.513 (6)	0.049 (6)*
H. australis	0.827 (6)	0.513 (6)	0.127 (6)	0.275 (6)	0.513 (6)	0.513 (6)	0.127 (6)	0.049 (6)*
N. americana	0.827 (6)	0.049(6)*	–	–	0.049 (6)*	0.513 (6)	0.049 (6)*	0.049 (6)*
N. fluviatilis	0.275 (6)	0.083 (5)	0.827 (6)	0.049(6)*	0.049 (6)*	0.513 (6)	0.513 (6)	0.275 (6)
M. furnieri	0.179 (8)	0.179 (8)	0.513 (6)	0.827 (6)	–	–	–	–
P. orbignyanus	–	–	0.999 (4)	0.121 (4)	–	–	–	–

Table 4. Seasonal comparison of $\delta^{13}C$ and $\delta^{15}N$ isotopic signals of selected organisms of Laguna de Rocha at north and south sites, separately. p: probability values resulting from the Kruskal-Wallis test; n: sample size; seasons: number of seasons in which the corresponding taxon was effectively found. *Significant differences with a probability level <5%. For full taxonomic names and acronyms, see Table 2

| Group | North | | | | | | South | | | | | |
| | $\delta^{13}C$ | | | $\delta^{15}N$ | | | $\delta^{13}C$ | | | $\delta^{15}N$ | | |
	p	(n)	Seasons	p	(n)	Seasons	p	(n)	Seasons	p	(n)	Seasons
POM	0.038*	(12)	4	0.042*	(12)	4	0.053	(12)	4	0.042*	(12)	4
SOM	0.546	(12)	4	0.099	(12)	4	0.094	(12)	4	0.086	(12)	4
Periphyton	0.014*	(12)	4	0.010*	(12)	4	–			–		
H. australis	0.264	(12)	4	0.121	(12)	4	0.347	(12)	4	0.024*	(12)	4
E. mactroides	0.099*	(12)	4	0.016*	(12)	4	0.023*	(12)	4	0.126	(12)	4
N. americana	0.837	(9)	3	0.051	(9)	3	0.024*	(12)	4	0.019*	(12)	4
N. fluviatilis	0.03*	(12)	4	0.047*	(11)	4	0.033*	(12)	4	0.644	(12)	4
Amphipoda	–		–	–		–	0.044*	(8)	3	0.044*	(8)	3
P. argentinus	0.103	(12)	4	0.396	(11)	4	–		–	–		–
C. sapidus	–		–	–		–	0.105	(11)	4	0.545	(11)	4
C. angulatus	–		–	–		–	0.082	(8)	3	0.986	(8)	3
Odontesthes sp.	–		–	–		–	0.827	(6)	2	0.513	(6)	2
B. aurea	–		–	–		–	0.050*	(6)	2	0.513	(6)	2
M. furnieri	0.301	(12)	3	0.670	(12)	3	0.393	(11)	3	0.472	(11)	3
P. orbignyanus	–		–	–		–	0.574	(8)	3	0.119	(8)	3

in $\delta^{15}N$ were important for periphyton (N) and POM (N and S). The $\delta^{15}N$ signal was higher in winter than in summer for most producers, with exception of *S. californicus*, which was more ^{15}N enriched in summer (Appendix 1).

Consumers

Mean $\delta^{13}C$ values for consumers ranged from –27.5 ± 0.9‰ for *Cyphocharax voga* to –17.6 ± 1.0‰ for *Nephtys fluviatilis*. Annual mean $\delta^{15}N$ values ranged from 5.9 ± 0.8‰ in an unidentified isopod to 14.3 ± 0.9‰ in *C. voga* (Fig. 2). $\delta^{13}C$ showed little spatial variability for species common to both sites (<1‰). Significant differences were restricted to *Erodona mactroides* and *N. fluviatilis* (spring), to and *Neomysis americana* (spring and summer) (Tables 3 & 4). $\delta^{15}N$ differed between sites for *E. mactroides* (autumn, winter and summer), *Heleobia australis* (summer), *N. americana* (autumn and summer) and *N. fluviatilis* (winter). Seasonal $\delta^{13}C$ variability in invertebrates was greater than in fish: at N, seasonal differences existed for *E. mactroides* and *N. fluviatilis* and, at S, for those 2 species plus *N. americana* and the amphipod (Appendix 1, Table 4).

Trophic levels

Mean $\delta^{15}N$ signature of reference organisms for TL = 2, *Erodona mactroides* and *Heleobia australis*, varied between 5.3 and 8.5‰ at N, corresponding to 0.9 TL units; at S, variability was narrower, between

6.6 and 7.7‰ (0.3 TL units) (Fig. 3). Maximum TL varied seasonally at both sites (K-W: H = 13.8, n = 19, p < 0.01 at N; H = 10.7, n = 20, p < 0.05 at S), but did not differ between sites for any of the seasons (M-W, autumn: U = 5, n = 9, p = 0.221; winter: U = 8, n = 11, p = 0.201; spring: U = 14, n = 12, p = 0.522; summer: U = 1, n = 7, p = 0.077). Trophic level of consumers tended to increase with organism size: a group of consumers with TL between 2 and 3 included the small planktonic and epibenthic crustaceans *Acartia tonsa*, *Pseudodiaptomus richardii*, *Palaemonetes argentinus*, *Neomysis americana*, *Callinectes sapidus* and *Cyrtograpsus angulatus*. TL between 3 and 4 was comprised by a heterogeneous assemblage of fish (*Odontesthes* sp., *Brevoortia aurea*, freshwater engraulid), polychaetes (*Nephtys fluviatilis*), and ctenophores present only in autumn (S) (Fig. 3). The mullet *Mugil* sp. was intermediate between that and the top trophic group, which was represented by the omnivorous or piscivorous fish *Micropogonias furnieri*, *Paralichthys orbignyanus* and *Hoplias malabaricus*. Seasonal variability in TL was evident for *M. furnieri* at N and S (Table 5), but none of the consumers showed differences in TL between sites (Table 6).

Identification of food sources by mixing models

Prey categories suggested for given consumers by mixing models were similar among seasons. Thus, comparison of food sources between sites was based on composite seasonal results (Table 7, see also Appendices 2 & 3 in the Electronic Supplementary
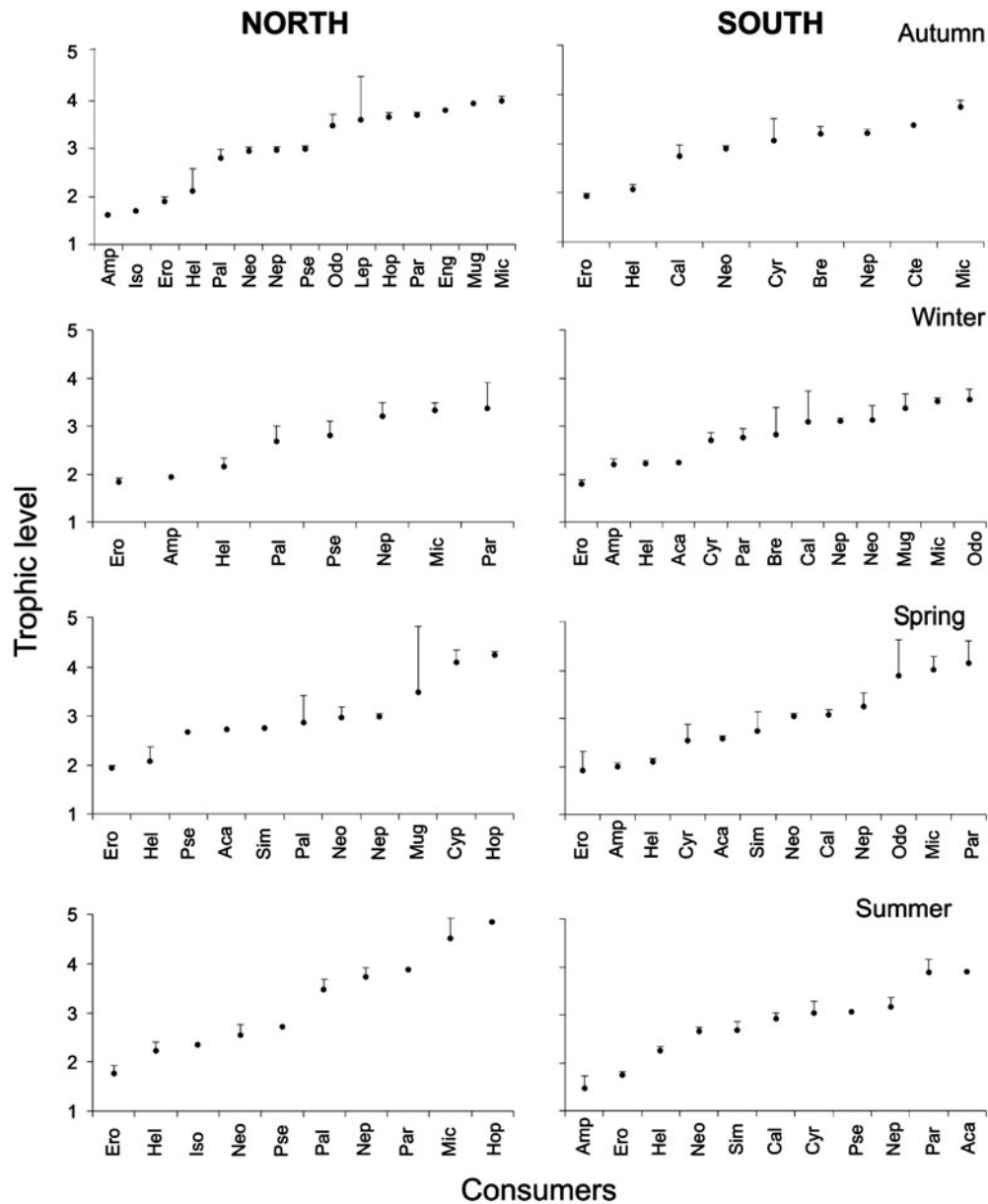
Fig. 3. Trophic position of consumers from Laguna de Rocha according to their δ^{15}N signature. Error bars indicate +1 SD. For full taxonomic names and acronyms, see Table 2

Material available at http://www.int-res.com/articles/suppl/m362p069_app.pdf). Using these data, PC (number of potential consumers) and CFE (contribution as food for the ecosystem) were calculated.

Among primary OM sources, highest scores for both PC and CFE corresponded to POM and SOM at both N and S. At N, macrophytes and periphyton were used by a small group of consumers (*Heleobia australis*, *Palaemonetes argentinus*, an unidentified amphipod and *Mugil* sp.; Table 7). Among invertebrates, several species evidenced high PC scores (sources for 5 to 7 different consumers), but, in terms of CFE, only a few

species appeared to be the most important: *P. richardii*, an unidentified amphipod, *Nephtys fluviatilis* and *Neomysis americana* (Table 7). Fish were prey for a limited number of other fish species, and no large differences existed within this group regarding PC or CFE. Cannibalism was potentially important for *Micropogonias furnieri*, *Hoplias malabaricus* and *Paralichthys orbignyanus* (Table 7).

At S, macrophytes were a potential food source for a wider assemblage of consumers, including the same grazer invertebrates at N plus *Callinectes sapidus*, *Cyrtograpsus angulatus*, an unidentified isopod and

Table 5. Trophic level of selected consumers at Laguna de Rocha: north versus south comparison. *U*: Mann-Whitney statistic; n: sample size; p: probability level. Comparisons made with pooled seasonal data as no seasonal differences existed (see 'Results; Trophic levels') except for *Micropogonias furnieri*. For full taxonomic names, see Table 2

Taxon	*U*	n	p
Amphipoda	6	10	0.602
N. americana	41	21	0.355
N. fluviatilis	56	23	0.568
P. orbignanus	17	13	0.661
M. furnieri (autumn)	1	8	0.053
M. furnieri (winter)	1	6	0.127

Table 6. Trophic level of selected consumers from Laguna de Rocha. Seasonal comparison for north and south sites, separately. *H*: Kruskal-Wallis statistic; N: number of seasons; n: sample size; p: probability level. For full taxonomic names, see Table 2

Taxon	——North——				——South——			
	H	N	n	p	*H*	N	n	p
Amphipoda	–	–	–	–	6.3	2	8	0.044
N. americana	4.4	2	9	0.113	7.62	3	12	0.055
N. fluviatilis	6.4	3	11	0.094	1.7	3	12	0.644
M. furnieri	9.5	2	9	0.039	7.5	2	11	0.024
P. orbignyanus	2.0	2	5	0.368	4.7	2	8	0.96

the fish *Micropogonias furineri* and *Brevoortia aurea* (Table 7). Among consumers, several species were potential food sources for numerous other consumers (between 7 and 9). *Nephtys fluviatilis* and *Neomysis americana* presented maximum CFE scores, followed by a group of lower importance that included *Erodona mactroides*, the unidentified amphipod, the polychaete *Heteromastus similis* and *Heleobia australis* (Table 7). Fish were prey for a few other fish species, with *Paralichthys orbignyanus*, *M. furineri* and *Odontesthes* sp. appearing to be the main contributors.

Diet composition: direct analysis of stomach contents in fish

Stomach content analyses showed the following items: *Odontesthes* sp. consumed *Neomysis americana*, *Heleobia australis*, mussels and unidentified insects; stomachs of *Micropogonias furnieri* contained amphipods, *Erodona mactroides*, crabs, *H. australis*, isopods, polychaetes, fish remains, mussels, tanaidaceans and plant remains; *Paralichthys orbignyanus* presented crab and unidentified Clupeidae as gut contents; *Hoplias malabaricus* stomachs had only fish remains, while SOM was the only food source found in *Mugil* sp. and in *Brevoortia aurea* (Table 8).

DISCUSSION

The main objective of the present study was to test the hypothesis that differences at contrasting sites along the main environmental gradient of Laguna de Rocha translate into different trophic structures. To our knowledge this represents a first attempt to use stable isotopes to simultaneously quantify the importance of both primary and secondary food sources for a large set of organisms within an ecosystem. We summarized environmental variability, the compositions of assemblages and their isotopic signals. Then, we evaluated whether descriptors of trophic structure matched expected results; the working hypothesis would be supported if differences between sites are evident in the relative importance of primary OM sources and intermediate consumers (based on CFE scores), and in maximum TL attained. Last, we discussed implications that intermediate consumers of highest trophic significance have for the functioning of coastal lagoons.

Environment, assemblage composition and its isotopic variability

The lagoon–ocean connection was interrupted for an unusually long period, but typical N–S environmental gradients persisted during most of the study. Nutrient levels, phytoplankton biomass and species compositions at N and S sites during the period considered here (Table 1) were in accordance with ranges and trends observed at LR during the last 10 to 15 yr (Aubriot et al. 2005, Bonilla et al. 2005). Biological assemblages only partially reflected differences in abiotic variables: the main differences involved a number of macrophyte species (a maximum of 7 at N versus 3 at S) and their biomass (up to 22-fold times higher at N; Table 2). Observed differences in the macrophyte assemblage between N and S sites were consistent with earlier measurements during the years from 2003 to 2004 (authors' unpubl. data) regarding species richness (8 at N versus 3 at S) and biomass (between 5- and 9-fold higher at N, depending on the season). Higher nutrient concentrations did not result in increased chlorophyll levels in the water, and 15 out of 39 microalgae identified were common to both sites, including 2 of the dominant taxa (*Melosira moniliformis* and *Paralia sulcata*). Invertebrate assemblages were also similar between sites, except the crabs *Callinectes sapidus* and *Cyrtograpsus angulatus*, which only occurred at S. Fish assemblages were also similar, but a few species only occurred at N (top predator *Hoplias malabaricus*), or only at S (*Brevoortia aurea*). These results closely agree with the quantitative and qualitative information available for LR, e.g. micro-

Table 7. Contribution of biological components at Laguna de Rocha as food sources, measured as number of potential consumers (PC), average contribution to the assimilated biomass of consumers (C_{avg}), and total contribution as food for the ecosystem (CFE = the product of PC and C_{avg}). POM: particulate organic matter; SOM: sediment organic matter; for acronyms, see Table 2

Sources	No. of PC	C_{avg} (%)	CFE	Potential consumers
North				
Ele	4	11	0.44	Amp, Hel, Mug, Pal
Myr	4	9	0.35	Amp, Hel, Mug, Pal
Per	4	10	0.42	Amp, Hel, Neo, Pal
POM	7	19	1.33	Amp, Hel, Eng, Neo, Nep, Odo, Pal
Pot	3	10	0.30	Hel, Mug, Pal
Rup	4	9	0.35	Amp, Hel, Mug, Pal
Sch	3	11	0.34	Amp, Hel, Pal
Zan	1	4	0.04	Hel
SOM	7	8	0.59	Amp, Eng, Hel, Neo, Nep, Pal, Odo
Aca	1	3	0.03	Pal
Amp	5	18	0.91	Eng, Hop, Mic, Nep, Odo
Ero	6	8	0.50	Eng, Mic, Mug, Nep, Odo, Par
Hel	5	9	0.47	Eng, Mic, Nep, Odo, Par
Iso	1	12	0.12	Par
Neo	7	13	0.89	Eng, Hop, Mic, Neo, Nep, Odo, Par
Nep	7	10	0.67	Eng, Mic, Mug, Neo, Nep, Odo, Par
Sim	2	11	0.21	Neo, Nep
Pal	5	8	0.39	Eng, Hop, Mic, Odo, Par
Pse	6	29	1.74	Eng, Mug, Neo, Nep, odo, Pal
Cyp	0	0	0	
Eng	2	8	0.17	Hop, Mic
Hop	2	7	0.14	Hop, Par
Lep	1	6	0.06	Hop
Mic	3	7	0.22	Hop, Mic, Par
Mug	2	8	0.16	Hop, Mic
Odo	3	11	0.33	Eng, Hop, Mic
Par	1	8	0.08	Par
South				
Ent	4	11	0.46	Amp, Cal, Cyr, Hel, Mug
POM	8	22	1.79	Amp, Bre, Cal, Cyr, Hel, Neo, Nep, Odo
Rup	6	14	0.81	Bre, Cal, Cyr, Hel, Mic, Mug
Zan	3	4	0.12	Amp, Cyr, Hel
SOM	10	12	1.12	Amp, Bre, Cal, Cyr, Hel, Mic, Mug, Neo, Nep, Odo
Aca	3	15	0.44	Bre, Neo, Odo
Amp	8	10	0.8	Bre, Cal, Cyr, Mic, Nep, Mug, Odo, Par
Cal	3	16	0.49	Bre, Mic, Par
Cte	2	9	0.18	Bre, Mic
Cyr	4	5	0.21	Bre, Cal, Mic, Par
Ero	8	12	0.95	Bre, Cal, Cyr, Mic, Mug, Nep, Par
Hel	8	9	0.75	Bre, Cal, Cyr, Mic, Mug, Neo, Odo, Par
Iso	0	0	0	
Neo	9	14	1.29	Bre, Cal, Cyr, Mic, Mug, Neo, Nep, Odo, Par
Nep	9	16	1.42	Bre, Cal, Cyr, Mic, Mug, Neo, Nep, Odo, Par
Sim	7	11	0.78	Cal, Cyr, Mic, Neo, Nep, Odo, Par
Pse	1	9	0.09	Nep
Bre	1	5	0.05	Mic, Par
Mic	2	11	0.22	Mic, Par
Mug	1	1	0.01	
Odo	2	10	0.20	Mic, Par
Par	1	23	0.23	Par

algae: Bonilla et al. (2005), benthos: Jorcin (1996) and Giménez et al. (2006), fish: Santana & Fabiano (2001), zooplankton: authors' unpublished information. We can thus safely assume that the 32 groups used for isotopic analyses were broadly representative of the biological community of LR.

Stable isotopic values of OM sources overlapped with ranges reported for other sites (Tay estuary, Thornton & McManus 1994; Tijuana estuary and San Dieguito lagoon, Kwak & Zedler 1997; Mediterranean lagoon, Vizzini & Mazzola 2003). An exception was *Ruppia maritima* which was δ^{13}C depleted with respect to Lagoa dos Patos (Brazil; Abreu et al. 2006) and to Australian lagoons (Boyce et al. 2001). Invertebrates also presented similar isotopic values to earlier studies, except for *Palaemonetes argentinus*, which was also δ^{13}C depleted (i.e. Kwak & Zedler 1997, Vizzini & Mazzola 2003, Connolly et al. 2005, Wissel & Fry 2005, Abreu et al. 2006). In turn, fish in LR tended to be δ^{13}C depleted, but δ^{15}N enriched with respect to other studies (Hughes & Sherr 1983, Kwak & Zedler 1997, Abreu et al. 2006, Garcia et al. 2007).

Macrophytes evidenced moderate seasonal changes in C and N isotopic signatures. Potential causes of differential ^{13}C fractionation include irradiance, reproductive condition, salinity stress and temperature (Grice et al. 1996, Hemminga & Mateo 1996, Anderson & Fourqurean 2003, Vizzini & Mazzola 2003). In winter most plants were more δ^{13}C depleted (expected pattern due to irradiance effects; Vizzini & Mazzola 2003) and ^{15}N enriched. The lowest conductivity in winter coincided with higher DIN and SRP levels, likely originating from increased influx from fertilizers in the basin and faster delivery of sewage from Rocha City during the rainy season. DIN from sewage and fertilizers has a higher δ^{15}N signature than natural DIN (Abreu et al. 2006), and thus the highest δ^{15}N signal of macrophytes in winter could result from the use of nitrogen from such sources. Seasonal changes in isotopic signals were less pronounced in

Table 8. Size range and stomach contents found in fish from Laguna de Rocha. n: number of specimens analysed for gut contents; J: juvenile; A: adult. For full taxonomic names, see Table 2

Taxon	n	Size (cm)	Stage	Stomach content
B. aurea	6	22–32.8	A	Sediments
C. voga	3	18.0–19.0	A	Sediments
Engraulid, unidentified	1	18.5	–	Empty
H. malabaricus	11	30.5–47.5	A	Unidentified fish remains
Leporinus sp.	2	15.5–17.0	J	Empty
M. furnieri	20	8.6–34.0	J, A	Amphipods, crabs, *E. mactroides*, *H. australis*, isopods, mussels, polychates, unidentified Clupeidae, unidentified fish remains, tanaedaceans
Mugil sp.	8	32.5–45.0	A	Sediment and plant remains
Odontesthes sp.	12	17.5–22.0	J, A	Gastropods, *H. australis*, insects, mussels, *N. americana*
P. orbygnianus	14	18.5–37.0	J, A	Crabs, unidentified Clupeidae

consumers, particularly in groups of larger individual body mass (fish less variable than invertebrates), and were consistent with the well-known allometric pattern of biomass turnover rate (Banse & Mosher 1980) also observed in earlier studies (Vizzini & Mazzola 2003).

Organic matter sources and trophic structure at north and south sites

The most important primary OM sources at both N and S were POM and SOM (Table 7). Direct use of macrophytes and macroalgae as food was minor at both sites, despite higher biomass and species-richness at N. This finding agrees with studies showing that vascular plants and macroalgae are not widely used as food within the system (Page 1997, Chanton & Lewis 2002, Fry 2006, Kang et al. 2007). Indirect use (as detritus) can be difficult to assess if isotopic signals change between living and decaying plant biomass. Decomposition of aquatic plants does not significantly fractionate carbon isotopes, so detritus $\delta^{13}C$ should not differ from $\delta^{13}C$ of the plant source (Fellerhoff et al. 2003). If plant detritus constituted the bulk of POM and SOM, their $\delta^{13}C$ should have reflected that of living plants, which was clearly not the case, suggesting that microalgae, protozooplankton and microzooplankton contributed more importantly to POM.

Consumers with highest CFE scores (i.e. the most important intermediary food sources) were a taxonomically heterogeneous group of invertebrates common to both sites that included the mysid *Neomysis americana*, the unidentified amphipod and the polychaete *Nephtys fluviatilis* (Table 7); the single difference between sites was the freshwater copepod *Pseudodiaptomus richardii*, a species normally absent at S. Similar assemblages of small consumers are also important trophic components in comparable ecosystems; for

example, mysids (*Mysis* sp.) and amphipods (*Gammarus salinus*) are central organisms in the trophic web of the Vaccarès coastal lagoon (Mediterranean; Persic et al. 2004); mysids, amphipods, isopods and polychaetes were most important in the food web leading to sand smelt *Atherina boyeri* in the shallow Stagnone di Marsala (Vizzini et al. 2002), as was *Neomysis integer* in the brackish Lake Ferring (Søndergaard et al. 2000). In shallow, temporally open South African estuaries, the zooplankton assemblage dominated by the copepods *Acartia natalensis* and *Pseudodiaptomus hessei* and the mysid *Gastrosaccus brevifissura* closely resembles that of LR and channel most of the primary production (Perissinotto et al. 2003).

Maximum TL ranged from 3.4 to 4.8, similar to values in comparable ecosystems, e.g. 4.4 in the brackish Vaccarès Lagoon (Persic et al. 2004) and >4.5 in the Tijuana Estuary (Kwak & Zedler 1997). No differences existed in TL between sites at LR, although there was a tendency for higher values at N and marginally significant differences between sites occurred during summer. In turn, maximum TL varied seasonally, with higher values during the most productive season at both N and S. Consistently, top predators *Micropogonias furnieri* (present at both sites) and *Hoplias malabaricus* (only at N) also evidenced higher TL in summer (Fig. 3), in line with the expected increase in the maximum length of energy pathways with increased system productivity (Vander Zanden et al. 1999).

Similarities in OM sources, intermediate consumers and maximum TL between N and S sites of LR were contrary to our prediction, indicating that in lagoon estuaries trophic structure does not always change along environmental gradients. In the present case, 2 factors contributed to the unexpected result: first, prevalence of marsh plants (*Schoenoplectus californicus* and *Potamogeton* sp.) at N was one key factor expected to generate diverse trophic structures between sites, but the trophic significance was similarly

low at both. Low trophic significance of marsh plants at N may be due to a low ratio of marsh to open water areas (<1), considering that the contribution of marsh plants to trophic processes tends to be insignificant when the above ratio is <2 (Fry 2006, and references therein). Second, the assemblages of consumers were highly similar between sites despite salinity differences. Distribution of organisms in estuaries along salinity gradients is mostly influenced by salinity variability, rather than average salinity (Attrill 2002). The stable hydrography of LR during the study period, i.e. a lentic body of water with a long retention time, little water exchange and no tidal regime, likely led to organisms extending their distribution over most of the salinity range.

Low trophic impact of macrophytes should not be interpreted as a lack of ecological significance. Marsh areas serve as refuge from predators to multiple vertebrates (larval and juvenile fish) and invertebrates (Day et al. 1989). Alterations in the extent of such areas may thus, hypothetically, still affect the presence and/or use of the lagoon by such taxa, and so ecosystem functions.

Implications of intermediate consumers for coastal lagoon processes

Intermediate consumers of high trophic importance (highest CFE scores; Table 7) shared several relevant traits: small size (ca. 1 mm for *Pseudodiaptomus richardii* to <10 cm for *Nephtys fluviatilis*), relatively low TL (between 2 and 3.2), tolerant to a wide range of conditions (i.e. salinity) that allowed it to inhabit both N and S sites, and a lifestyle adapted to the benthic–pelagic interface. The latter implies not only living close to the sediments—the case by force in such a shallow environment—but that they move or have functional links (i.e. feed) in both realms. *Neomysis americana* migrates daily between surface sediments during light hours to the water column by night (Calliari et al. 2001), and feeds at both (Takahashi 2004). Amphipods live on sediments and associated vegetation, making short excursions in the water column; during our study, they were collected with the epibenthic sledge, but also with the plankton net, as were occasionally polychaetes. In turn, *P. richardii* belongs to a genus of calanoid copepods with demersal affinities to move between the benthos and the water column (Rios 1998). In LR, *P. richardii* lives associated with sediments or vegetation, as revealed by the high numbers found in trawl samples and low abundance or absence in plankton tows. These intermediate consumers thus constituted a core structural element common to both ends of the environmental gradient, and their trophic role proved robust under the observed

variability range. It could be conjectured that a different hydrographical setting, i.e. more distinct marine or freshwater conditions at the extremes of the environmental gradient, would likely alter the assemblage of intermediate consumers; furthermore, that changes in water column depth may modulate their trophic significance, for instance, by favouring pelagic (i.e. copepods) over benthic organisms as the main intermediaries in trophic fluxes.

Significant coupling between benthic and pelagic subsystems has also been observed in comparable ecosystems—i.e. temporally open estuaries in South Africa—where copepods and mysids rely on benthic production during the closed phase (Perissinotto et al. 2003). Such coupling is likely driven by adaptations of key intermediate consumers and shallow depth that favours resuspension–sedimentation of microalgae, as indicated here by similar $\delta^{13}C$ signals of SOM and POM and the presence of benthic diatoms in the water column, a frequent observation at LR (Bonilla et al. 2005). Shallowness also grants benthic feeders direct access to living plankton, i.e. abundant and high-quality food, while nekton predators can readily consume both pelagic and benthic prey (Vizzini & Mazzola 2003), as indicated here for *Micropogonias furnieri*, *Paralichthys orbygnanus* and *Odontesthes* sp. by isotopes and direct diet analyses.

Stomach contents of fish generally confirmed results from isotopic mixing models, even in unexpected cases. For instance, stomachs of silverside *Odontesthes* sp., considered a zooplanktivore–piscivore predator (Sagretti & Bistoni 2001, Froese & Pauly 2006), contained mysids, gastropods, mussels and insects, in close agreement with isotopic results and with observations of predation on benthic invertebrates at Laguna Merín, a nearby shallow lagoon (Piedras & Pouey 2005). For *Micropogonias furnieri*, a mixing model suggested a generalist benthic–piscivorous diet, matching our findings in stomach contents as well as earlier results at LR (Olsson et al. 2003) and other areas (Puig 1986). Adult *Paralichthys orbygnianus* at LR (25 to 65 cm length) had been classified as ichthyophagous, preying on *Odontesthes argentiniensis*, *Brevoortia aurea*, *M. furnieri* and younger co-specifics (Norbis & Galli 2004); present results from isotopic and stomach analyses confirmed those and also evidenced carcinophagy. Finally, the genus *Mugil* is iliophagous during the adult stage, feeding on OM-rich sediments (Pillay 1953), but there are few detailed descriptions of detrital matter composition in their stomachs (Oliveira & Soares 1996, Blaber 1997). Stomach contents of *Mugil* sp. in the present study revealed only detrital matter and plant remains, but isotopes suggested that plants and small benthic invertebrates could contribute to its diet. That is consistent with reports of small benthic invertebrates in the

diet of *Mugil* sp. (gastropod molluscs and harparticoid copepods; Blaber 1997).

In summary, the main differences recorded at LR between N and S sites expected to turn into distinct trophic web structures were: (1) dominance of submerged macrophytes and exclusive presence of marsh plants at N and (2) the salinity gradient between fresh- and seawater ends of the lagoon. A minor contribution of marsh plants and macrophytes to the diet of the local fauna and the along-year hydrological stability probably allowed a fairly homogeneous biological assemblage and food web structures between both sites. Those results indicate that, in coastal lagoons, differences in the physical environment may not necessarily translate into spatially diverse trophic structures.

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