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Testing the relationship between primary production and *Acartia tonsa* grazing pressure in an estuarine lagoon

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Flow of carbon to large consumers in marine environments is mediated by copepods. Globally, copepod grazing removes a small fraction of pelagic primary production, and that fraction decreases from oligotrophic to productive ecosystems. Such a pattern should result from mechanisms whose validity has not been explicitly tested. We analysed the relationship between primary production and copepod herbivory pressure (HP) in a subtropical lagoon under the hypothesis that HP is higher during periods of low, compared to periods of high productivity. On 18 occasions during 2 years, we estimated primary production as ^{14}C incorporation, and herbivorous grazing (as gut fluorescence) and egg production rates for the dominant zooplankter Acartia tonsa. Primary production varied between 18 and 407 mg C m^{-2} day^{-1}; A. tonsa HP was low (max. of ca. 5% or 18%, depending on assumptions) and followed a non-linear negative pattern with primary production consistent with expectations. The herbivorous fraction of A. tonsa diet was usually $<50\%$, suggesting strong trophic links with microbial processes. Despite sustained high fecundity (11–83 eggs $female^{-1}$ day^{-1}), population density of A. tonsa was moderate or low, which contributed to low HP. Top-down control on copepods, also suggested by earlier studies in this ecosystem, may be one factor constraining fluxes via the herbivory pathway.

INTRODUCTION

The flow of organic carbon from primary producers to large-sized consumers in the marine pelagic ecosystem is largely mediated by copepods. Relevant questions on the functioning of this ecosystem involve, among others, elucidating the fraction of primary production consumed by copepods, the efficiency of its conversion into secondary production available to large consumers, and how these processes respond to environmental and biological forcing.

The fraction of primary production channelled by copepods is often moderate or low. Meta-analyses considering widely diverse ecosystems have shown that on average copepods remove nearly 22%, but most frequently only 6% of primary production (Calbet, 2001). The main pelagic grazers are a heterogeneous group of protozoans <200 μm (nano- and micro-sized heterotrophic flagellates, dinoflagellates and ciliates) and metazoan microplankton generally referred to as microzooplankton. Microzooplankton grazing accounts for ca. 67% of daily autotrophic growth as a global average, but can occasionally remove $>100\%$ and their impact changes little from less productive to more productive areas (Calbet and Landry, 2004). In contrast, the fraction of primary production consumed by copepods tends to be lower in highly productive waters (coastal areas and estuaries) (Calbet, 2001), and so the relative importance of the herbivorous pathway decreases from oligotrophic

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to productive ecosystems. Thus, productive systems may not be more efficient, as traditionally thought, at transferring energy from producers to large predators; but the matter is open to debate.

Such global pattern would result from mechanisms that operate at individual and ecosystem level: a size threshold for food particle capture by copepods, and a mismatch between the typical response times of larger phytoplankton and their mesoplankton grazers which allows prey to escape predation control (Kiørboe, 1993; Irigoien *et al*., 2005). Growth of pico- and nanophytoplankton is paralleled by consumption and growth rates of nano- and micro-sized grazers, so ultimately primary production by such size fractions is limited by biological control (Irigoien *et al*., 2005). High productivity can result from short-lived events of increased growth of nano- to micro-sized species (blooms of cells >10 μm) (Kiørboe, 1993) that escape grazing control of microzooplankton based on size, or other defence mechanisms (Irigoien *et al*., 2005). Copepods graze efficiently on large cells (>5 μm, Bartram, 1980; Berggreen *et al*., 1988) and may take advantage of blooms to increase consumption and fecundity rates. However, long generation times (>1 month) mean that their population increase is slow, and grazing pressure during blooms remains low. Therefore, most of the primary production during blooms may never enter the herbivorous pathway (Kiørboe, 1993). The fraction taken by copepods would thus be proportionally lower in ecosystems where episodic productivity peaks contribute a significant fraction of annual production. Despite several studies which have simultaneously evaluated phytoplankton production and grazing by different zooplankton fractions, to our knowledge the functional relationship between these two biological processes has not been explicitly evaluated in marine systems. We hypothesized that during periods of low productivity, herbivorous grazing by mesozooplankton should remove a higher fraction of phytoplankton production compared to periods of high productivity, so both biological processes should be negatively correlated within a given ecosystem.

To evaluate the above hypothesis, we analysed the relationship between planktonic primary production and copepod herbivorous grazing using a lagoon estuary on the subtropical South-West Atlantic as a study case. We report here that herbivorous consumption by the strongly dominant mesozooplankton species was independent of primary production; consequently herbivorous grazing pressure decreased along the same production gradient supporting the proposed hypothesis.

METHOD

Study site

Laguna de Rocha is a temporally open lagoon estuary on the Atlantic coast of Uruguay, South America, at 34°37'S, 54°17'W. The lagoon is largely pristine, moderately eutrophic and productive (Conde *et al*., 2002); it constitutes a nursery for high-valued crustaceans and fish (e. g. pink shrimp *Farfantopenaeus paulensis*, blue crab *Callinectes sapidus*, white croaker *Micropogonias furnieri*, black drum *Pogonias chromis*, flounder *Paralichthys orbignyanus*), and a breeding or feeding site for >100 species of resident and migratory birds including charismatic ones (e.g. black-necked swan *Cygnus melancoryphus*, white swan *Coscoroba coscoroba*, Chilean flamingo *Phoenicopterus chilensis*). A detailed description of the study site can be found in Rodríguez-Graña *et al*. (Rodríguez-Graña *et al*., 2008).

Sampling design and data collection

Sampling was performed during six visits in September (Winter) and October (Spring) 2004, January (Summer), April (Autumn) and December (Summer) 2005, and April (Autumn) 2006; and on each visit, identical sampling and experimental protocols were repeated on three consecutive days, totalling 18 observations. Such a design covered the four seasons but its primary goal was to capture a wide range of environmental and biological conditions. Sampling was performed at one station during the first hour following sunset; preliminary investigations suggested that only small differences exist in copepod feeding activity between day and night hours at Laguna de Rocha. Variables routinely measured *in situ* were: water depth (m), temperature (°C, Horiba OM-14) and conductivity (mS cm^{-1}, Horiba D-24). Salinity values were derived from conductivity and temperature measurements using UNESCO International Equation of State for Seawater. Photosynthetic available radiation (PAR) in the water column was measured before sunset with a LI-192SA 4 π quantum sensor and LI-250 (Li-Cor) data logger every 5 or 10 cm, and at each depth irradiance values were averaged for 10 s. Light attenuation coefficient k_d (m^{-1}) was estimated by linear regression of $\log_e$-transformed PAR values versus depth.

Surface water samples were taken for chlorophyll-*a* (chl), nitrate, ammonium, soluble reactive phosphorus and reactive silicate concentration. Nutrients were analysed according to standard methods (APHA, 1995); chl was measured following Wasmund *et al*. (Wasmund *et al*., 2006) by filtering 50 mL onto GF/F filters (three replicates), extracting in 96% ethanol for 24 h in the dark at 4°C and reading the fluorescence of the extract on a

Turner 111 fluorometer before and after acidification (1.2 N HCl). Phytoplankton composition and abundance were recorded from surface bottle samples; cells >3 μm were identified under an inverted microscope with phase contrast at 1000× from Lugol preserved (ca. 1% Lugol added in amounts to render a light brown colour) and formaldehyde-preserved (ca. 3%) replicate samples using sedimentation chambers of 5 or 10 mL, depending on cell concentration. Biovolume (μm^3 mL^{-1}) of dominant groups was estimated by counting and measuring individual cells according to the set of geometric shapes and corresponding equations proposed by Hillebrand *et al.* (Hillebrand *et al.*, 1999). Primary production was estimated from surface samples in the laboratory as ^{14}C incorporation (Gätcher *et al.*, 1984): ca. 3 μCi (as HCO_3^-) were added to each of 18 20 mL scintillation vials containing *in situ* water and incubated at *in situ* temperature (±1°C) under a gradient of irradiance levels. After 30 min, photosynthesis was stopped by adding 500 μL of analytic grade formaldehyde; 100 μL pure HCl was further added and subsequently samples were bubbled for 30 min in a hood to eliminate excess HCO_3^-. Finally, the radioactivity of the samples was measured on a Beckman LS 6000 counter after addition of 5 mL of ReadyGel Beckman® scintillation cocktail. Primary production experiments were started <1 h after the sample was taken.

Mesozooplankton was sampled by horizontal subsurface tows of a 36 cm mouth diameter and 160 μm pore-size net equipped with a flow meter; this net samples adult and juvenile stages of small copepods (i.e. *Acartia tonsa*) but likely underestimates abundance of naupliar stages (see Discussion section). On each occasion, three successive tows were performed: the first was intended for taxonomic identification and counting, for which the sample was preserved with formaldehyde 4% (final concentration); the second was intended for estimation of copepod gut pigment contents and the third to collect live animals for egg production incubations in the laboratory. Mesozooplankton from preserved samples was identified to the lowest possible taxonomic level (usually species for copepods). Copepods were staged, sexed (adults) and measured (cephalothorax length, ca. 20 organisms per species, stage and sample) to estimate individual carbon content from length–weight regressions (Berggreen *et al.*, 1988).

To estimate gut fluorescence (GF) of copepods, the contents of the cod-end were sieved through a 200 μm mesh and immediately frozen (−20°C) until further processing within 1 week. In the laboratory, the sample was thawed and adult females of dominant species (usually only *Acartia tonsa*) were picked under dim light conditions, put into small vials containing 5 mL of 96% ethanol and left to extract for 24 h at 4°C in the dark. Three to five replicates were taken for each sampling day, each replicate consisting of 15 to 30 individuals. The fluorescence of the extract was read on the Turner 111 before and after acidification (1.2 N HCl). Chl and phaeopigment concentrations were estimated as recommended by Dam and Peterson (Dam and Peterson, 1988) and added together to express GF as ng pigments $individual^{-1}$. Background fluorescence of animal tissue was estimated from starved individuals kept in 0.7 μm-filtered *in situ* water for 24 h, and the value obtained subtracted from the previous estimate. Pigment evacuation rates were measured on two occasions (when conditions were most favourable, i.e. availability of clean and abundant zooplankton samples and the manpower for the extra work). Briefly, copepods were collected in short tows and the catch diluted in a 20 L temperature-insulated bucket containing unfiltered *in situ* water. After few minutes, animals were transferred to a similar volume of *in situ* 0.45 μm filtered water at the same temperature. Samples (three replicates) were then taken at times 0, 5, 10, 15, 20 and 30 min after transfer, quickly sieved and frozen for later estimation of GF as described above. Instantaneous pigment evacuation rate k was derived from fit of model $GF_t = GF_0 * \exp(-k * t)$, where GF_t is GF at time t, and GF_0 is initial GF. Pigment ingestion rates were estimated as $I = k * GF$ (Båmstedt *et al.*, 2000). Temperature-dependent values of k can be obtained from Dam and Peterson (Dam and Peterson, 1988) empirical models, but the temperature range in our study extended beyond that considered in Dam and Peterson's database. Instead, we estimated evacuation rates from our own regression of k versus temperature obtained by pooling values from this study and those in Calliari *et al.* (Calliari *et al.*, 2004), obtained using same protocol and same species in a nearby estuary (Table I).

Tows for live plankton were short (ca. 3 min) and slow (ca. 0.5 $m\ s^{-1}$) to minimize damage, and the catch was immediately diluted in a 20 L temperature

Table I: Gut evacuation rates (k, min^{-1}) of non-feeding individuals of Acartia tonsa on different dates by fitting of a negative exponential model $Gt = G0 * \exp(-k * t)$

Date	Temperature	k	r^2	Source
16 January 2003	27.0	0.0440	0.82	Calliari *et al.*, 2004
14 February 2003	23.5	0.0300	0.60	Calliari *et al.*, 2004
07 September 2004	15.5	0.0203	0.37	This study
25 April 2006	16.5	0.0336	0.26	This study

Model fit and parameter values were significant at P-level of 5% in all cases.

insulated bucket. Egg production experiments started within 2 h after sample collection; healthy-looking females of dominant species (usually only *Acartia tonsa*) were sorted under a dissecting microscope and transferred in batches of 3–6 individuals to each of 3–5 replicate 625 mL blue-cap bottles filled with 50 μm filtered *in situ* water; that represents 5 to <10 copepods L^{-1}, a moderate copepod density in estuaries and similar to that found in the study site. Bottles were incubated standing in the dark at *in situ* temperature (±1°C); after 24 h, the contents were sieved through a 50 μm mesh, the condition of the females checked and the number of eggs recorded. Egg production rates (EPR) were expressed as eggs fem^{-1} day^{-1}. *Acartia tonsa* can sometimes cannibalize its own eggs; incubating females in standing bottles helps to minimize such effects (as compared to bottles mounted on a rotating wheel), since eggs rapidly sink out of the copepods reach; cannibalism can be accounted for when calculating EPR by counting crumpled eggshells at the end of incubations (Calliari *et al.*, 2006), but during this study such a phenomenon was very rare.

Data analysis

Photosynthesis versus irradiance responses were described with equation:

$$P^{B} = P^{B}_{m} * (1 - \exp(-\alpha * I/P^{B}_{m})),$$

where P^{B} is biomass normalized primary production (mg C mg chl^{-1} h^{-1}), P^{B}_{m} is the maximum specific production rate at light saturation, i.e. the plateau of the P–I curve, α is the initial slope (mg C mg chl^{-1} h^{-1} W^{-1} m^{-2}) and I is light intensity (W m^{-2}) (Harrison and Platt, 1986). *In situ* daily primary production for each sampling day was calculated by graphical integration of P^{B} in the vertical (10 cm depth-intervals) using PAR values calculated from measured vertical extinction coefficient k_d, and for the whole day using hourly surface irradiance for the corresponding Julian day from the Bird and Hulstrom (Bird and Hulstrom, 1991) model corrected with *in situ* light measurements.

Phytoplankton carbon content was estimated from measured biovolume according to Montagnes *et al.* (Montagnes *et al.*, 1993), and resulting carbon: chlorophyll ratios used to convert copepod pigment ingestion rates to carbon units. EPR was converted to carbon assuming an egg carbon content for *Acartia tonsa* of 45.7 ng egg^{-1} (Kiørboe and Sabatini, 1995). Total herbivory consumption by *A. tonsa* population was estimated by expanding weight-specific phytoplankton carbon ingestion rates of adult females to the population, and the fraction of primary production consumed by *A. tonsa* as herbivorous grazing [herbivorous pressure (HP) expressed as percentage] resulted from dividing herbivory consumption by estimated primary production.

Measured ingestion and EPR were combined to estimate an herbivory index H for *Acartia tonsa* and the fraction of the primary production required (PPR) to be ingested to sustain measured EPR. We note that H and PPR are not expected to provide accurate estimates but constitute useful indicators of general trends. Herbivory index H was defined as (Peterson and Dam, 1996): $H = \mathrm{GGE}/(\mathrm{EPR}/I)$, where GGE is herbivorous-based gross growth efficiency, and EPR and I are measured on the same species and date; H ranges from 1 for a completely herbivorous diet, to 0 for a completely carnivorous diet, i.e. a diet based on heterotrophic organisms only. To estimate PPR, we first calculated the fraction of *A. tonsa* production based on carnivory $(1-H)$ as: $\mathrm{EPR}^* = \mathrm{EPR} * (1-H)$; the ingestion rate of autotrophic biomass needed to account for EPR* was calculated assuming animal prey of *A. tonsa* were herbivorous (i.e. trophic level 2) as: $I^* = \mathrm{EPR}^*/\mathrm{GGE}^2$; GGE of 37% (Peterson and Dam, 1996) was assumed constant for both *A. tonsa* and for the heterotrophic prey, and the exponent=2 stands for the 2-step conversion of biomass along this pathway (phytoplankton to microzooplankton to copepod); thus, total ingestion of autotrophic biomass needed to sustain observed EPR was: $I_T = I + I^*$.

The direct approach to explore the relationship between HP and primary production has the aim of assessing the correlation between them. In the present case, the hypothesis is defined in terms of a ratio of variables; i.e. the dependent variable (HP) is a quotient whose denominator is the independent variable itself (primary production); that might lead to what has been termed "spurious correlation", i.e. a correlation that bears no meaning by itself as it arises of mathematical necessity (Atchley *et al.*, 1976; Praire and Bird, 1989). One way around that problem is to follow an indirect approach and evaluate the correlation between herbivorous consumption (based on GF) and primary production: in that context a slope <1 (or zero) indicates that the fraction of primary production consumed diminishes with increasing productivity (i.e. Calbet, 2001). But inexorable "spuriousness" of correlations involving a common term has been strongly questioned. Several authors argue in favour of their validity when hypotheses to be tested were originally formulated as ratios, as in the present case (Kuh and Meyer, 1955; Praire and Bird, 1989; Sokal and Rohlf, 1995); in these cases, correlations sharing a common term are logical consequences of variable formulations and there are no theoretical reasons to disregard them, provided their

formulation is deliberate and well-considered (Praire and Bird, 1989; Sokal and Rohlf, 1995). Here we make use of both alternative approaches to evaluate the dependency of HP on primary production.

RESULTS

Environmental conditions

Temperature ranged from 13.8 (September 2004) to 26.1°C (January 2005) (Fig. 1). Salinity varied over a wide range: moderate initial values ca. 15 in September 2004 decreased to a range between 4.9 and 7.4 that prevailed from October 2004 through April 2005; salinity increased again in December 2005 and reached a maximum ca. 24 in April 2006. Nutrient concentration were moderate to high throughout the study and did not show clear temporal trends (Fig. 1); dissolved inorganic nitrogen ranged between 1 and 11.2 μM and phosphate between 0.2 and 1.4 μM. Silicate (not shown) was always very high between 24 and 99 μM. PAR vertical extinction coefficient varied between 0.35 and 3.6 m^{-1} (average 1.8 m^{-1}). Chlorophyll varied

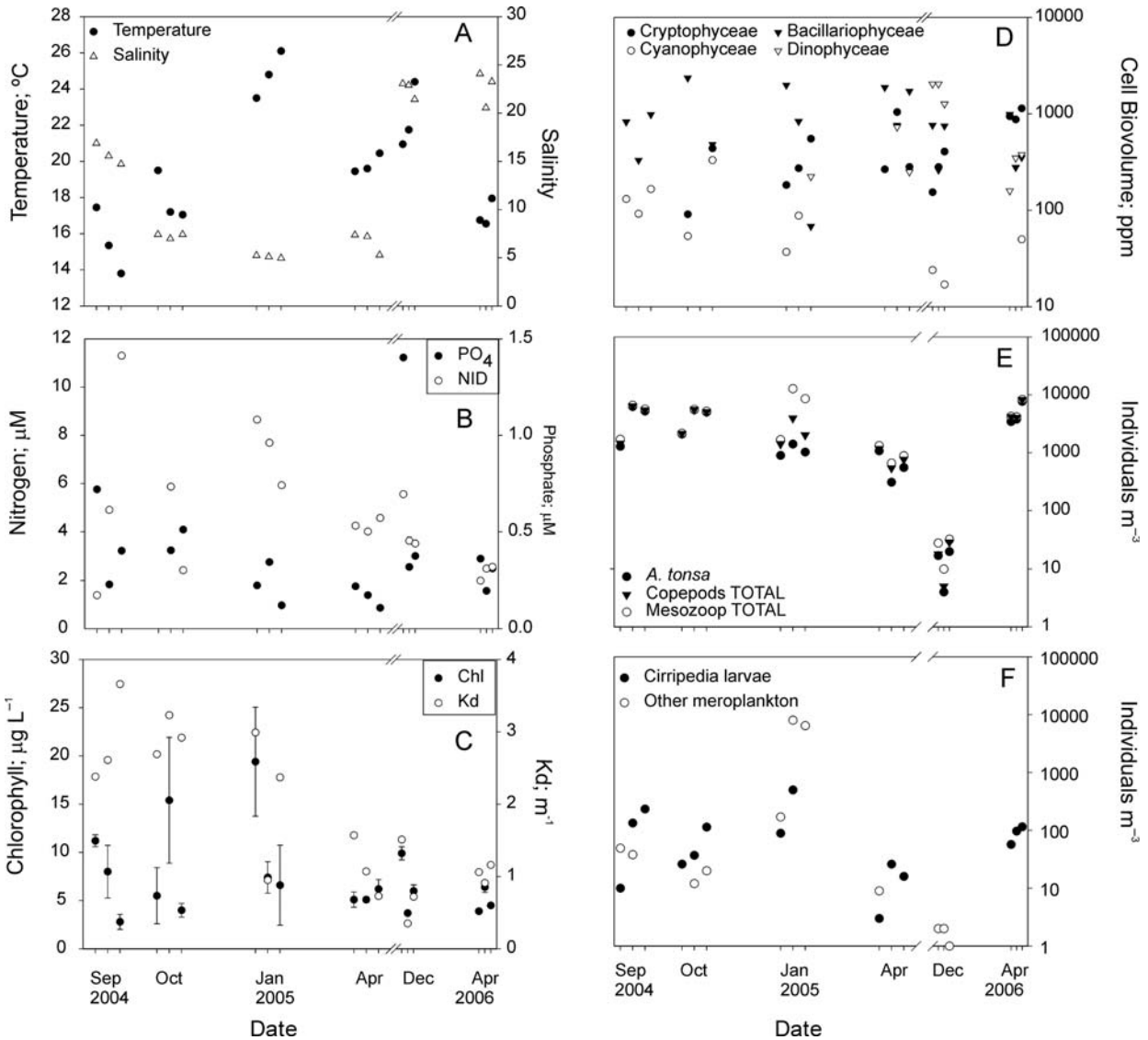

Fig. 1. Environmental variables and planktonic assemblages in Laguna de Rocha during the study period. (**A**) Temperature and salinity; (**B**) concentration of dissolved inorganic nitrogen (NID = $NO_3 + NH_4$) and reactive soluble phosphorus; (**C**) chlorophyll concentration and light attenuation coefficient Kd; (**D**) biovolume of microalgae grouped by Class; (**D**) Abundance of *Acartia tonsa*, total copepods and total mesozooplankton; (**E**) Abundance of meroplankton.

between 3.7 and 19.4 μg L^{-1} and showed remarkable day to day variability, but no overall temporal trend during the study period (Fig. 1).

Biological assemblages

The autotrophic assemblage was represented by 29 taxa. Bacillariophyceae and Chlorophyceae were best represented with nine and eight species, respectively; Chlorophyceae was the least represented with only one species (*Pseudopedinella* sp.). The picoplanktonic cyanophyte *Aphanotece* aff. *minutissima* was numerically dominant during most of the study period reaching 10^5 cells mL^{-1}. Chlorophytes were also abundant mostly represented by *Pseudodictiosphaerium* sp. (up to 10^5 cells mL^{-1}), *Monoraphidium minutum* (up to 10^4 cells mL^{-1}) and *M. caribeum* (up to 10^3 cells mL^{-1}). Small autotrophic flagellates <10 μm reached moderate abundance (10^2–10^3 cells mL^{-1}), as did diatoms *Melosira moniliformis* and *Paralia sulcata*, and autotrophic dinoflagellates *Gymnodinium* sp. and *Prorocentrum minimum*. Diatoms were usually dominant in terms of biovolume due to larger individual size, as were occasionally dinoflagellates and the cryptophyte *Plagioselmis nannoplanctica* (Fig. 1).

Copepods constituted >80% of mesozooplankton numbers during the whole period except in January 2005 when meroplankton were similarly abundant (Fig. 1). *Acartia tonsa* numbers (adult and juvenile stages combined) ranged between 1×10^3 and 5×10^3 ind. m^{-3} except in April (ca. 10^2 ind. m^{-3}) and December 2005 (10^1 ind. m^{-3}) when abundance of all mesoplankton groups was at minimum (Fig. 1) coincident with the occurrence of the ctenophore *Mnemiopsis leydii* (whose abundance was not properly quantified). *Acartia tonsa* typically comprised >90% (in numbers) of the copepod assemblage with exception of January 2005 when it comprised slightly >50%; ingestion and production rates estimates were consequently referred to *A. tonsa*. Other copepod species occasionally present were *Paracalanus parvus*, *Pseudodiaptomus richardii*, *Oncaea* sp., *Euterpina acutifrons* and *Temora* sp.

Production and consumption

Photosynthesis–irradiance responses of the autotrophic assemblage did not show photoinhibition effects at high light intensities in any of the experiments, but biomass-specific production (P^B_m) showed considerable variability on a day to day scale and during the study period (range: 0.21–14.33 mgC $mgChl^{-1}$ h^{-1}), as did the estimated PP (range: 18–407 mgC m^{-2} day^{-1}) (Figs 2 and 3).

GF of *Acartia tonsa* ranged between 0.1 and 2.6 ng pigm $ind.^{-1}$ (mean = 0.56, Table II) and EPR between 11 and 83 eggs fem^{-1} day^{-1} (mean = 38), without clear temporal trends except higher EPR on the last two sampling dates (December 2005 and April 2006; Fig. 4). HP by *A. tonsa* was consistently low during the study period between nearly zero (9×10^{-4} %) and 4.7% (average 1.2%, Fig. 4). Abundance of all *A. tonsa* juvenile stages was corrected by a factor of five to compensate for underestimation due to escape through the mesh (see Discussion for details). Re-calculation of HP based on corrected abundances was still low, ranging between 2.8×10^{-3} and 17.5% (average 4.3%). The herbivory index *H* for *A. tonsa* ranged between 0.03 and 0.95, and in most cases was <0.5 (Fig. 4) suggesting that a large fraction of its diet was made up of heterotrophic prey (ca. 75% on average).

Herbivorous consumption by *Acartia tonsa* was independent of primary production as indicated by a marginally positive but non-significant slope (Fig. 5), implying that the fraction of primary production consumed decreased with increasing primary production. Direct analysis showed the expected negative correlation of HP versus PP, although data were rather scattered (Fig. 5B and D). This suggested that even if the expected relationship was valid, further variables also affected HP response. Subsequent multiple regression indicated that primary production, *A. tonsa* ingestion rate and *A. tonsa* biomass accounted for 72% of variance in HP (Table III). The PPR to sustain EPR by *A. tonsa* ranged from 3×10^{-2} to 58% of primary production (mean = 14.3%).

DISCUSSION

Environmental and biological variability

Several environmental variables such as temperature, salinity, nutrients and even biological ones like chlorophyll and production showed important variability at both short- and long-time scales (i.e. between days and during the whole study period). Coastal lagoons are a particular type of estuary characterized by high surface to volume ratios and intermittent connection to the sea (Day *et al.*, 1989); these ecosystems often exhibit strong environmental fluctuations driven by hydrographic perturbations (freshwater runoff and marine water penetration), as well as by wind forcing acting on shallow water columns that resuspend fine sediments, microphytobenthic algae and sedimented phytoplankton cells. Specifically at Laguna de Rocha, high frequency variability imposed by hydrographic processes and wind

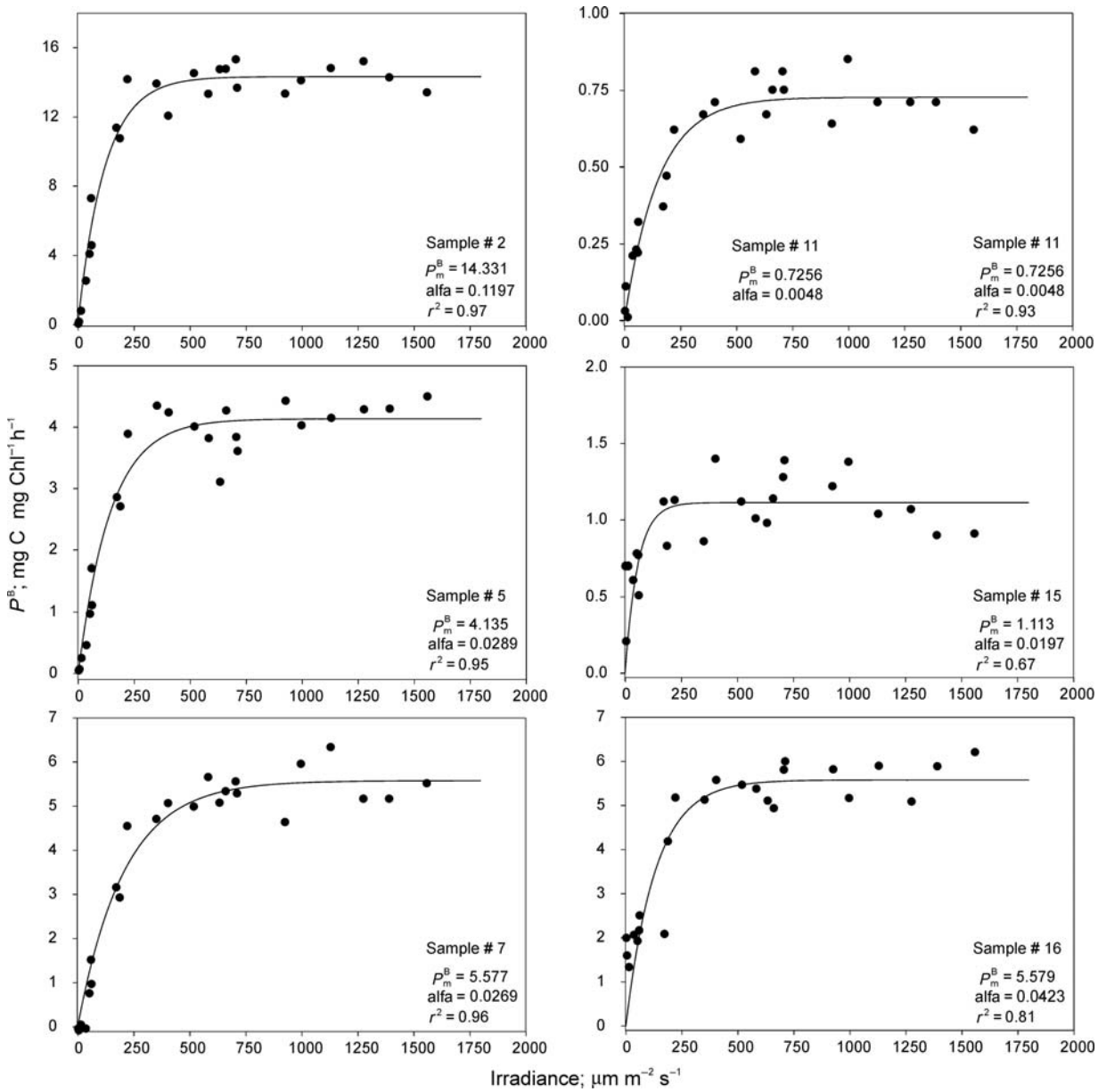

Fig. 2. Photosynthesis–irradiance response of microalgal assemblages in the water column of Laguna de Rocha during the study period (one selected example from each visit). Lines correspond to model $P^B = P^B_m * (1 - \exp(-\text{alfa} * I/P^B_m))$. Model fit and parameters were significant at a *P*-level of 5% in all cases, and are indicated in the inset in each plot.

tends to mask any seasonal pattern in nutrient availability, phytoplankton biomass (chlorophyll-*a*) and assemblage structure (Conde *et al.*, 2002; Bonilla *et al.*, 2005).

Gut fluorescence and potential bias for feeding estimation

GF of *A. tonsa* during the present study was comparable to earlier reports for the same or congeneric species (Table II). Limitations of the GF approach to estimate zooplankton feeding refer primarily to the fact that it only considers ingestion of autotrophic prey, and secondarily to potential pigment degradation to non-fluorescent forms during gut passage. The approach is still unique in providing real *in situ* estimates and allowing high replication with minimal manipulation of copepods, avoiding incubations with altered predator and prey concentration, unrealistic small-scale distribution, micro turbulence levels and other factors known

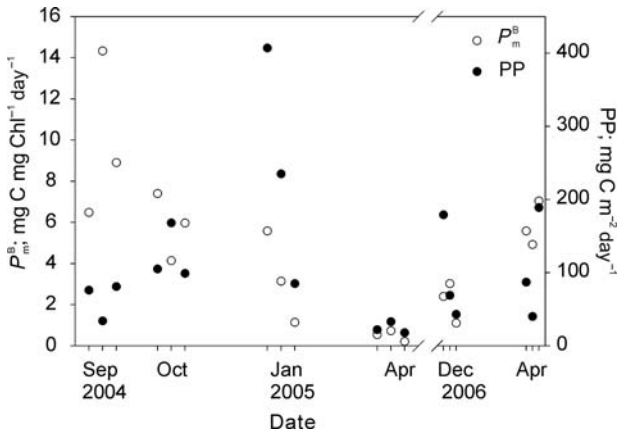

Fig. 3. P^B_m parameter of the P–I response curve and total daily primary production estimated in Laguna de Rocha during the study period.

Table II: Gut pigment contents reported for Acartia tonsa and closely related species in comparable estuarine ecosystems

Taxon	Gut pigments	Environment	Reference
A. tonsa	0.2–2.7	Río de la Plata, Uruguay	Calliari *et al.*, 2004
A. tonsa	0.4–1.5	Newport, USA	Stearns *et al.*, 1987
A. tonsa	0.2–1.0	Newport and Skidaway, USA	Stearns *et al.*, 1989
A. tonsa	0.41–6.97	Chesapeake Bay, USA	Durbin *et al.*, 1990
A. bifilosa	0.1–0.4	Mundaka and Gironde, France	Burdloff *et al.*, 2002
A. spinicauda	0.71–1.5	Pearl River, China	Tan *et al.*, 2004
A. natalensis	0.12–0.45	Mpenjati, South Africa	Kibirige and Perissinoto, 2003
A. tonsa	0.1–2.6	Laguna de Rocha, Uruguay	This study

In the study by Durbin *et al.* in Chesapeake Bay copepods were fed suspensions of the diatom *Thalassiossira weissflogii*.

to affect copepod feeding and general behaviour. But since heterotrophy can contribute a significant fraction of ingested biomass, clearly GF may only represent the herbivorous fraction of total ingestion (Peterson and Dam, 1996), and as such it will be considered here.

The significance of pigment degradation has been extensively debated (Kiørboe *et al.*, 1982; Conover *et al.*, 1986; Kiørboe and Tiselius, 1987; Dam and Peterson, 1988; Peterson and Dam, 1996). Reported fluorescence loss is frequently around 30%, e.g. 34% (Shuman and Lorenzen, 1975), 33% (Helling and Baars, 1985), 35% or 10–30% depending on the method and assumptions (Kiørboe and Tiselius, 1987). Thus, a 33% correction was suggested when no *ad hoc* estimates exist (Dam and Peterson, 1988). Estimates of pigment degradation ca. 90% are also found (Conover *et al.*, 1986) and may be considered unrepresentative for copepods (Dagg and Grill, 1980; Kiørboe *et al.*, 1982; Kiørboe and Tiselius, 1987). The uncertainty associated with the GF approach is likely within the factor of 1.5–2 (Peterson and Dam, 1996), comparable to expected error in standard plankton counts (Boltovskoy, 1981). Grazing estimated from GF is equivalent to approaches such as particle-volume removal (Kiørboe et. al., 1985). Thus, while no consensus has been reached, continued use of gut fluoresce is supported by strong experimental evidence (Kiørboe *et al.*, 1985) and on theoretical grounds (Durbin and Campbell, 2007). Another factor that may add uncertainty to consumption rates estimated from GF measured in adult females is that different development stages may graze on algae of different size ranges (Berggreen *et al.*, 1988). However, this difference should not introduce an important bias in most situations given the general pattern of cell size distribution in natural environments (Berggreen *et al.*, 1988).

Impact of *Acartia tonsa* grazing in Laguna de Rocha

The consumption of herbivorous biomass by *Acartia tonsa*, the dominant and occasionally exclusive copepod species in LR was independent of primary production rate, and HP was actually very low (<5%) and decreased nonlinearly with increasing primary production. Thus, at Laguna de Rocha primary production levels modulated the efficiency of copepods to transfer organic matter from producers. Similar analyses at other locations will reveal whether the present results

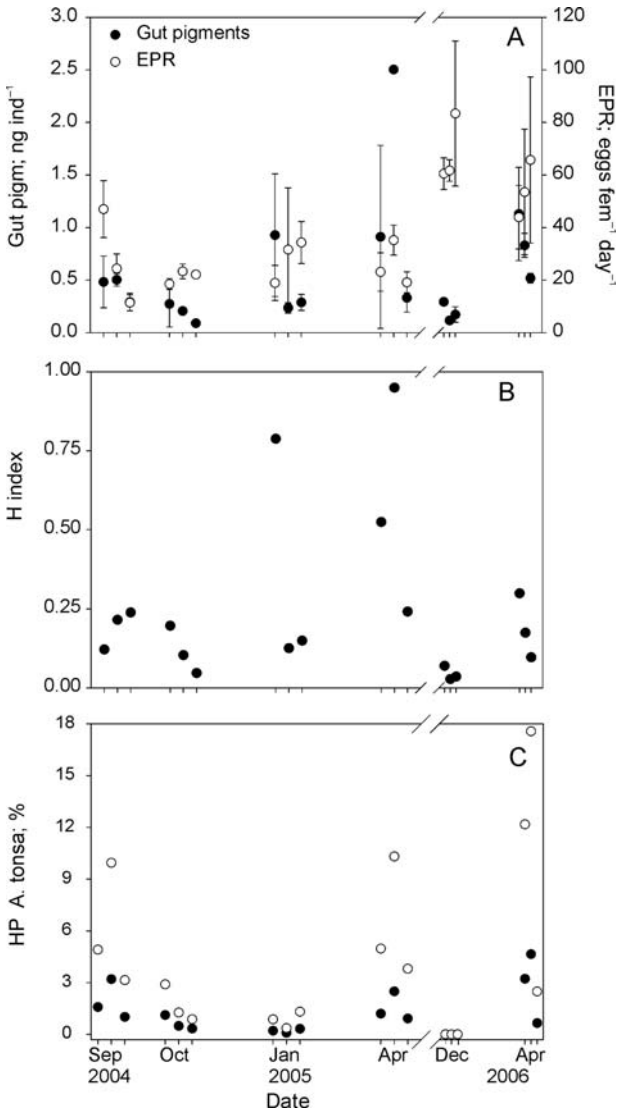

Fig. 4. *Acartia tonsa.* (**A**) Gut fluorescence and egg production rate; (**B**) herbivory index H; and (**C**) herbivory pressure in Laguna de Rocha during the study period: filled circles correspond to results without correction of *A. tonsa* abundance due to escape, open circles incorporate such correction based on Chisholm and Roff (1990a) (see Discussion for details).

are generally valid, or if they may only apply to some particular type of ecosystems. For instance, shallow coastal lagoons may show a proportionally much higher influence of benthic feeders on planktonic assemblages and organic matter fluxes.

Coastal lagoons rank among the most productive ecosystems on Earth (Harrison and Parsons, 2000), so a low direct impact of *Acartia tonsa* grazing was actually expected in the light of the global pattern of decreasing HP with increasing productivity. It could be argued that the present results for HP are underestimated due to under-sampling of larval and early copepodid stages that can escape through the 160 μm mesh used in this study. An approximation of the extent of such underestimation can be deduced from the comparison of parallel collections by 63 and 200 μm nets in estuarine waters (Chisholm and Roff, 1990a), which suggested a 5-fold difference by numbers for copepodid stages. Underestimation in the present case must have been less that 5-fold as our mesh was finer than Chisholm and Roff's (160 versus 200 μm), and because *A. tonsa* during our study were bigger (mean cephalotorax length ca. 750 μm for adult females) than dominant copepod species during the reference study (*P. parvus*, *Oithona oculata* and *O. plumifera*, which constituted ca. 90% of total copepod numbers, with mean cephalothorax lengths of 643, 298 and 542 μm, respectively; Chisholm and Roff, 1990b). Recalculating abundance

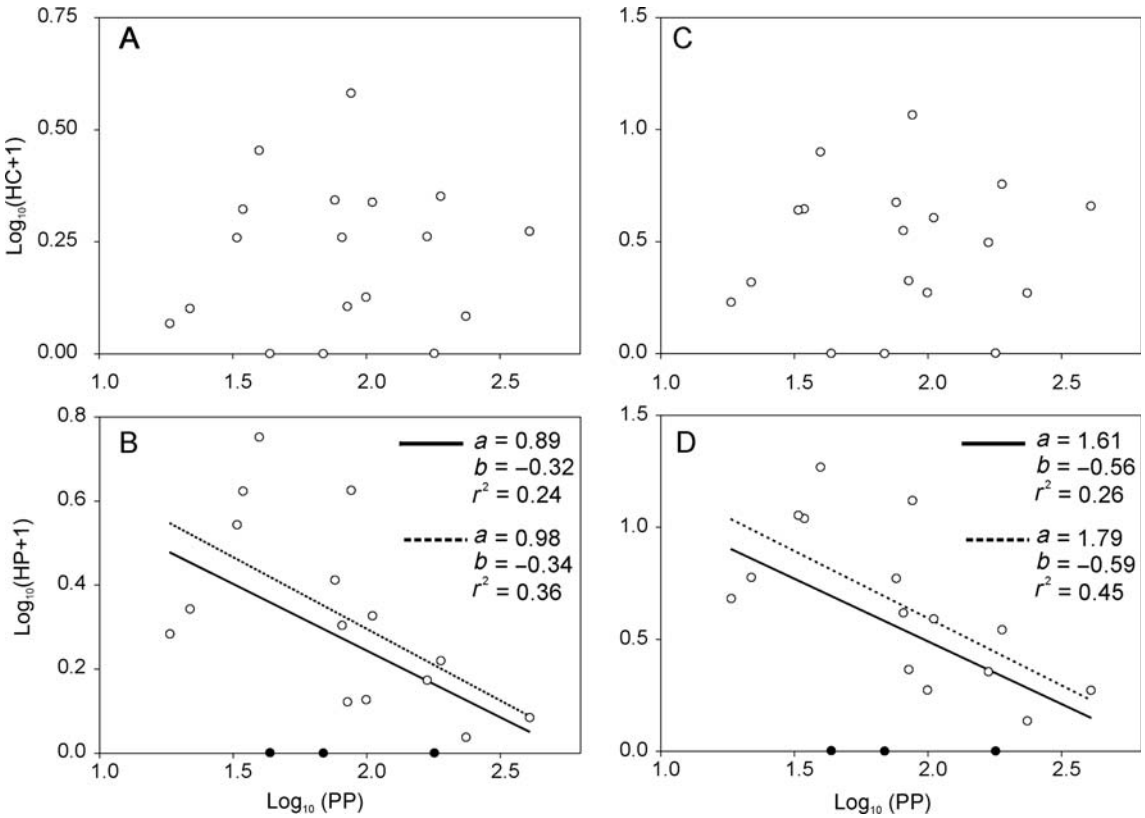

Fig. 5. Log–log scatter-plots of herbivorous consumption by *Acartia tonsa* versus primary production (**A** and **C**), and herbivory pressure versus primary production (**B** and **D**). Panels (A) and (B) correspond to results without correction of *A. tonsa* abundance due to escape; (C) and (D) incorporate such correction based on Chisholm and Roff (1990a) (see Discussion for details). In (B) and (D) lines correspond to linear regression $y = a + b * x$ including all data points (solid line) and without the three sampling days when zooplankton biomass was lowest (filled circles, dotted line). Parameter values are indicated in the inset to corresponding plot. In all cases, regression line and parameters are significant at a *P*-level 5%.

and grazing under the assumption of a 5-fold underestimation for all *A. tonsa* copepodid stages, HP in Laguna de Rocha ranges between 2.8×10^{-3} and 17.6% with a mean of 4.3%. Present estimates of HP without correcting for under-sampling (1.2%) and estimates considering such correction (4.3%) likely represent lower and upper limits for *A. tonsa* mean HP in Laguna de Rocha during the study period. Importantly, the pattern of herbivore consumption and HP versus productivity is equally valid under both scenarios (Fig. 5).

Current HP estimates are lower than the world-wide average of copepod HP (6%) and to the average of ca. 10% for productive systems (Calbet, 2001). They are comparable, albeit also lower, to estimates in analogous systems like South African temporally open estuaries in the same latitudinal range as Laguna de Rocha (ca. 30° to 34°S). For instance, in Kariega Estuary (Froneman, 2001) during autumn and winter, mesozooplankton HP was similarly as low as the present estimates (1–2% and 1–4%, respectively), but much higher in Spring and Summer (7–37%, and ca. 12–58%, respectively); in the Kasouga, estuary HP ranged from 1.8 to ca. 46% (Froneman, 2004), and in the Mpenjati from 17 to 69% (this one referred to biomass removal only, Kibirige and Perissinotto, 2003). As a reference for a different nearshore environment, in Narragansett Bay HP by *A. clausi* rarely exceeded 5%, except when nutrient limitation of phytoplankton production combined with high temperatures that favour rapid copepod population growth during late Spring (Deason, 1980).

Some differences in the physical setting between Laguna de Rocha and South African estuaries mentioned include the surface area of the water body and drainage basin, which are approximately one order of magnitude larger at Laguna de Rocha. However, lower HP in our case may rather result from key differences in food webs attributes, like the biomass ratio of phytoplankton to zooplankton. Interestingly, South African estuaries show concurrent low phytoplankton (as chl, 0.1–11 $\mu g\ L^{-1}$) and high zooplankton biomass (8–2600 mg DW m^{-3}) (Perissinotto *et al.*, 2003). For comparison, ranges at Laguna de Rocha

Table III: ANOVA for the multiple regression analysis on Acartia tonsa herbivorous pressure without abundance correction due to escape (above), and incorporating such correction (below)

	Factor	SS	DF	MS	*F*	*P*-value
Non-corrected	Intercept	0.310	1	0.310	16.5	<0.01
	PP	0.230	1	0.230	12.3	<0.01
	BAt	0.326	1	0.326	17.4	<0.01
	IR	0.152	1	0.152	8.1	<0.05
	Error	0.263	14	0.019		
Corrected	Intercept	0.803	1	0.803	27.4	<0.01
	PP	0.664	1	0.664	22.7	<0.01
	BAt	1.085	1	1.085	37.1	<0.01
	IR	0.439	1	0.439	15.0	<0.01
	Error	0.409	14	0.029		

Fitted model is: HP = 1.004(±0.247) − 0.327(±0.093) * PP + 0.149(±0.036) * BAt − 0.103(±0.036) * IR. Model fit: $r^2 = 0.72$ (non-corrected results) and HP = 1.688(±0.322) − 0.554(±0.116) * PP + 0.243(±0.039) * BAt − 0.175(±0.045) * IR. Model fit: $r^2 = 0.85$ (corrected results). HP, herbivory pressure (%); PP, primary production (mg C m^{-2} day^{-1}); BAt, biomass of *A. tonsa* population (mg C m^{-2}); IR, biomass-specific phytoplankton ingestion rate (ng pigment μgC^{-1} min^{-1}). Values between brackets correspond to standard error of estimates.

were 3.7–19.4 μg L^{-1} (but can reach 55 μg L^{-1}) and 0.03–17.1 mg DW m^{-3}, considering all copepod species combined.

Acartia tonsa biomass was a significant factor explaining low HP in Laguna de Rocha. Production rates by *A. tonsa* were high (average 32, max. 83 eggs fem^{-1} day^{-1}) but population abundance was generally moderate (10^3 ind. m^{-3}) or low (10^1–10^2 ind. m^{-3}). Diverse evidence suggests that top-down control by planktivore fish and mysid *Neomysis americana* (as well as ctenophore *Mnemiopsis leydi*, when present) plays a significant role in controlling copepod biomass: *N. americana* females may ingest the equivalent to ca. 5 adult *A. tonsa* $mysid^{-1}$ h^{-1} (28 μg DW $ind.^{-1}$ h^{-1}, at saturation level) when feeding on the natural mesozooplankton assemblage of Laguna de Rocha (own unpublished results). Consistently, dual stable isotopes analyses indicated that *A. tonsa* is an important food item for *N. americana* in Laguna de Rocha (Rodríguez-Graña *et al.*, 2008). This situation, where the mysid component is a zooplanktivore predator contrasts with the trophic role of the mysid *Gastrosaccus brevifissura* in South African estuaries, where they feed primarily on microphytobenthic algae (Kibirige *et al.*, 2002, Perissinotto *et al.*, 2003). Also, fish like *Micropogonias furnieri*, *Brevoortia aurea*, *Odontesthes* sp. and others than that use Laguna de Rocha as nursery area feed on the mesozooplankton during early juvenile and/or adult stages (Giangiobbe and Sanchez, 1993; Goncalves *et al.*, 1999; Froese and Pauly, 2008).

Significance of the link between copepod and microzooplankton pathways in Laguna de Rocha

Herbivory by *A. tonsa* provided a relatively small fraction (ca. 25%) of the carbon required to sustain measured EPR. Facultative resort to carnivory is a well-known trait, particularly in *Acartia* species. Switching from autotrophic to (motile) heterotrophic prey in *A. tonsa* involves a shift from suspension feeding to raptorial behaviour (Kiørboe *et al.*, 1996) determined by relative abundance of prey and environmental variables like small-scale turbulence where copepods choose the feeding mode that maximizes energy intake (Saiz and Kiørboe, 1995; Kiørboe *et al.*, 1996). Selective feeding of copepods on microzooplankton exists in most types of ecosystems (Calbet and Saiz, 2005), including estuaries (Gifford and Dagg, 1988; Pagano *et al.*, 2006). For example, in San Francisco, estuary heterotrophic prey contributed at least 50 to 60% of *Acartia* spp. diet (Bollens and Penry, 2003).

The percentage contribution of heterotrophic prey to copepods' diet indicates the significance of the copepod-microzoan link that connects herbivory and microbial food webs (Bollens and Penry, 2003; Calbet and Saiz, 2005). Such a link implies that the actual amount of energy channelled through the copepod assemblage (and potentially available to larger consumers) is higher than the figure resulting from considering HP alone. For instance, we estimated that if carnivory is taken into account, the participation of *Acartia tonsa* in trophic flows increases by one order of magnitude from 1.12 to 14.3% of primary production (average values). A cautionary note is in order; given the assumptions involved in estimations to account for carnivory (constant GGE and that heterotrophic preys of *A. tonsa* belong to trophic level 2) one should regard such figure cautiously as indicative of the actual order of the corresponding flows. For instance, food absorption efficiency and GGE can change according to the type and quality of food ingested (Calliari and Tiselius, 2005; Thor *et al.*, 2007) and environmental conditions like salinity (Calliari *et al.*,

2006). But even if the current estimate is crude (i.e. to the order of magnitude), the increase is still substantial. These results suggest a planktonic network structure at Laguna de Rocha characterized by strong intra-guild predation (*sensu* Polis *et al.*, 1989), where *A. tonsa* and its prey (microzooplankton) share the phytoplankton as common resource. The dynamics of these systems (copepod-heterotrophic protozoan-alga) are strongly dependent on the biology and feeding behaviour of both intermediate and top predators (Gismervick, 2006), and constitute interesting models that deserve further study. In the present case, the system would be further complicated by the presence of mysid *N. americana* that may consume copepods, microzooplankton and probably even phytoplankton (Rodríguez-Graña *et al.*, 2008, own unpublished results).

In summary, the present results suggest that HP by *A. tonsa* at Laguna de Rocha decreased with increasing primary production but was always low. Production by *A. tonsa* was usually high and apparently sustained through carnivory to a large extent, implying a strong link between classical and microbial food webs. We speculate that top-down control by predatory zooplankton and micronekton may contribute to the low HP by *A. tonsa*, and constitutes an aspect that deserves further attention.

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