Sticholonche zanclea (PROTOZOA, ACTINOPODA) IN FECAL PELLETS OF COPEPODS AND *Euphausia* sp. IN BRAZILIAN COASTAL WATERS

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(With 8 figures)

ABSTRACT

Fecal pellets produced by mesozooplanktonic copepods (*Centropages velificatus* and *Paracalanus parvus*) and macrozooplanktonic Euphausiacea (*Euphausia* sp.) were examined using scanning electron microscopy. Fragments of the protozoan *Sticholonche zanclea* were found in both copepod and in *Euphausia* sp. fecal pellets, even when the abundance of the protozoan in the water was low. The results suggest that *S. zanclea* is an important food resource for different trophic levels, including meso- and macrozooplankton, in Brazilian coastal waters.

Keywords: zooplankton, fecal pellets, feeding, protozoa.

RESUMO

Sticholonche zanclea (Protozoa, Actinopoda) em pelotas fecais de copépodos e *Euphasia* sp. em águas costeiras brasileiras

Pelotas fecais produzidas por copépodos mesozooplanctônicos (*Centropages velificatus* e *Paracalanus parvus*) e por Euphasiacea macrozooplanctônico (*Euphasia* sp) foram examinadas em microscópio eletrônico de varredura. Fragmentos do protozoário *Sticholonche zanclea* foram encontrados nas pelotas fecais dos copépodos e de *Euphasia* sp, mesmo quando a abundância do protozoário no ambiente foi bastante reduzida. Os resultados sugerem que *S. zanclea* pode representar um importante recurso alimentar para diferentes níveis tróficos, incluindo meso e macrozooplâncton, em águas costeiras brasileiras.

Palavras-chave: zooplâncton, pelotas fecais, alimentação.

INTRODUCTION

The role of protozooplankton in the trophic structuring of marine ecosystems has become a key focus in studies of the zooplankton food web (Sherr *et al.*, 1989; Kleppel *et al.*, 1991; Tackx *et al.*, 1995; Kivi *et al.*, 1996; Hwang & Hetah, 1997). Protozoans are abundant in both nano- and microplankton, and constitute an important food source for many zooplanktonic organisms and fish larvae (Pomeroy, 1974; Turner, 1984a; Porter *et al.*, 1985; Stoecker & Capuzzo, 1990; Zeldis *et al.*, 2002).

There is little published information on predation on protozoans under natural conditions. Evidence of the ingestion of protozoans comes, mostly, from predation experiments done *in situ* or in the laboratory (Paffenhöfer *et al.*, 1995), through the use of biomarkers (Kivi & Setala, 1995; Li *et al.*, 1996), filming (Verity & Paffenhöfer, 1996), or through identification of hard parts such as loricae and skeletal material in gut contents or in fecal pellets (Conover, 1982).

A previous investigation (Eskinazi-Sant'Anna, 2000) showed that the protozoan *S. zanclea* forms

part of the natural diet of mesozooplanktonic cope-pods such as *Parvocalanus crassirostris*, *Paracalanus quasimodo*, *Temora stylifera*, and *T. turbinata* in the São Sebastião Channel (SSC). The aim of the present study was to verify whether *S. zanclea* is also utilized as a food resource by other zooplankters in the SSC. In order to achieve this, the contents of fecal pellets of the copepods *Paracalanus parvus* and *Centropages velificatus* and of the euphausiacean *Euphausia* sp. were examined under Scanning Electron Microscopy (SEM). These organisms have distinct feeding habitats and body sizes, representing different trophic niches in the SSC.

STUDY AREA

The São Sebastião Channel is located on the northeast coast of São Paulo State (from 23° 41' S – 45° 10' W to 23° 53' S – 45° 30' W). The channel is bounded on the west by the slopes of the Serra do Mar, and on the east by São Sebastião Island (Fig. 1). The funnel-shaped channel is curved along its 25 km length, with the narrowest part

(2 km wide) in the center, widening toward both ends to 7.2 km at the southern entrance and 5.6 km at the northern entrance. Depths along the channel range from approximately 20 m at the entrances down to 50 m in the middle part (Gianesella *et al.*, 1999). Water temperature and salinity vary annually from 18 to °C and 35 to 37‰, respectively. Total chlorophyll (chl *a* + pheopigments) averages 4.3 mg Chl-*a* m^{-3} and can vary annually from 0.16 to 6.42 mg Chl-*a* m^{-3} (Gaeta *et al.*, 1995). The temperature in the region is warm year-round, with typical monthly means from 19.5 °C in winter to 25.5 °C in summer. Annual precipitation oscillates between 1500 and 2000 mm, with a dry period from June through August and a rainy period from January through March.

MATERIAL AND METHODS

Sampling campaigns were carried out in January and July 1997, July 1998, and January 1999, at two stations (P1 and P2) in the SSC, Brazil. Surface-water samples (250 mL) for phytoplankton and protozooplankton analyses
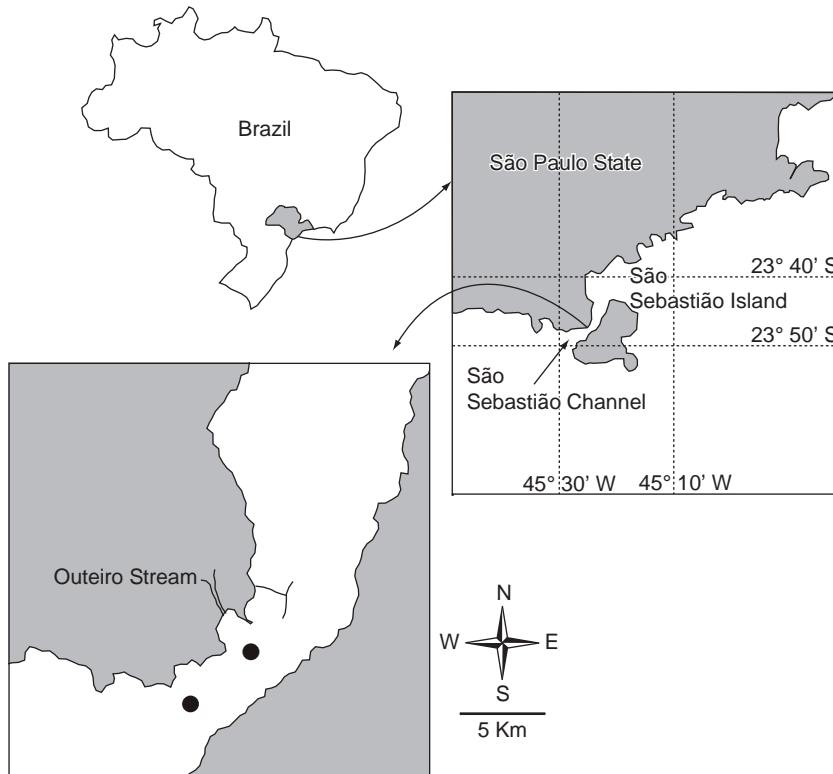

Fig. 1 — São Sebastião Channel, at the Northern coast of São Paulo State, and the position of the sampling points.

were taken with a van Dorn bottle (5 L). All samples were fixed with Lugol's solution. Phytoplankton and protozooplankton were enumerated using the inverted microscope method (Lund *et al.*, 1958) under magnifications of 40x, 60x and 100x. Meso and macrozooplankton were collected during 2 min surface tows with a 150 μm mesh net. Adult females of the copepods *Centropages velificatus* and *Paracalanus parvus*, and individuals of *Euphausia* sp. were sorted under a WILD M8 stereomicroscope, isolated within 20 min of collection, and transferred to Petri dishes containing collected surface sea water, and then left for 30 to 120 min to produce fecal pellets. The expelled fecal pellets (15 from *C. velificatus*, 22 from *P. parvus*, and 25 from *Euphausia* sp.) were individually removed by pipette and placed in petri dishes containing a mixture of filtered sea water (Nucleopore cellulose acetate membrane filters 20 μm) and 10 μm screened surface sea water. The pellets were then left for 24 h at 24 °C (± 2 °C) for microbial stripping of their peritrophic membranes (Turner, 1984b,c). All pellets were subsequently preserved in 4% formalin-seawater-glutaraldehyde solution. Ashore, preserved pellets were individually removed by pipette, transferred to Nucleopore filters, and then left to adhere. Filters with attached pellets were washed in distilled water for salt elimination, dehydrated in a graded ethanol series (50, 60, 70, 80, 90, 95, and 100%), left to dry at room temperature, and then coated with gold. The fecal pellets were examined with a Zeiss DSM-950 Scanning Electron Microscope (SEM).

RESULTS

Analysis of the fecal pellets of *C. velificatus*, *P. parvus*, and *Euphausia* sp. indicated the presence of *S. zanclea* fragments and remains of diatoms, mainly *Chaetoceros* sp., *Thalassiotrix frauenfeldii*, and *Thalassionema nitzschioides*. Unidentified centric and pennate nanoplanktonic cells (< 20 μm) were also observed. Fecal pellets of *Euphausia* sp. contained dinoflagellate remains (Fig. 2) and amorphous material, which was also present in the fecal pellets of *C. velificatus* (Fig. 3). Overall, the diatom remains observed in the fecal pellets of copepods and *Euphausia* sp. did not reflect the composition of the available phytoplankton. Phytoplankton abundance in SSC ranged from

25.4 to 717×10^3 cells.L^{-1}. The assemblages were dominated mainly by flagellates and diatoms (mainly *Cerataulina pelagica*, *Leptocylindrus danicus*, *Skeletonema costatum*, and *Cylindrotheca closterium*). In January 1997, phytoplankton assemblages at station P1 were dominated mainly by flagellates and large diatoms (> 50 μm), represented by *L. danicus*. At station P2, the same pattern of phytoplankton assemblage composition was observed, together with the occurrence of *C. closterium* and *Asterionella japonica* (Fig. 4).

In fecal pellets of *C. velificatus* and *P. parvus*, fragments of diatoms (*Thalassionema nitzschioides* and *Thalassiotrix frauenfeldii*) and a protozoan (*S. zanclea*) were observed. Remains of *T. nitzschioides* and *S. zanclea* were detected in the fecal pellets of *Euphausia* sp. In July 1997, flagellates comprised almost 90% of the phytoplankton community at P1, although in the fecal pellets of all three zooplankters, the main fragments found were of *S. zanclea* and diatoms (mainly *T. frauenfeldii*).

Identification of *S. zanclea* fragments was only possible under SEM, which revealed their characteristic ornamentation, consisting of a series of fringes along the spine (Fig. 5). These spines were the only item identified in some pellets (Fig. 6). Fragments of *S. zanclea* were found in more than 80% of the pellets of the species studied, during both summer and winter. These were present even when the relative abundance of this protozoan corresponded to less than 10% of the total microzooplankton in the SSC (Fig. 7), comprising a minimal fraction of the microzooplankton density which ranged from 5 to 101 org.L^{-1} (Fig. 8).

DISCUSSION

All the zooplankters examined fed *in situ* upon *S. zanclea*. In the fecal pellets of the copepods, the proportion of *S. zanclea* spines was in some cases higher than the proportion of diatom frustules. The same composition pattern was observed in the fecal pellets of *Euphausia* sp. *S. zanclea* is apparently an important food resource for different trophic levels in the SSC.

Grassé (1953) described the species *S. zanclea* as a peculiar organism. It was considered as an intermediary form between heliozoarians and radiolarians, which did not have a defined taxonomic
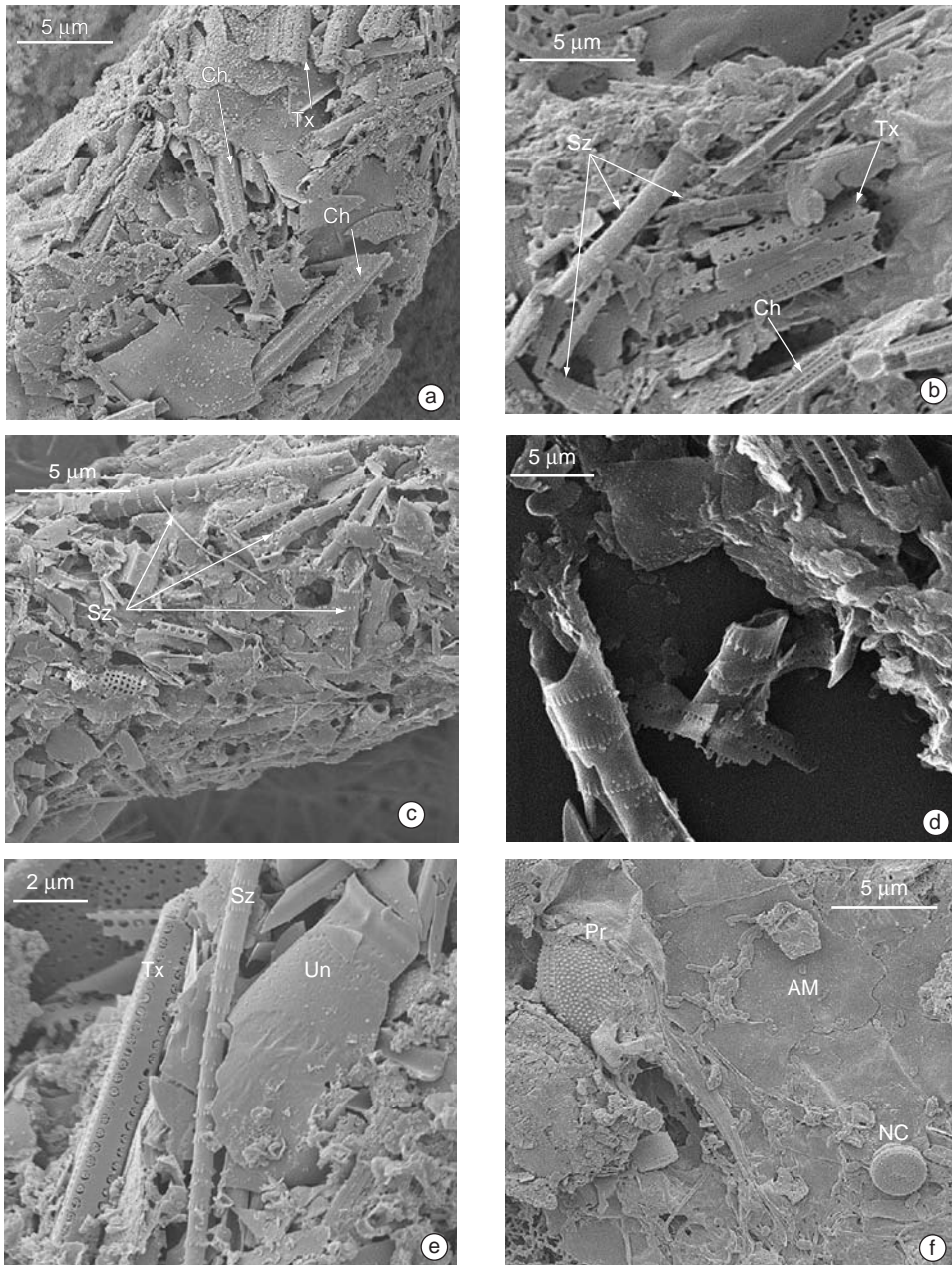
Fig. 2 — Contents of fecal pellets from the copepods *C. velificatus* (a and b) and *P. parvus* (c and d) and the Euphasiacea *Euphasia* sp (e and f) at P1, Jan/97. Ch = *Chaetoceros* sp; Tx = *Thalassiotrix frauenfeldii*; Sz = *Sticholonche zanclea*; AM = Amorphous material; Pr = *Prorocentrum* sp; UN = Unknown; NC = Nanoplanktonic cell.

position at that time. *S. zanclea* is common in the plankton of the Mediterranean Sea (Tregouboff & Rose, 1957). However, in Brazilian waters its distribution seems to be limited to the southeast coast, since there are no published records of its occurrence in other areas of Brazil.

Diatoms were the most abundant phytoplankton cells in the feces of the copepods *C. velificatus* and *P. parvus*, and also of *Euphausia* sp. Remains of *Thalassiotrix frauenfeldii*, *Thalassionema nitzschioides*, and *Skeletonema costatum* were common in all fecal pellets at all times, suggesting
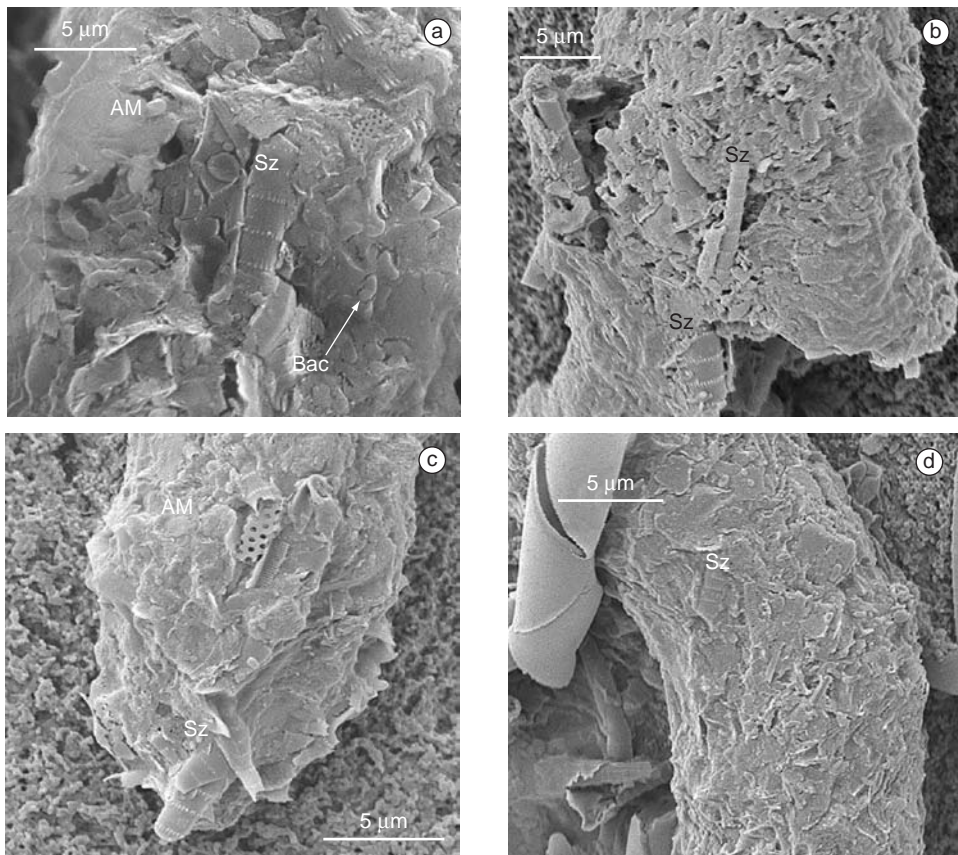
Fig. 3 — Contents of fecal pellets from the copepods *C. velificatus* (a) and *P. parvus* (b) and the Euphasiaceae *Euphasia* sp (c and d) at P2, January/99. Sz = *Sticholonche zanclea*; AM = Amorphous material.

that these species are present during all seasonals in the SSC. Also, they seem to be a preferential food source for the meso- and macrozooplankton, even when they are available in low abundance. They may be preferred because of the small size of their cells (< 50 µm).

Remains of flagellates were not identified in the fecal-pellet contents of either copepods or *Euphausia* sp. This is surprising, because flagellates may represent a reasonable food source, in view of their small size and ease of digestion because of the lack of a shell or rigid cell walls. Rapid processing of these cells within the digestive tract probably accounts for the difficulty in identifying their fragments in the fecal pellets, even with SEM. Although fecal-pellet analysis is considered a reliable method to determine food preference, there are some limitations to the technique, such as the difficulty of appropriately preserving for

identification, particles lacking hard parts or composed only of soft tissue, *e.g.*, flagellates. The amorphous material observed in the fecal-pellet contents of *C. velificatus* and *P. parvus* and *Euphausia* sp. may indicate that these soft-celled organisms were indeed ingested.

Another important observation made through analysis of fecal pellets of copepods and *Euphausia* sp. was the presence, within a single fecal pellet, of remains of phytoplankton cells and protozoan fragments ranging over two orders of magnitude in their longest dimension. According to Turner (1986), the presence of food fragments of different sizes could be indicative of selective grazing on large particles. Therefore, ingestion of *S. zanclea* by the meso- and macrozooplankton in SSC seems to indicate that these zooplankters are actively selecting this protozoan, because of its size (> 300 µm) and its low abundance in the environment.
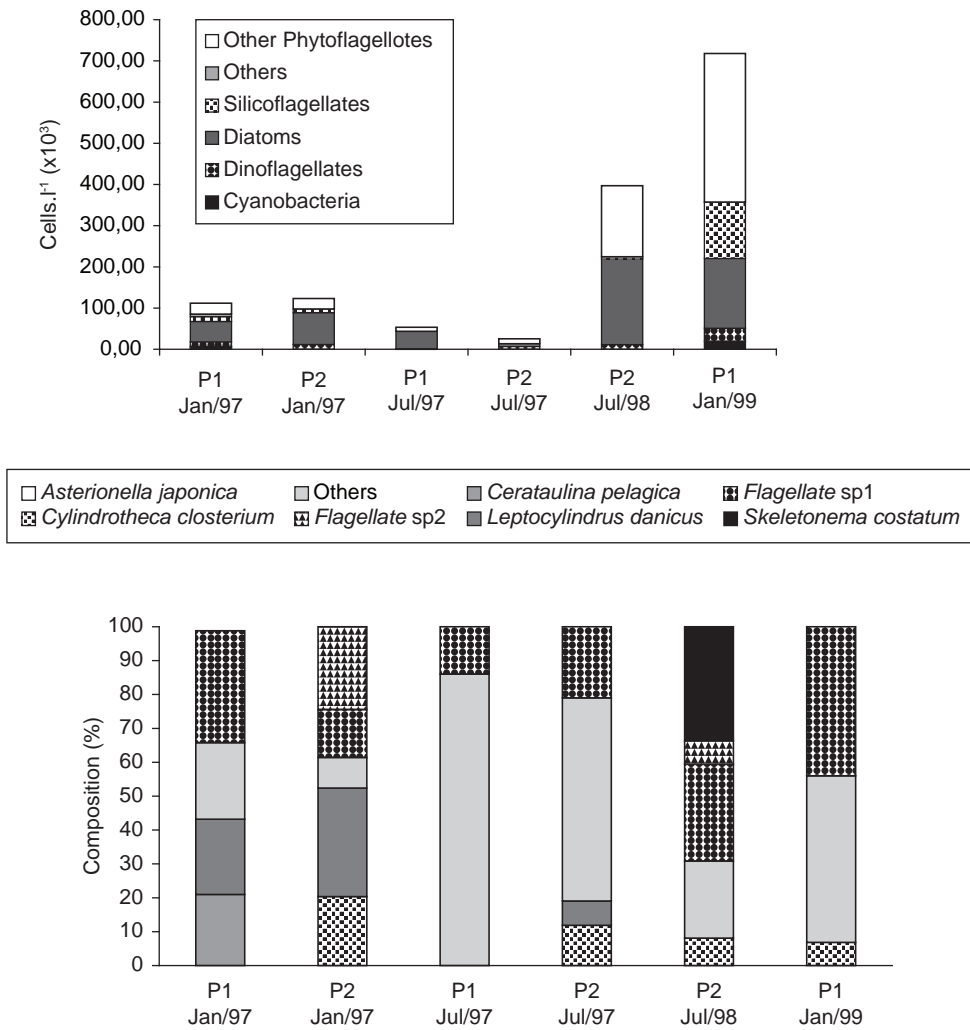
Fig. 4 — Abundance (upper) and composition (lower) of the phytoplankton in the São Sebastião Channel where fecal pellets of zooplankton were collected.
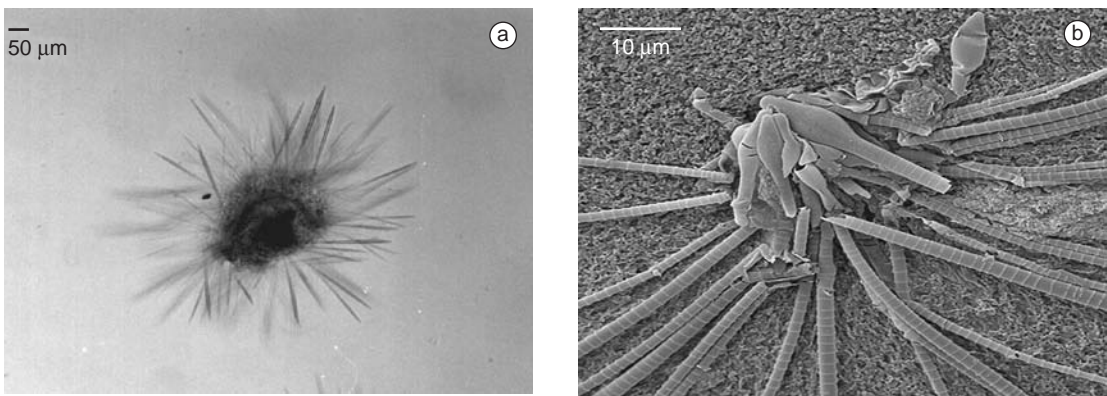

Fig. 5 — Optical (a) and SE microscopy (b) of Sticholonche zanclea collected in the São Sebastião Channel.
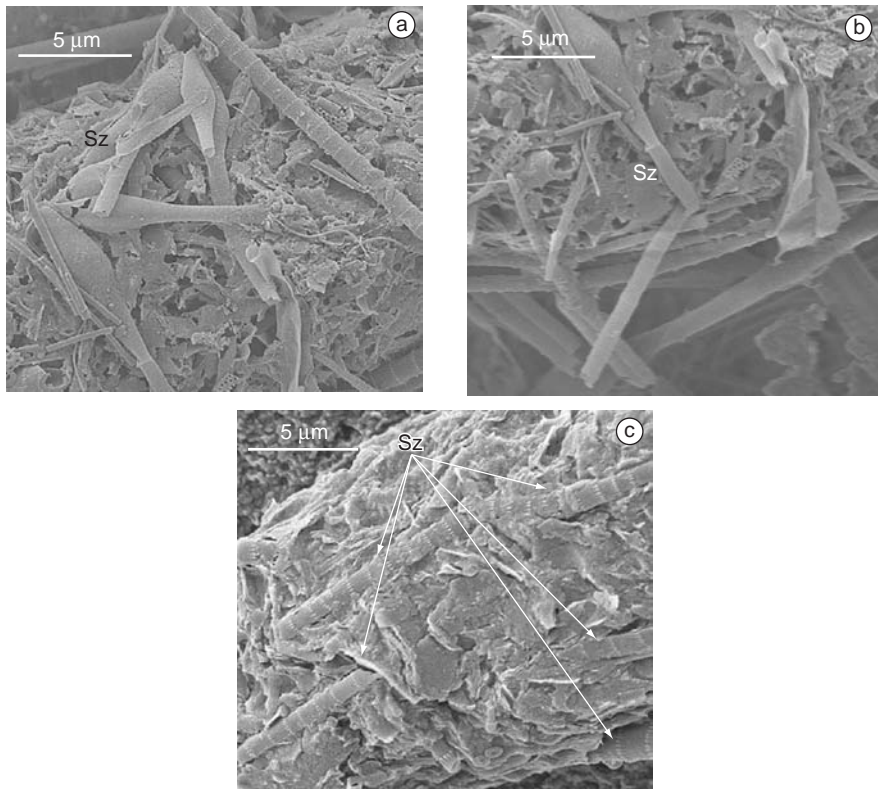
Fig. 6 — Contents of fecal pellets from the copepods *C. velificatus* (a) and *P. parvus* (b) and *Euphasia* sp (c) showing remains of *S. zanclea* (Sz).
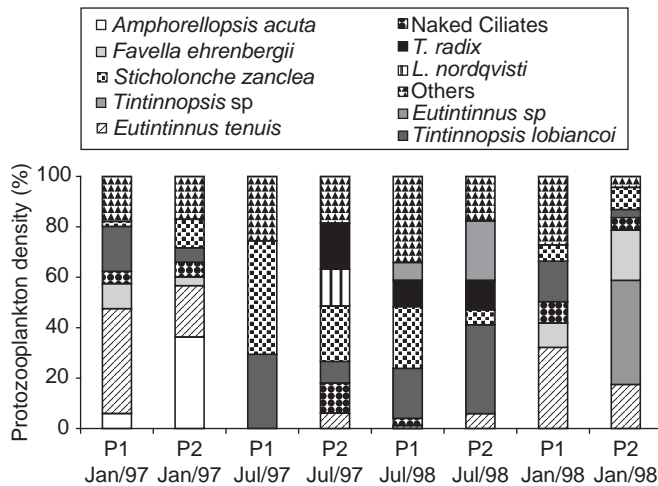

Fig. 7 — Abundance (%) and composition of the protozooplankton in the SSC.

However, corroboration of this conclusion requires more conclusive experimental treatments.

Predator-prey interactions are important for identifying the links of the planktonic food chains, as well as for interpreting seasonal changes in the structure and species composition of the aquatic community. Protozoans are likely to be an important alternative food resource for
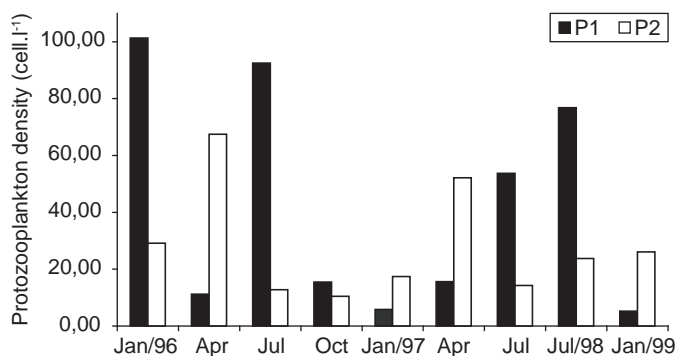
Fig. 8 — Protozooplankton density (Cell.L^{-1}) in the SSC.

zooplankton, when phytoplankton abundance is low or when phytoplankton quality is reduced (*i.e.*, during periods of nutrient limitation) (Schmidt *et al.*, 1998). The phytoplankton standing-stock in the SSC is similar to levels observed in other tropical coastal areas (Eppley *et al.*, 1978; Webber *et al.*, 1996) and to those reported by Turner & Tester (1989) and Kleppel *et al.* (1996) in previous studies on the natural diet of copepods.

Because phytoplankton is available in the SSC, questions that arise are: why is *S. zanclea* predated on by such different zooplanktonic organisms, with different food habits and at high frequency? Also, what would be the competitive advantage for the active capture of a species that is relatively large (> 300 µm) and rare in the environment?

The search for additional food sources can occur when phytoplankton biomass falls to very low levels, or when drastic changes in phytoplankton composition occur (Smetacek, 1980). The SSC is dominated by Coastal Water (CW: T > 20 °C; S > 36‰), but during spring and summer a distinct flow of nutrient-rich, high-salinity, low-temperature water (Central Water from the South Atlantic, CWSA: T < 20 °C; S > 35‰) can be detected in the deepest layers. The CW is superficial, with oligotrophic characteristics, marked by the supremacy of flagellates, which comprise around 70% of the total number of organisms, and enriched seasonally by intrusion of the CWSA (Gianesella-Galvão *et al.*, 1997). Diatoms need high nutrient concentrations, and for this reason are scarce in oligotrophic waters. Because diatoms are the main food item ingested by copepods (Turner, 1991), the quantitative dominance of flagellates in the SSC waters, also confirmed in the present

investigation, may be the determining factor for incorporation of heterotrophic food sources, such as the protozoan *S. zanclea*, into the algal diet by the local zooplankton.

Utilization of varied food sources may also be associated with specific micronutrient requirements, which would help maintain the nutritional status of individuals (Stoecker & Egloff, 1987; Gifford & Dagg, 1988). Previous studies (Verity & Langdon, 1984; Claustre *et al.*, 1988) showed that there are significant differences in the concentration of proteins, carbohydrates, and lipids in planktonic particles. Protozoans are richer in proteins and lipids than diatoms, which may be why they are targets of such intense predation.

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