

Herbivorous and microbial grazing pathways of metazooplankton in the Senegal River Estuary (West Africa)

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Abstract

The trophic relationships between metazooplankton and natural particles were studied in May 2002 in the Senegal River Estuary (16°N, 16°W) in low water conditions (dry season). Environmental factors, micro-organism and metazooplankton were analyzed through sampling at a fixed station. Gut fluorescence measurements of and field experiments on zooplankton metabolism were also performed. Chlorophyll *a* concentrations ranged from 6.5 to 10.2 $\mu\text{g l}^{-1}$. The phytoplankton was dominated by picoplanktonic cells (83–94% of total numbers). The particulate organic carbon (1.2–2.7 mg l^{-1}) originated for a large amount from organic detritus (20–70%). The zooplankton biomass was dominated by Cirripedia larvae and calanoid copepods (*Acartia clausi*, *Temora stylifera* and *Paracalanus* spp.). These taxa showed diel vertical migrations and maximal gut fluorescence at night, independently of tidal effects. Metabolic budgets show that their daily ingestion rates on phytoplankton (27–55% of body carbon weight) were insufficient to balance their respiration needs (40–51% of body carbon) and suggest that a selective feeding upon micro-heterotrophs (Heterotrophic NanoFlagellates, HNF) and/or detritus would be necessary to complete their energetic needs. The daily grazing pressure of metazooplankton represented only 5% of the in situ chlorophyll *a* and 14% of the primary production, but this pressure was mainly orientated towards nanophytoplankton. The daily recycling of nutrients by the metazooplankton excretion was rather high (83 and 46% of the in situ $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations, respectively). Therefore, the impact of metazooplankton on phytoplankton through top-down (grazing) and bottom-up (nutrient recycling) processes seemed substantial in this tropical estuary.

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1. Introduction

Grazing exerted by zooplankton is one of the key factors controlling composition and dynamics of phytoplankton communities in aquatic ecosystems (Sterner, 1989; Kagami et al., 2002). Abundance, production, size and nature of phytoplankton cells available for feeding are also known to control strongly the dynamics of zooplankton communities (Mauchline, 1998). In this two-way relationship, planktonic microbial

organisms (bacteria, micro-heterotrophs) and organic detritus are now considered as very important because they may influence the main trophic way(s) and the transfer efficiency between micro-organisms (autotrophs or heterotrophs) and zooplankton (Winkler et al., 2003; Dagg et al., 2004; Stibor et al., 2004). Microbial components can be alternate food sources when non-edible phytoplanktonic species are blooming (Buskey et al., 2003). Incorporation of organic detritus by zooplankton can be important in turbid and detritus-rich environments like river plumes (Pagano et al., 1993; Dagg et al., 2004) or estuaries (Heinle et al., 1977; Tackx et al., 2003), where phytoplankton cells represent a minor fraction of particulate organic carbon (POC). If inorganic resource inputs drive the limits for primary production, the herbivorous zooplankton

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can also regulate this production through direct consumption (Calbet et al., 2003) and nutrient recycling. Excretion could offset grazing effects by stimulating phytoplankton, particularly in nutrient-limited conditions (Elser and Goldman, 1991).

In estuarine and coastal areas, although clear relationships were evidenced between nutrient load and primary production, studying phytoplankton dynamics requires information on biotic and abiotic factors and on their interactions (Pinckney et al., 2001). Most ecological studies on these ecosystems were performed on temperate areas (Capriulo et al., 2002; Kimmmerer, 2002), whereas studies on tropical or subtropical estuaries are scarce (Murrell and Lores, 2004). In West Africa, in situ and experimental studies performed in the Senegal River Estuary (SRE) during a low nutrient period showed the high abundance of picophytoplankton and the high abundance and activity of bacterioplankton (Troussellier et al., 2004). Bouvy (pers. comm.) revealed the high carbon transfer through the microbial loop and showed experimentally that the metazooplankton could have cascading effects on microbial organisms through direct consumption and/or predation. This suggests that in the SRE, the heterotrophic pathway could be more important for sustaining metazooplankton production than the autotrophic way. Nutrient limitation conditions for phytoplankton, and particularly for nanophytoplankton, were also evidenced experimentally (Troussellier et al., 2005) and therefore, metazooplankton excretion could be an important nutrient source for phytoplankton.

In this context, our study based on a combination of field and experimental works aims (1) to assess the trophic transfers between the main constituents of the SRE planktonic ecosystem, (2) to compare the relative importance of autotrophic and heterotrophic pathways for metazooplankton and (3) to evaluate the impact of metazooplankton on micro-organisms through grazing and nutrient recycling.

2. Methods

2.1. Study site and sampling strategy

The SRE is a 50 km long water body globally orientated north–south along the Atlantic coast between the Diama dam and the river mouth in the Atlantic Ocean (Fig. 1). After the flood period (July–November), the dam is closed to prevent salinity increase of upstream water. Then, salinity in the estuary increases progressively as the thermo-haline stratification vanishes. At the end of the low water period (May–June), the estuary is mainly under tidal influence and a homogeneous water-body oscillates between the river mouth and the dam. The site was studied in low water conditions, from May 15 to May 29, 2002 at a fixed station located near Saint-Louis in the middle of the estuary (16°N, 16°W). The survey began in ending spring tide conditions (average tidal coefficient of 71 from May 15 to 17), continued in neap tide (average coefficient of 65 from May 18 to 23) and ended in spring tide (average coefficient of 92 from May 24 to 29). Environmental factors and biological variables (phytoplankton,

micro-heterotrophs and zooplankton abundance, gut fluorescence of zooplankton taxa) were measured:

- (1) daily between 08:00 and 10:00 from May 15 to May 29, (“**daily survey**”); sampling occurred at ebb tide until May 21 and at flood tide afterwards (Fig. 2A),
- (2) at a 3-h interval from May 26 at 09:00 to May 27 at 09:00, (“**24-h cycle**”); sampling was homogeneously distributed between ebb and flood tides and between dark and light periods (Fig. 2B).

Additional gut fluorescence measurements were performed at night between 20:00 and 22:00 in five occasions (May 20, 21, 24, 25 and 28) in order to complete the comparison of day and night feeding activity. Five experiments (May 16, 21, 23, 25 and 28) were conducted to measure the respiration and excretion rates of metazooplankton.

2.2. Environmental factors

Current velocity and direction were measured using a downward-looking 3 MHz Sontek ADP (Acoustic Doppler Profiler) installed on a buoy anchored in the river bed (average depth = 7 m). The profiler has three acoustic beams oriented at an angle of 25° to the vertical. It was configured to integrate for 300 s during the whole deployment. The cell size was fixed to 0.2 m, with the first bin at a depth of 0.4 m, the last one 0.5 m above the bottom. Due to the tide variation, 28 (low tide) to 30 (high tide) cells were measured. The ADP compass was corrected for the local magnetic declination in post-processing. Speed of sound at the transducer was calculated using measured temperature and a constant salinity of 28.

Temperature, salinity and fluorescence were measured on vertical profiles using a Sea Bird Electronics recorder (SBE19 CTD). Water samples were collected using a Niskin bottle at three depths: –0.5, –4 and –6.5 m. Ammonia concentrations were measured spectrophotometrically and nitrate, nitrite and phosphate concentrations were measured with a Technicon AutoAnalyser according to the methods detailed in Strickland and Parsons (1972).

Suspended solids were determined on pre-weighted Whatman GF/F filters. After filtration, filters were rinsed twice with distilled water, dried (105 °C, 48 h) and then weighted. Particulate Organic Matter (POM) was determined after subtracting the ash-weight measured on the same filters after combustion at 550 °C during 10 h. POC was deduced from POM using a ratio of 0.4 (Bamstedt et al., 2000).

2.3. Primary producers

Chlorophyll *a* concentrations (cells retained on Whatman GF/F filters) were determined after methanol extraction (Yentsch and Menzel, 1963) on a Turner Design AU-5 fluorometer. Phytoplanktonic cells were enumerated by flow cytometry following Troussellier et al. (1999) while phytoplanktonic groups were discriminated according to their fluorescence and scatter characteristics as described in Troussellier et al.

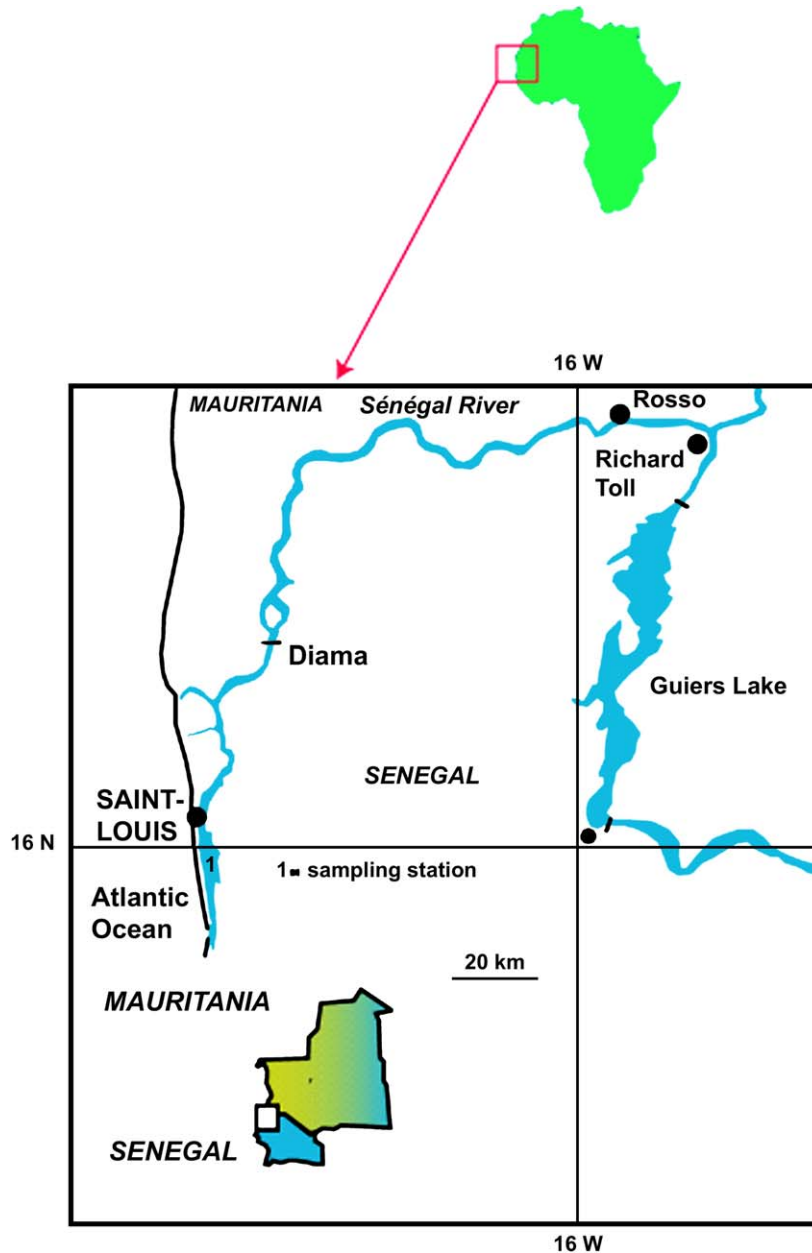


Fig. 1. The Estuary of the Senegal River. Map of the study site.

(1993). Cells excited at 488 nm were detected and enumerated according to their right angle light scattering properties and their orange (585/42 nm) and red fluorescence (>650 nm) emissions related to phycoerythrin and chlorophyll pigments, respectively. Fluorescent beads (diameter: 0.94 μm and 2 μm ; Polysciences Inc., Warrington, PA) were added to each sample for calibration. Thus, the ratio of mean fluorescence of a cellular population to that of 0.94 μm fluorescence beads was used to normalize cell fluorescence emission and scatter values between samples. Acquisition and analysis of flow cytometry data were done using CellQuest software (Becton Dickinson).

Potential phytoplankton production was estimated daily around noon using the ^{14}C -uptake method. Replicate

125-ml light and dark glass bottles filled with surface water (since the water column was not stratified) and $\text{NaH}^{14}\text{CO}_3$ (specific activity 3.7 MBq mmol^{-1} , CEA, France) were incubated in situ for 2 or 3 h. After incubation, samples were filtered onto Whatman GF/F filters, rinsed with 2% HCl and then with filtered estuarine water. Radioactivity was measured by liquid scintillation on a Beckman LS 5000, equipped with external standard. Gross productivity was integrated using the trapeze method over the reconstituted depth from the light attenuation coefficient measured in the field. Hourly production was extrapolated to daily values using daily irradiance (pyranometer records, unpublished data) and the ratio [light received during incubation time]/[light received over the total day].

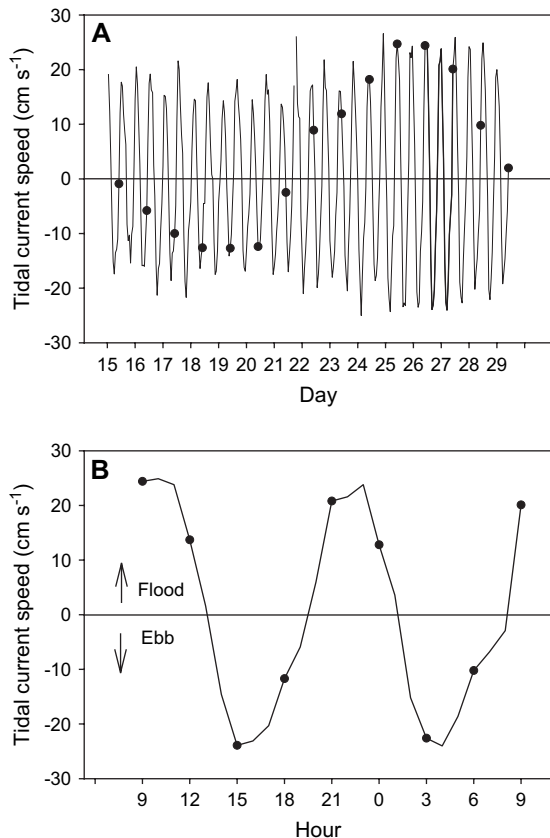


Fig. 2. Meridian component (V_n) of the tidal current speed during the daily survey (A) and during the 24-h cycle (B). The black circles indicate sampling times.

2.4. Microbial components

Abundance and biomass of bacteria and heterotrophic nanoflagellates (HNF) were determined by standard techniques using the DAPI fluorochrome and epifluorescence microscopy. Mean bacterial and HNF volumes were determined by measurement of up to 100 cells using an Olympus DP 50 camera (ANALYSYS software, Soft Imaging System, GmbH) mounted on an Olympus BX 60 epifluorescence microscope equipped with a Plan Neofluar 100 \times /1.3 oil immersion objective. For volume calculation, bacteria were considered as shaped like cylinders with hemispheric ends and flagellates as ellipsoid. Ciliates were determined from Lugol's fixed samples by inverted microscopy at 200–400 \times magnification; their volumes were estimated from standard geometric shapes. Biomass of the different organisms was calculated using literature conversion factors: bacteria, 0.2 pg C μm^{-3} (Simon and Azam, 1989); heterotrophic flagellates, 220 fg C μm^{-3} (Borsheim and Bratbak, 1987); ciliates, 190 fg C μm^{-3} (Putt and Stoecker, 1989).

2.5. Metazooplankton

Metazooplankton was collected using a cylindro-conical net (30 cm aperture, 100 cm height, 64- μm mesh size) equipped with a General Oceanics flowmeter and towed obliquely. Samples were preserved in 5% buffered formaldehyde.

Organisms were identified from subsamples using wide bore piston pipettes (0.5–5 ml). At least 300 individuals of the main taxa were counted on each subsample; the rarest taxa were estimated on the whole sample. Individual weights of most taxa were estimated from their size measured under a dissecting microscope (objective 50 \times , ocular 10 \times). Organism carbon weights were estimated using literature length–weight relationships (Uye, 1982; Mauchline, 1998).

Grazing rates of the dominant metazooplankton taxa were estimated using the gut fluorescence technique (Mackas and Bohrer, 1976). Animals were collected using a cylindro-conical net (30 cm aperture, 100 cm height, 200- μm mesh size) towed obliquely. The use of a 200- μm mesh size (which collects efficiently most of the dominant taxa) facilitated the quick constitution of animal sets. Immediately after collection, the organisms were anaesthetized by addition of CO_2 -saturated filtered water. At the laboratory (i.e. 1 or 2 h after sample collection), Cirripedia larvae, *Acartia clausi*, *Temora stylifera* and *Paracalanus* spp. were sorted and grouped in taxonomic sets of 20–250 individuals according to their size. Gut pigments were then extracted in 90% acetone for 12 h, and measured after centrifugation (2500 rpm) on a Turner Design AU-5 fluorometer. The pigment gut contents (G) were expressed as ng chlorophyll a equivalent ind. $^{-1}$ (or $\mu\text{g C}^{-1}$), assuming a molar conversion efficiency of chlorophyll a to phaeophorbide of 67% (Dam and Peterson, 1988). Short-term ingestion rates ($I = G \times k \times 60$) expressed as $\mu\text{g Chl} a \text{ ind.}^{-1} \text{ h}^{-1}$, were calculated from G values and the gut filling rates (k , min^{-1}) were estimated from the k vs temperature relationship established by Dam and Peterson (1988) and modified by Dam (in Bamstedt et al., 2000). The ingestion rates of the other metazooplankton taxa (I in $\mu\text{g Chl} a \text{ ind.}^{-1} \text{ h}^{-1}$) were estimated from their average individual weight (W in $\mu\text{g C}$) using a I vs W relationship established from the average values obtained for the four dominant taxa during the 24-h cycle: $I = 0.211W + 0.150$, $r = 0.98$, $p = 0.035$.

The daily ingestion rate ($\text{ng Chl} a \mu\text{g C}^{-1} \text{ d}^{-1}$) was obtained by integrating discrete values over the 24-h cycle. Since only daytime values were calculated during the daily survey, the ingestion rates for each taxa were corrected for diel variations using the [mean value]/[9 a.m. value] ratio calculated during the 24-h cycle. The daily ingestion rates were also expressed in $\mu\text{g C} \mu\text{g C}^{-1} \text{ d}^{-1}$ using a carbon:chlorophyll a ratio of 60 (Bamstedt et al., 2000).

The clearance rates ($F = I \times \text{Chl}$), expressed in $\text{ml ind.}^{-1} \text{ h}^{-1}$, were estimated from ingestion rates (I) and in situ chlorophyll a concentration (Chl) assuming a 100% filtering efficiency on chlorophyll particles.

The daily metazooplankton community grazing rate ($\mu\text{g Chl} a \text{ l}^{-1} \text{ d}^{-1}$) was obtained by summing the grazing rates (ingestion rate \times abundance) calculated for each taxa. Abundance values for the daily survey were corrected for diel variations using the [mean abundance]/[9 a.m. abundance] ratio of the 24-h cycle. The community grazing rate was compared to the in situ depth-integrated chlorophyll a concentration or to the primary production consumed.

2.6. Zooplankton metabolism experiments

The experiments were performed with metazooplankton collected after sunset using the 200 μm mesh-sized net. We used the $>200 \mu\text{m}$ metazooplankton fraction ($>90\%$ of the biomass) to minimize the introduction of non-zooplankton items in the experimental sets. After collection and sorting, homogeneous sets of metazooplankton were incubated for 24 h in 300 ml flasks filled with water from the sampling station, filtered through a 0.2 μm membrane to remove biological particles which could interfere with the ammonia or phosphorus dynamics during the incubation time. Two experimental flasks (with animals) and two controls (same water without animals) were prepared and incubated in the dark, at ambient temperature. At the end of the experiment, subsamples were siphoned for O_2 measurements (YSI 57 probe corrected for salinity) and for $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ analyses (as described above). Animals from experimental flasks were transferred in a 5% formaldehyde solution for enumeration and measurements in order to calculate the zooplankton carbon biomass as described above. Excretion and respiration rates were calculated from the differences between experimental and control bottles. Metabolic rates were expressed per day and per zooplankton carbon weight units.

The daily metazooplankton community nutrient regeneration rate ($\mu\text{M PO}_4\text{-P}$ or $\text{NH}_4\text{-N l}^{-1} \text{d}^{-1}$) was calculated by multiplying mean excretion rates by the metazooplankton

abundance (corrected for diel variations using the [mean abundance]/[9 a.m. abundance] ratio of the 24-h cycle). It was also expressed as a percentage of the in situ concentration of nutrients.

2.7. Data processing

Two-way ANOVA was used to test the day–night or tidal effects on organism abundance or gut fluorescence during the 24-h cycle. Bravais–Pearson correlation coefficient, computed after logarithmic transformation of variables [$\log(x+1)$] to tend towards normal distribution (Kolmogorov–Smirnov test), allowed to describe the relationship between the different variables.

3. Results

3.1. Environmental factors

During the sampling period, the river flow was nil and the current only resulted from wind and tidal effects; its velocity varied from 0 to 22 cm s^{-1} during the neap tide and between 0 and 27 cm s^{-1} during the spring tide, with comparable maximal values at ebb and flood tides (Fig. 2). Temperature (25–27 $^\circ\text{C}$) and salinity (28.8–31.3) showed low vertical stratification. Nutrient concentrations were low (Table 1). $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations ranged from 0 to 0.45 $\mu\text{M l}^{-1}$ and

Table 1

Mean, minimum and maximum values for main environmental variables, primary production and abundance and biomass of micro-organisms during the daily survey and the 24-h cycle. UD, undetectable; ND, not measured

| Data | Unit | Daily survey | | | 24-h cycle | | |
|-------------------------------|------------------------------------|--------------|------|------|------------|------|------|
| | | Mean | Min | Max | Mean | Min | Max |
| Salinity | | 28.7 | 27.4 | 29.8 | 28.9 | 28.6 | 29.3 |
| Temperature | $^\circ\text{C}$ | 24.2 | 23.1 | 25.7 | 25.6 | 25.1 | 25.9 |
| $\text{PO}_4\text{-P}$ | μM | 0.08 | UD | 0.39 | 0.04 | UD | 0.11 |
| $\text{NH}_4\text{-N}$ | μM | 0.18 | UD | 0.45 | 0.58 | 0.30 | 1.45 |
| $\text{NO}_3\text{-N}$ | μM | 0.13 | UD | 0.43 | 0.03 | UD | 0.07 |
| $\text{NO}_2\text{-N}$ | μM | 0.07 | UD | 0.13 | 0.08 | 0.07 | 0.10 |
| Suspended solids | mg l^{-1} | 15.9 | 8.4 | 27.8 | 23.1 | 18.3 | 27.8 |
| Inorganic fraction | % | 68.0 | 59.6 | 72.6 | 73.8 | 72.1 | 75.7 |
| Organic fraction | % | 32.0 | 27.4 | 40.4 | 26.2 | 24.3 | 27.9 |
| POC | mg l^{-1} | 1.90 | 1.18 | 2.71 | 2.38 | 2.05 | 2.71 |
| Primary production | $\text{mg C m}^{-2} \text{d}^{-1}$ | 1259 | 740 | 1761 | 1266 | | |
| Phytoplankton | 10^6 ind. l^{-1} | 77.3 | 66.2 | 95.0 | ND | | |
| %Picophytoplankton | | 58.9 | 54.8 | 64.9 | | | |
| Chlorophyll <i>a</i> | $\mu\text{g l}^{-1}$ | 10.6 | 8.1 | 12.6 | 9.0 | 7.7 | 10.3 |
| Bacteria | $10^6 \text{ cells ml}^{-1}$ | 6.2 | 3.0 | 10.9 | 7.6 | 5.4 | 10.9 |
| | $\mu\text{g C l}^{-1}$ | 104 | 50 | 183 | 127 | 91 | 183 |
| HNF | 10^6 ind. l^{-1} | 68 | 38 | 95 | 70 | 38 | 104 |
| | $\mu\text{g C l}^{-1}$ | 59 | 33 | 83 | 61 | 33 | 90 |
| Ciliates | ind. ml^{-1} | 39 | 39 | 40 | ND | | |
| | $\mu\text{g C l}^{-1}$ | 70 | 69 | 70 | ND | | |
| Autotrophic C ^a | $\mu\text{g C l}^{-1}$ | 638 | 487 | 753 | 543 | 464 | 615 |
| Living organisms ^b | $\mu\text{g C l}^{-1}$ | 845 | 634 | 959 | ND | | |
| | %POC | 47 | 29 | 81 | | | |

^a Chlorophyll *a* $\times 60$.

^b Sum of bacteria + HNF + ciliates + autotrophic C.

from 0 to $0.39 \mu\text{M l}^{-1}$, respectively; both variables featured their highest concentration on May 18. Suspended solids varied from 8 to 28 mg l^{-1} and included 60–73% of inorganic matter.

3.2. Phytoplanktonic organisms

Two classes of phytoplanktonic cells were determined by flow cytometry. Picoplanktonic cells (diameter $< 2 \mu\text{m}$, class 1) were the most abundant, representing 83–94% of total abundance (Table 1). This size-distribution on the three sampling depths showed little variation during the survey. Phytoplankton total abundance varied between 66 and $95 \times 10^6 \text{ cells l}^{-1}$, and chlorophyll *a* concentration between 8.1 and $12.6 \mu\text{g l}^{-1}$ (Table 1). Primary production ranged from 700 to $1800 \text{ mg C m}^{-2} \text{ d}^{-1}$ (Table 1). The highest values were recorded at the end of the survey (May 25–29).

Important variations in chlorophyll *a* concentrations were observed during the 24-h cycle, with an obvious decrease at night (from 18:00 to 03:00) followed by an increase after dawn (Fig. 3A). A two-way ANOVA revealed that both day–night and tidal effects could explain this pattern (Table 2).

3.3. Heterotrophic micro-organisms

During the daily survey, the bacterial abundance varied from 3×10^6 to $11 \times 10^6 \text{ cells ml}^{-1}$, which corresponded to biomass

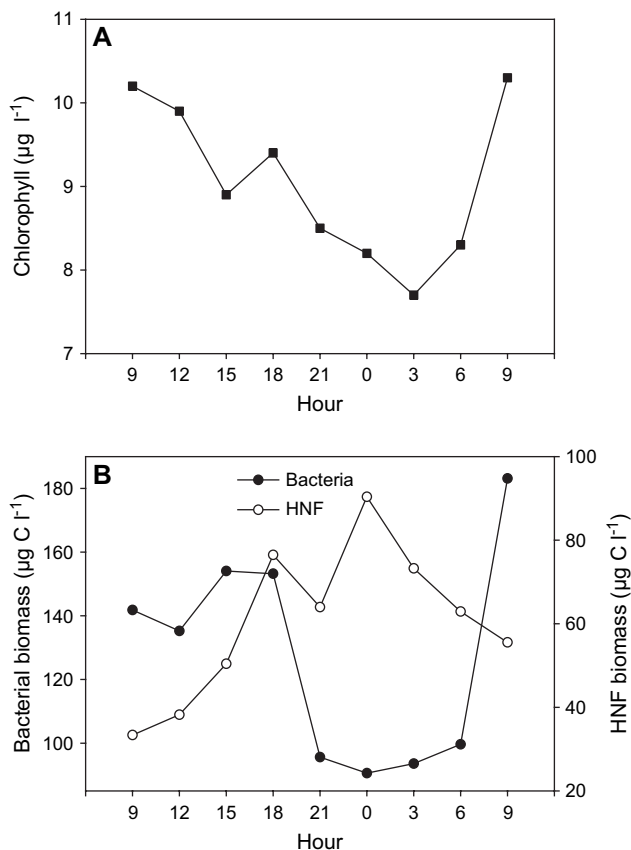


Fig. 3. Time-variations during the 24-h cycle of chlorophyll *a* concentration (A) and of bacterial and heterotrophic nanoflagellates (HNF) (B).

Table 2

Two-way ANOVA on day–night and tidal effects on biological variables during the 24-h cycle. Risk (*p*) values for *F*. Day values are from 09:00 to 18:00 (on May 26) and night values from 21:00 to 06:00. *N* = 8, degrees of freedom are 1 for the tested factors and 4 for the error. Bold characters indicate significant effect (*p* < 0.05)

| Variable | Day–night | Tide | Day–night × tide |
|----------------------------|--------------|--------------|------------------|
| Chlorophyll <i>a</i> | 0.003 | 0.048 | 0.362 |
| Bacteria | 0.000 | 0.042 | 0.235 |
| HNF | 0.043 | 0.203 | 0.083 |
| Bacteria/HNF | 0.006 | 0.404 | 0.137 |
| Abundance | | | |
| Total zooplankton | 0.016 | 0.055 | 0.408 |
| <i>Acartia clausi</i> | 0.396 | 0.598 | 0.432 |
| <i>Temora stylifera</i> | 0.025 | 0.021 | 0.568 |
| <i>Paracalanus</i> spp. | 0.094 | 0.293 | 0.314 |
| <i>Oithona brevicornis</i> | 0.100 | 0.096 | 0.779 |
| Cirripedia larvae | 0.002 | 0.015 | 0.060 |
| Gut fluorescence | | | |
| <i>Acartia clausi</i> | 0.032 | 0.788 | 0.942 |
| <i>Temora stylifera</i> | 0.003 | 0.781 | 0.617 |
| <i>Paracalanus</i> spp. | 0.098 | 0.401 | 0.715 |
| Cirripedia larvae | 0.002 | 0.562 | 0.776 |

comprised between 50 and $180 \mu\text{g C l}^{-1}$ (Table 1). The HNF density ranged from 38 to $95 \times 10^3 \text{ ind. l}^{-1}$ and their biomass ranged between 30 and $90 \mu\text{g C l}^{-1}$. HNF and bacteria showed opposite variations. The lowest HNF values and the highest bacteria numbers were observed during the spring tide. Abundance of heterotrophic ciliates which was measured only on two samples (39 and 40 ind. l^{-1} on May 21 and 29, respectively) corresponded to a biomass close to $70 \mu\text{g C l}^{-1}$. Average biovolumes of bacteria, HNF and ciliates were: $0.084 \pm 0.008 \mu\text{m}^3$, $3.95 \pm 0.85 \mu\text{m}^3$ and $9332 \pm 500 \mu\text{m}^3$, respectively.

During the 24-h cycle, there was a clear decrease of the bacterial biomass and a slight increase of the HNF biomass at night (Fig. 5B). Bacterial biomass changes were explained by both day–night and tidal effects, whereas HNF variations were only linked to day–night effect (Table 2).

3.4. Particulate organic carbon (POC)

POC varied between 1.2 and 2.7 mg C l^{-1} (Table 2). The sum of the living components (phytoplankton, bacteria, HNF and ciliates) represented between 630 and $940 \mu\text{g C l}^{-1}$, i.e. between 30 and 80% of the POC.

3.5. Metazooplankton

Metazooplankton was dominated by Cirripedia larvae and the copepods *Oithona brevicornis*, *Acartia clausi*, *Temora stylifera* and *Paracalanus* spp. which represented more than 80% of the zooplankton biomass (Table 3). During the daily survey, the total abundance varied from 5 to 50 ind. l^{-1} and the biomass from 3 to $26 \mu\text{g C l}^{-1}$. Generally, total zooplankton biomass as well as the biomass of the main taxa was lower during the neap tide period than during the spring tide period, with the lowest and highest values recorded on May 20 and May 27, respectively. During the survey, metazooplankton had no correlation

Table 3
Mean values (and range) for zooplankton abundance and biomass during the daily survey and mean day and night biomass during the 24-h cycle

| | Daily survey | | | | 24-h cycle | | |
|----------------------------|---|--------------|---|--------------|--------------------------------|----------------------------------|--------------------|
| | Day (9 a.m.) (ind. l ⁻¹) | | Day (9 a.m.) (μg C l ⁻¹) | | Day (μg C l ⁻¹) | Night (μg C l ⁻¹) | Night/day ratio |
| Total | 15.50 | (4.51–50.45) | 8.71 | (3.04–26.39) | 15.02 | 66.72 | 4.4 |
| Copepod nauplii | 3.64 | (0.13–10.71) | 0.36 | (0.01–1.07) | 0.71 | 1.66 | 2.3 |
| <i>Paracalanus</i> spp. | 0.97 | (0.11–2.37) | 1.13 | (0.12–2.76) | 1.20 | 3.85 | 3.2 |
| <i>Temora stylifera</i> | 0.18 | (0.03–0.58) | 0.84 | (0.15–2.70) | 0.80 | 2.57 | 3.2 |
| <i>Acartia clausi</i> | 0.08 | (0.01–0.36) | 0.25 | (0.02–1.07) | 0.29 | 0.51 | 1.8 |
| <i>Candacia</i> spp. | <0.01 | (0.00–<0.01) | <0.01 | (0.00–0.01) | <0.01 | 0.02 | 6.2 |
| <i>Oithona brevicornis</i> | 7.46 | (1.79–29.14) | 1.63 | (0.37–6.47) | 3.13 | 8.77 | 5.5 |
| <i>Euterpina</i> sp. | 0.01 | (0.00–0.06) | 0.01 | (0.00–0.03) | 0.01 | 0.03 | 2.7 |
| Chaetognath | 0.15 | (0.00–0.48) | 1.07 | (0.00–3.37) | 3.08 | 7.12 | 2.3 |
| Appendicularian | 0.04 | (0.00–0.39) | 0.01 | (0.00–0.05) | 0.01 | 0.02 | 1.9 |
| Larvae | | | | | | | |
| Cirripedia | 2.95 | (0.14–10.03) | 3.40 | (0.44–11.28) | 5.78 | 42.12 | 31.8 |
| Gasteropod | <0.01 | (0.00–<0.01) | <0.01 | (0.00–<0.01) | <0.01 | 0.01 | 57.9 |
| Polychaete | <0.01 | (0.00–0.04) | <0.01 | (0.00–0.02) | <0.01 | <0.01 | 1.4 |

with total phytoplankton chlorophyll and HNF, but displayed significant relationships with nanophytoplankton ($r = -0.67$, $p < 0.01$, $n = 15$) and bacteria ($r = 0.63$, $p < 0.05$, $n = 15$).

During the 24-h cycle, all zooplankton taxa showed their highest biomass at night, with night/day ratios ranging from 1.4 (polychaete larvae) to 57.8 (gastropod larvae) (Table 3). Among the most abundant copepods, the three calanoids (*Acartia clausi*, *Temora stylifera* and *Paracalanus* spp.) peaked at 03:00 (Fig. 6A), whereas the small cyclopoid *Oithona brevicornis* peaked at 06:00 (Fig. 4B). Two peaks of abundance were observed at 21:00 and 03:00 for the Cirripedia larvae (Fig. 4B). The two-way ANOVA showed only a significant day–night effect for total zooplankton, whereas both day–night and tidal effects were significant for Cirripedia larvae and *T. stylifera* (Table 2). No significant interaction was observed between tidal and day–night effects.

3.6. Gut fluorescence and feeding rates of the main metazooplankton taxa

During the daily survey, the mean gut fluorescence of *Acartia clausi*, *Temora stylifera*, *Paracalanus* and Cirripedia larvae measured each day between 09:00 and 11:00 ranged from 0.02 to 0.05 ng Chl μg C⁻¹ (Table 4) and showed no clear temporal variation. The night values were significantly higher than the corresponding day values (t -test on paired data, $p < 0.05$, $n = 5$). Clear diel variations were noted during the 24-h cycle, with maximal gut fluorescence at night (Fig. 5). The significance of day–night variations was confirmed by the two-way ANOVA for *A. clausi*, *T. stylifera* and Cirripedia larvae, whereas no significant tidal effect was found (Table 2).

Either during the 24-h cycle or over the daily survey, the Cirripedia larvae displayed higher average specific gut fluorescence than copepods. The lowest values were observed for *Temora stylifera* (Table 4).

During the 24-h cycle, the average specific chlorophyll *a* ingestion rates ranged from 5.6 (*Temora stylifera*) to 9.4

(Cirripedia larvae) ng Chl μg C⁻¹ d⁻¹ (Table 4). Daily carbon ingestion rates on autotrophic organisms represented 34–56% of body carbon weight. Clearance rates ranged from 0.7 to 1.1 ml μg C⁻¹ d⁻¹. Similar average rates were found for the daily survey based on daytime values corrected for diel variations.

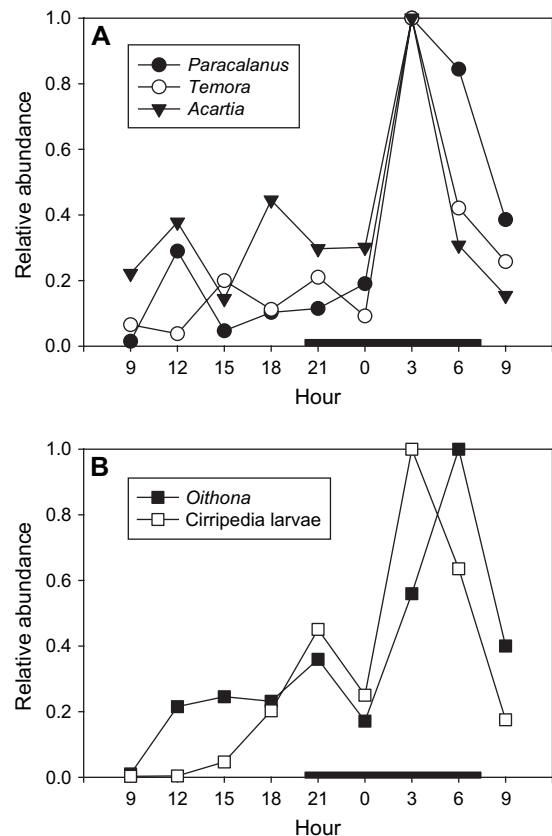


Fig. 4. Time-variations of the relative abundance of the main taxa during the 24-h cycle: calanoid copepods (A), *Oithona brevicornis* and Cirripedia larvae (B).

Table 4
Mean (\pm standard deviation) values for specific gut fluorescence, chlorophyll *a* ingestion rate, autotrophic carbon ingestion rate and clearance rate of the four studied taxa. *N*, number of data

| Period | <i>N</i> | <i>A. clausi</i> | <i>T. stylifera</i> | <i>Paracalanus</i> | Cirripedia larvae |
|--|----------|------------------|---------------------|--------------------|-------------------|
| Gut fluorescence (ng Chl $\mu\text{g C}^{-1}$) | | | | | |
| 24-h cycle | 9 | 0.06 \pm 0.01 | 0.04 \pm 0.03 | 0.05 \pm 0.02 | 0.07 \pm 0.04 |
| Survey (day) | 15 | 0.05 \pm 0.04 | 0.02 \pm 0.01 | 0.04 \pm 0.02 | 0.05 \pm 0.02 |
| Survey (night) | 5 | 0.08 \pm 0.04 | 0.06 \pm 0.02 | 0.07 \pm 0.03 | 0.19 \pm 0.11 |
| Chlorophyll <i>a</i> ingestion rates (ng eq Chl $\mu\text{g C}^{-1} \text{d}^{-1}$) | | | | | |
| 24-h cycle | 9 | 7.19 \pm 1.83 | 5.59 \pm 3.08 | 6.04 \pm 2.87 | 9.37 \pm 5.06 |
| Survey | 15 | 7.30 \pm 4.62 | 4.50 \pm 1.73 | 4.63 \pm 2.35 | 9.15 \pm 2.85 |
| Carbon ingestion rates ($\mu\text{g C } \mu\text{g C}^{-1} \text{d}^{-1}$) | | | | | |
| 24-h cycle | 9 | 0.43 \pm 0.11 | 0.34 \pm 0.18 | 0.36 \pm 0.17 | 0.56 \pm 0.30 |
| Survey | 15 | 0.44 \pm 0.28 | 0.27 \pm 0.10 | 0.28 \pm 0.14 | 0.55 \pm 0.17 |
| Clearance rates ($\text{ml } \mu\text{g C}^{-1} \text{d}^{-1}$) | | | | | |
| 24-h cycle | 9 | 0.82 \pm 0.27 | 0.65 \pm 0.42 | 0.69 \pm 0.36 | 1.09 \pm 0.68 |
| Survey | 15 | 0.73 \pm 0.59 | 0.43 \pm 0.18 | 0.45 \pm 0.25 | 0.88 \pm 0.30 |

The influence of phytoplanktonic food abundance (chlorophyll *a* and phytoplankton numbers) on the gut fluorescence (*G*) for the four taxa was tested using correlation coefficients. During the daily survey, there was no significant correlation between *G* and phytoplankton abundance (total, pico or nanophytoplankton). Correlation was negative between chlorophyll *a* and *G* for *Acartia clausi* ($r = -0.53$, $p = 0.043$, $n = 15$) but not significant for the other taxa ($p > 0.05$). During the 24-h cycle, significant negative relationships were found between chlorophyll *a* and *G* for *A. clausi* ($r = -0.67$, $p = 0.038$), *Temora stylifera* (-0.87 , $p = 0.002$) and Cirripedia larvae ($r = -0.84$, $p = 0.005$).

3.7. Respiration and excretion rates of metazooplankton

The assemblages incubated in the experimental jars were dominated by Cirripedia larvae and copepods (Table 5). Respiration rates ranged from 1.1 to 1.6 $\mu\text{g O}_2 \mu\text{g C}^{-1} \text{d}^{-1}$. When expressed as carbon values using a respiratory quotient (RQ) of 0.9 (Mauchline, 1998), these rates represented 35–54% of body carbon per day. Excretion rates ranged from 3.5 to 5.1 nM $\text{NH}_4\text{-N } \mu\text{g C}^{-1} \text{d}^{-1}$ and from 0.39 to 0.51 nM $\text{PO}_4\text{-P } \mu\text{g C}^{-1} \text{d}^{-1}$. Atomic metabolic ratios showed

low variability, with mean values of 20 for O: $\text{NH}_4\text{-N}$, 9.1 for $\text{NH}_4\text{-N}:\text{PO}_4\text{-P}$ and 183 for O: $\text{PO}_4\text{-P}$.

3.8. Metabolic budgets

When compared to the respiration rates calculated for the total zooplankton and expressed in the same unit, the ingestion rates on autotrophic material for the three dominant copepod species appeared insufficient to balance their metabolic requirements for respiration (Fig. 6A). For Cirripedia larvae, the average ingestion rate was slightly higher than the upper limit of the confidence interval for the respiration rates, but the confidence intervals of the two rates overlapped. If we assume that metazooplankton organisms could incorporate other available food sources (such as HNF, ciliates or organic detritus) at the same rate (i.e. with the same clearance rate *F*) as phytoplankton, we can estimate, for each taxa, the ingestion rates on these food sources, by multiplying the *F* values (summarized on Table 4) by the biomass of each food category (summarized in Table 1). Ingestion hardly balanced respiration when considering a ration based on phytoplankton and micro-heterotroph (Fig. 6B). The budget was fully balanced for Cirripedia larvae and, at a lower degree, for *Acartia clausi* in the case of a diet including the organic detritus (Fig. 6C). However, even in this most favorable case, the lower confidence limit for carbon ingestion of *Temora stylifera* and *Paracalanus* spp. was within the range of the confidence interval of respiration, suggesting that the budget was not always fully balanced for these species.

3.9. Grazing pressure

The cumulated zooplankton grazing during the 24-h cycle corresponded to 5.1% of the average in situ chlorophyll *a* concentration and to 14% of the average primary production (Table 6). During the daily survey, the zooplankton grazing rate ranged from 0.06 to 1.13 $\mu\text{g Chl l}^{-1} \text{d}^{-1}$, which corresponded to 0.5–11% of the in situ chlorophyll *a*, and 2.7–33% of the primary production per day. This grazing pressure could also remove 0.5–11% of the HNF or ciliate

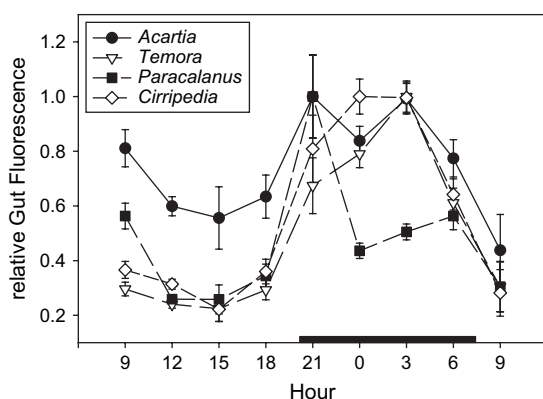


Fig. 5. Time-variations of the gut fluorescence during the 24-h cycle.

Table 5

Metabolism experiments: zooplankton biomass and composition per experimental flask. Daily specific rates for respiration (Resp.), ammonia (E_{NH_4}) and phosphate (E_{PO_4}) excretion. Atomic ratios measured in each jar during the five experiments. Cir, cirriped larvae; Cop, copepods

| Date | Experimental zooplankton | | | Metabolic rates ($\mu\text{g C}^{-1} \text{d}^{-1}$) | | | Atomic ratios (at/at) | | |
|--------|--------------------------|------|------|---|--|--|--------------------------|------|-------|
| | $\mu\text{g C l}^{-1}$ | %Cir | %Cop | Resp. ($\mu\text{g O}_2$) | E_{NH_4} (nM NH_4) | E_{PO_4} (nM PO_4) | O/N | N/P | O/P |
| 16-May | 582 | 76.3 | 19.0 | 1.60 | 5.06 | 0.51 | 19.8 | 10.0 | 198.0 |
| | 769 | 60.7 | 36.9 | 1.43 | 4.36 | 0.48 | 20.5 | 9.0 | 185.0 |
| 21-May | 100 | 70.9 | 29.1 | 1.05 | 3.40 | 0.39 | 19.3 | 8.7 | 168.0 |
| | 95 | 63.0 | 37.0 | 1.38 | 4.46 | 0.44 | 19.3 | 10.2 | 197.0 |
| 23-May | 128 | 45.5 | 54.5 | 1.48 | 4.36 | 0.51 | 21.1 | 8.6 | 181.2 |
| | 165 | 63.3 | 36.7 | 1.36 | 4.66 | 0.47 | 18.3 | 9.8 | 179.7 |
| 25-May | 473 | 85.1 | 12.2 | 1.28 | 4.00 | 0.44 | 20.0 | 9.0 | 180.3 |
| | 421 | 89.0 | 8.5 | 1.32 | 4.08 | 0.47 | 20.3 | 8.6 | 175.2 |
| 28-May | 502 | 63.5 | 34.8 | 1.17 | 3.57 | 0.42 | 20.6 | 8.6 | 176.3 |
| | 594 | 55.4 | 43.9 | 1.16 | 3.47 | 0.39 | 20.9 | 8.8 | 184.0 |
| Mean | 383 | 67.3 | 31.2 | 1.32 | 4.14 | 0.45 | 20.0 | 9.1 | 182.5 |
| SD | 243 | 13.3 | 14.3 | 0.16 | 0.54 | 0.04 | 0.9 | 0.6 | 9.3 |

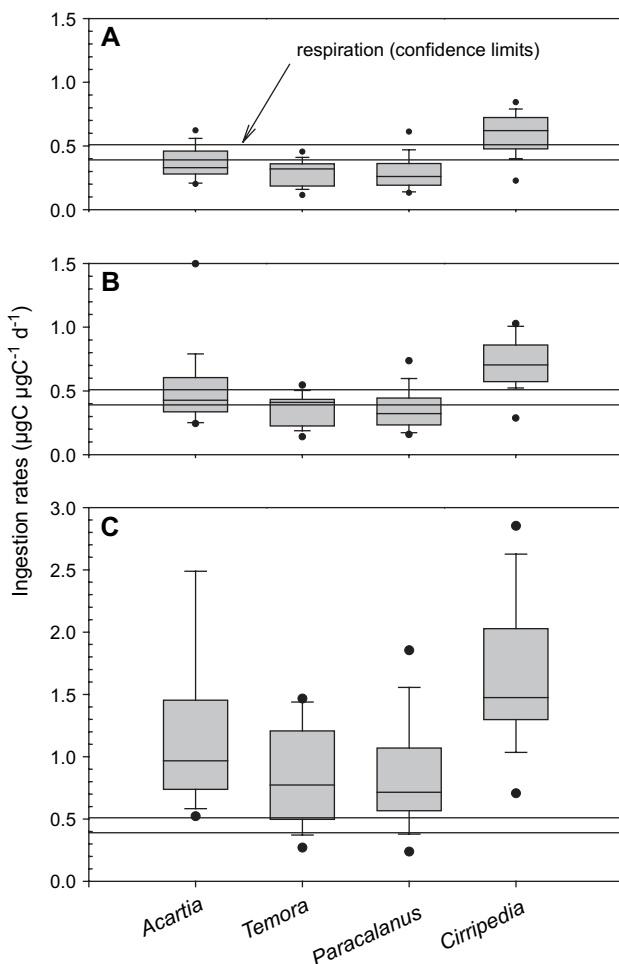


Fig. 6. Box plots of the weight specific ingestion rates of the main zooplankton taxa calculated for rations based on phytoplankton (A), phytoplankton + microbial components (B) phytoplankton + microbial components + organic detritus (C). The horizontal lines indicate the confidence limits of metabolic expenditure (respiration) of total zooplankton expressed in the same unit.

biomass, if we assume (as above) that the clearance rate on these organisms and on micro algae are equivalent. The zooplankton grazing rate was not correlated with chlorophyll *a*, primary production or HNF but it was negatively correlated with the abundance ($r = -0.63$, $p = 0.016$, $n = 15$) or the percentage ($r = -0.67$, $p = 0.012$, $n = 15$) of nanophytoplankton and positively correlated with the percentage of picophytoplankton ($r = 0.56$, $p = 0.038$, $n = 15$).

3.10. Nutrient recycling

During the 24-h cycle, the daily integrated nutrient recycling through zooplankton excretion was equivalent to 30 and 59% of the in situ $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations, respectively (Table 6). During the daily survey, zooplankton excretion ranged from 0.03 to 0.30 $\mu\text{M NH}_4\text{-N}$ and from 0.00 to 0.03 $\mu\text{M PO}_4\text{-P d}^{-1}$, which represented 11–450% (mean = 83%) and 1–205% (mean = 47%) of the in situ $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations, respectively. In any of the two considered periods, the recycling rates of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were correlated with the respective concentrations in these nutrients ($p > 0.05$).

Table 6

Zooplankton grazing pressure and nutrient recycling. Average estimates during the 24-h cycle and the daily survey

| | 24-h cycle | Daily survey | | |
|---|------------|------------------|------|-------|
| | | Mean | Min | Max |
| Daily grazing pressure | | | | |
| $\mu\text{g Chla l}^{-1} \text{d}^{-1}$ | 0.42 | 0.34 ± 0.25 | 0.06 | 1.13 |
| %Phytoplankton biomass | 5.1 | 3.2 ± 2.5 | 0.5 | 11.1 |
| %Primary production | 14.4 | 6.7 ± 4.8 | 1.7 | 22.6 |
| Daily nutrient recycling | | | | |
| $\mu\text{M NH}_4\text{-N d}^{-1}$ | 0.16 | 0.10 ± 0.07 | 0.03 | 0.30 |
| %In situ $\text{NH}_4\text{-N}$ | 30.3 | 83.0 ± 109.2 | 11.2 | 450.2 |
| $\mu\text{M PO}_4\text{-P d}^{-1}$ | 0.02 | 0.01 ± 0.01 | 0.00 | 0.03 |
| %In situ $\text{PO}_4\text{-P}$ | 59.5 | 46.9 ± 62.5 | 1.4 | 205.8 |

4. Discussion

4.1. Diel vertical migrations and feeding rhythms

Clear diel variations of abundance were observed during the 24-h cycle since most taxa were present with very little numbers in the water column during the day. These variations could reflect tidal effects as observed by Dauvin et al. (1998), who showed that the semi-diurnal tidal current was the dominant factor in determining the short-term changes in zooplankton abundance and composition in the Seine River Estuary (France). They could also reflect diel vertical migration (DVM); in this case, during the day, the animals were probably located very close to (or burrowed into) the sediment as observed in other shallow brackish tropical environments (Kouassi et al., 2001). Finally, these variations could also result from both phenomena as observed in other estuarine environments (Barans et al., 1997; Kimmerer et al., 2002; Hampel et al., 2003). In our study, it is difficult to analyze this aspect in detail on the basis of only one 24-h sampling period, performed in a given tidal context. However, our results show that the DVM effect was globally more important than the tide effect (significant day–night effect but no tidal effect for total zooplankton in the ANOVA). In addition, the absence of significant interaction between tidal and day–night effects suggests that the tidal vertical migration, described by Dauvin et al. (1998) probably did not occur in our study.

The main zooplankton taxa (*Acartia clausi*, *Temora stylifera*, *Paracalanus* spp. Cirripedia larvae) showed a clear diel pattern in gut fluorescence, characterized by a nocturnal increase. Therefore, they featured diel feeding rhythms (DFR) as classically observed for most herbivorous zooplankton species in various environments (Mauchline, 1998). However, there was no clear association between DFR and DVM and between these patterns and the vertical distribution of food particles in the water column. Indeed, during the 24-h cycle (May 26–27) the algal food was vertically distributed rather homogeneously and no significant positive relationships were observed between in situ chlorophyll *a* concentration and gut pigment content. Therefore, the diel variability observed in gut contents for these organisms in the SRE was unlikely to result from continuous feeding in vertically stratified food environments as also evidenced by Dam and Peterson (1993) for the copepod *Temora longicornis*. It probably resulted from an increase of feeding activity at night, which could reflect endogenous rhythms.

4.2. Feeding rates and carbon requirement by zooplankton and possible trophic links

Expressed as carbon unit, the average daily specific ingestion rates on phytoplankton for *Acartia clausi*, *Temora stylifera*, *Paracalanus* spp. and Cirripedia larvae (0.44, 0.27, 0.28 and 0.56 $\mu\text{g C } \mu\text{g C}^{-1} \text{d}^{-1}$, respectively) were within the range of values summarized by Mauchline (1998) for co-generic copepod species (0.14–1.50 $\mu\text{g C } \mu\text{g C}^{-1} \text{d}^{-1}$). They seemed low as compared to those obtained by Kouassi et al. (2001) for *A. clausi* in a tropical brackish lagoon, with the same gut

fluorescence technique (0.37–1.66 $\mu\text{g C } \mu\text{g C}^{-1} \text{d}^{-1}$). Generally, ingestion rates of zooplankton in tropical waters are in average higher than in temperate waters, partly because the metabolic requirements of these poikilotherm organisms are higher at high temperature. In our study, the daily respiration rates of total zooplankton were high and represented 40–51% of body carbon weight. These high rates are in agreement with other high values reported for tropical zooplankton. For example, Gerber and Gerber (1979) and Pagano and Saint-Jean (1994) reported respiration values for mixed zooplankton from tropical lagoons representing 55–88% of body C d^{-1} .

In our study, the four studied taxa did not balance their metabolic needs for respiration with a ration containing only phytoplankton. These metabolic budgets were hardly better balanced when considering a ration including heterotrophic micro-organisms (ciliates and HNF), grazed at the same clearance rate (no food selectivity). Therefore, in order to meet their metabolic needs and produce organic matter, these organisms have probably to ingest organic detritus in abundance, and/or to feed selectively upon micro-heterotrophs. Any of these assumptions suggest the existence of a strong omnivory within the metazooplankton in this estuarine ecosystem. The low herbivory level is probably linked to the phytoplankton composition, dominated by picoplanktonic cells, which are not easily edible for the large zooplankton considered in our study (copepodites and adults of calanoids, Cirripedia larvae). A parallel experiment performed on the same organisms (Bouvy, pers. comm.) showed that picophytoplankton cells were poorly grazed by *Acartia clausi*, *Temora stylifera* and Cirripedia larvae but were actively ingested by flagellates, which in turn were consumed by ciliates that clearly served as prey for *T. stylifera*. These experimental results argue for a selective feeding of copepods upon micro-heterotrophs (HNF, ciliate and even bacteria) in field conditions. The fact that metabolic budgets were not always balanced, when considering a mixed ration including phytoplankton, micro-organisms and detritus grazed at the same clearance rate, also argues in this sense. In the same way, metabolic O:N ratio < 24, indicating a protein-oriented metabolism (Ikeda, 1974), suggested a degree of carnivory for these metazoan organisms. The microbial and the detrital trophic ways appear complementary for the herbivorous chain in the SRE. The role of micro-heterotrophs in the metazooplankton ration (as well as selective feeding upon these prey) was shown in several studies dealing with estuarine environments (Gasparini and Castel, 1997; Vincent and Hartmann, 2001; Rollwagen Bollens and Penry, 2003). Bacteria are probably partly incorporated by smaller metazoans (e.g. nauplii of copepods), as shown for example by Turner and Tester (1992) and Bouvy et al. (1994). Finally, the role of the organic detritus as food complement for copepods was evidenced in highly loaded detrital environments (Poulet, 1976; Heinle et al., 1977).

4.3. Grazing impact and nutrient recycling by zooplankton

The daily grazing pressure of metazooplankton (0.5–11% of the in situ chlorophyll *a* concentrations and 2–23% of the

primary production) seemed low in comparison with values mentioned for other African estuaries. Kibirige and Perissinotto (2003) have reported zooplankton grazing rates corresponding to 17–69% of chlorophyll *a* per day in a South African temporarily open estuary. Therefore, the impact of zooplankton grazing on the dynamics of the phytoplankton in the SRE would be low, as also suggested by the absence of significant relationship between grazing and chlorophyll *a* during the daily survey. Other mortality factors would explain the variations of phytoplankton abundance, as predation by microzooplankton which can remove 12–131% of the phytoplankton stock and up to 265% of the primary production in coastal waters (references summarized by Sherr and Sherr, 2002). Protozooplankton can have a higher grazing impact on phytoplankton than metazooplankton, particularly in coastal or estuarine waters (Froneman, 2002; Calbet et al., 2003; Liu and Dagg, 2003). In our study, the low grazing pressure of metazooplankton on algae is probably due to the size-structure of the phytoplankton community, which is dominated by picoplanktonic organisms, too small to be grazed efficiently, as also observed by Froneman (2002) in a temperate South African estuary. In the SRE, the estimated grazing pressure was mainly orientated towards large cells, as suggested by the negative correlation between the zooplankton grazing rate and the abundance (or relative abundance) of nanophytoplankton. Thus the zooplankton impact on the size-composition of the phytoplankton was probably effective. Assuming that the zooplankton consumption is exerted exclusively (or mostly) on the nanophytoplankton which represented 6–17% of cell numbers (or 30–50% of the biomass), the daily grazing pressure would represent up to 35% of the biomass of this edible fraction. This estimate is in agreement with other studies carried out in coastal or estuarine waters, showing the strong grazing impact on the nano or the microphytoplankton. For example, Liu and Dagg (2003) estimated that up to 86% of the production of >20 µm phytoplanktonic cells was consumed by mesozooplankton in a station off the plume of the Mississippi River.

Recycling of nutrients by metazooplankton excretion was not negligible since this process represented, on average per day, 83% (11–450%) and 46% (1–205%) of the in situ concentrations for NH₄-N and PO₄-P, respectively. When applying Redfield's ratios to the values of primary production, these contributions could satisfy 10% of the N requirements and 75% of the P requirements for the phytoplankton growth. Estimates of nutrient regeneration through excretion by zooplankton, particularly for coastal or estuarine tropical waters, are scarce in the literature. Atkinson and Whitehouse (2001) estimated that copepods and small euphausiids excrete at least one third of the ammonium potentially required by phytoplankton in the upper mixed layer of Antarctic waters. Bode et al. (2004) estimated that the phytoplankton demand for ammonium was closely matched by the regeneration rates of microplankton, whereas mesozooplankton contributed on average to less than 10% to this process. Our estimated values seem high compared to the data from literature. In addition, the contribution of microzooplankton excretion to nutrient recycling is undoubtedly high, owing to the fact that the microbial loop here is very efficient (Troussellier et al., 2004). Then, in the SRE and in the studied

conditions, the primary production seems primarily linked to the recycling related to the excretion of the planktonic organisms (dominant regenerated production). On the other hand, the stoichiometry of the excreted nutrients appears to reflect a nitrogen limitation of the phytoplankton and could thus be favorable to nitrogen-fixing organisms like cyanobacteria.

The zooplankton activity could, in addition, stimulate the microbial loop, as suggested by the positive correlations observed between bacterial abundance and zooplankton biomass (or grazing rate) during the survey. This should reflect the recycling, through sloppy feeding, of dissolved organic matter (DOM) available for bacteria (Moller et al., 2003) and cascading trophic effect, i.e. predation upon bacteria predators (HNF). Torrèton et al. (1994) explained the close coupling between free-living bacteria and zooplankton abundance in a eutrophic tropical lagoon in Côte d'Ivoire (West Africa) by the same hypothesis.

In conclusion, at the end of the dry season (low water period), the SRE is characterized by a relative stability of environmental conditions, by a relatively low external nutrient inputs (as compared to those recorded during the flood period) and by the dominance of picophytoplankton. At this period, the impact of metazooplankton on phytoplankton through top-down (grazing) and bottom-up (nutrient recycling) processes seems important. Metazooplankton ingestion upon phytoplankton, primarily directed towards the nanophytoplankton, is not sufficient to satisfy its energetic needs. This implies the exploitation of organic detritus and/or of other prey such as HNF and ciliates, whose intensive consumption by the metazooplankton could support the proliferation of picoplanktonic organisms (picophytoplankton and bacteria). Our results argue also in favor of a strong coupling between herbivorous chain and microbial loop (and/or detrital chain). This coupling is also supported by experimental observations performed during the same survey and demonstrating a significant grazing effect of zooplankton (mainly *Temora stylifera*) on ciliates involving a trophic cascade within the microbial loop (Bouvy, pers. comm.). However, further investigations are necessary to clarify the nature of the picophytoplankton and the causes of its dominance in the SRE.

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References

- Atkinson, A., Whitehouse, M.J., 2001. Ammonium regeneration by Antarctic mesozooplankton: an allometric approach. *Marine Biology* 139, 301–311.
- Bamstedt, U., Gifford, D.J., Irigoien, X., Atkinson, A., Roman, M.R., 2000. Feeding. In: Harris, R.P., Wiebe, P., Lenz, J., Skjoldal, H.R., Huntley, M. (Eds.), *ICES Zooplankton Methodology Manual*. Academic Press, London, pp. 297–380.

- Barans, C.A., Stender, B.W., Holliday, D.V., Greenlaw, C.F., 1997. Variation in the vertical distribution of zooplankton and fine particles in an estuarine inlet of South Carolina. *Estuaries* 20, 467–482.
- Bode, A., Barquero, S., Gonzalez, N., Alvarez Ossorio, M.T., Varela, M., 2004. Contribution of heterotrophic plankton to nitrogen regeneration in the upwelling ecosystem of A Coruna (NW Spain). *Journal of Plankton Research* 26, 11–28.
- Borsheim, K.Y., Bratbak, G., 1987. Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from sea water. *Marine Ecology Progress Series* 36, 171–175.
- Bouvy, M., Arfi, R., Guiral, D., Pagano, M., Saint-Jean, L., 1994. Role of bacteria as food for zooplankton in a eutrophic tropical pond (Ivory Coast). *Netherlands Journal of Aquatic Ecology* 28, 167–174.
- Buskey, E.J., Deyoe, H., Jochem, F.J., Villareal, T.A., 2003. Effects of mesozooplankton removal and ammonium addition on planktonic trophic structure during a bloom of the Texas 'brown tide': a mesocosm study. *Journal of Plankton Research* 25, 215–228.
- Calbet, A., Vaque, D., Felipe, J., Vila, M., Sala, M.M., Alcaraz, M., Estrada, M., 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. *Marine Ecology Progress Series* 259, 303–309.
- Capriulo, G.M., Smith, G., Troy, R., Wikfors, G.H., Pellet, J., Yarish, C., 2002. The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. *Hydrobiologia* 475, 263–333.
- Dagg, M., Benner, R., Lohrenz, S., Lawrence, D., 2004. Transformation of dissolved and particulate materials on continental shelves influenced by large rivers: plume processes. *Continental Shelf Research* 24, 833–858.
- Dam, H.G., Peterson, W.T., 1988. The effect of temperature on the gut clearance rate constant of planktonic copepods. *Journal of Experimental Marine Biology and Ecology* 123, 1–14.
- Dam, H.G., Peterson, W.T., 1993. Seasonal contrasts in the diel vertical distribution, feeding behavior, and grazing impact of the copepod *Temora longicornis* in Long Island Sound. *Journal of Marine Research* 51, 561–594.
- Dauvin, J.C., Thiebaut, E., Wang, Z.X., 1998. Short-term changes in the mesozooplanktonic community in the Seine ROFI (Region of Freshwater Influence) (eastern English Channel). *Journal of Plankton Research* 20, 1145–1167.
- Elser, J.J., Goldman, C.R., 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnology and Oceanography* 36, 64–90.
- Froneman, P.W., 2002. Trophic cascading in an oligotrophic temperate estuary, South Africa. *Journal of Plankton Research* 24, 807–816.
- Gasparini, S., Castel, J., 1997. Autotrophic and heterotrophic nanoplankton in the diet of the estuarine copepods *Eurytemora affinis* and *Acartia biflosa*. *Journal of Plankton Research* 19, 877–890.
- Gerber, R.P., Gerber, M.B., 1979. Ingestion of natural particulate organic matter and subsequent assimilation, respiration and growth by tropical lagoon zooplankton. *Marine Biology* 52, 33–43.
- Hampel, H., Cattrijsse, A., Vincx, M., 2003. Tidal, diel and semi-lunar changes in the faunal assemblage of an intertidal salt marsh creek. *Estuarine, Coastal and Shelf Science* 56, 795–805.
- Heinle, D.R., Harris, R.P., Ustach, J.F., Flemer, D.A., 1977. Detritus as food for estuarine copepods. *Marine Biology* 40, 341–353.
- Ikeda, T., 1974. Nutritional ecology of marine zooplankton. *Memoirs of Faculty of Fisheries, Hokkaido University* 22, 1–97.
- Kagami, M., Yoshida, T., Gurung, T.B., Urabe, J., 2002. Direct and indirect effects of zooplankton on algal composition in situ grazing experiments. *Oecologia* 133 (3), 356–363.
- Kibirige, I., Perissinotto, R., 2003. In situ feeding rates and grazing impact of zooplankton in a South African temporarily open estuary. *Marine Biology* 142, 357–367.
- Kimmerer, W.J., 2002. Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages? *Marine Ecology Progress Series* 243, 39–55.
- Kimmerer, W.J., Burau, J.R., Bennett, W.A., 2002. Persistence of tidally-oriented vertical migration by zooplankton in a temperate estuary. *Estuaries* 25, 359–371.
- Kouassi, E., Pagano, M., Saint-Jean, L., Arfi, R., Bouvy, M., 2001. Vertical migrations and feeding rhythms of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) in a tropical lagoon (Ebrié, Cote d'Ivoire). *Estuarine, Coastal and Shelf Science* 52, 715–728.
- Liu, H.B., Dagg, M., 2003. Interactions between nutrients, phytoplankton growth, and micro- and mesozooplankton grazing in the plume of the Mississippi River. *Marine Ecology Progress Series* 258, 31–42.
- Mackas, D.L., Bohrer, R., 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. *Journal of Experimental Marine Biology and Ecology* 25, 77–85.
- Mauchline, J., 1998. The biology of calanoid copepods. In: Blaxter, J.H.S., Southward, A.J., Tyler, P.A. (Eds.), *Advances in Marine Biology*. Academic Press, London, 710 pp.
- Moller, E.F., Thor, P., Nielsen, T.G., 2003. Production of DOC by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* through sloppy feeding and leakage from fecal pellets. *Marine Ecology Progress Series* 262, 185–191.
- Murrell, M.C., Lores, E.M., 2004. Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *Journal of Plankton Research* 26, 371–382.
- Pagano, M., Gaudy, R., Thibault, D., Lochet, F., 1993. Vertical migrations and feeding rhythms of mesozooplanktonic organisms in the Rhone River plume area (North-West Mediterranean Sea). *Estuarine, Coastal and Shelf Science* 37, 251–269.
- Pagano, M., Saint-Jean, L., 1994. In situ metabolic budget for the calanoid copepod *Acartia clausi* in a tropical brackish water lagoon (Ebrié Lagoon, Ivory Coast). *Hydrobiologia* 272, 147–161.
- Pinckney, J.L., Paerl, H.W., Tester, P., Richardson, T.L., 2001. The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives* 109 (Suppl. 5), 699–706.
- Poulet, S.A., 1976. Feeding of *Pseudocalanus minutus* on living and non-living particles. *Marine Biology* 34, 117–125.
- Putt, M., Stoecker, D.K., 1989. An experimentally determined carbon:volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. *Limnology and Oceanography* 34, 1097–1104.
- Rollwagen Bollens, G.C., Penry, D.L., 2003. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). *Marine Ecology Progress Series* 257, 139–158.
- Sherr, E.B., Sherr, B.F., 2002. Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology* 81, 293–308.
- Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Marine Ecology Progress Series* 51, 203–213.
- Sterner, R.W., 1989. The role of grazers in phytoplankton succession. In: Sommer, U. (Ed.), *Plankton Ecology: Succession in Plankton Communities*. Springer Verlag, Berlin, pp. 107–170.
- Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Hantzsche, F., Katechakis, A., Lippert, B., Loseth, K., Peters, C., Roederer, W., Sandow, M., Sundt Hansen, L., Olsen, Y., 2004. Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. *Ecology Letters* 7, 321–328.
- Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. *Fisheries Research Board of Canada* 167, 1–311.
- Tackx, M.L.M., Herman, P.J.M., Gasparini, S., Irigoien, X., Billiones, R., Daro, M.H., 2003. Selective feeding of *Eurytemora affinis* (Copepoda, Calanoida) in temperate estuaries: model and field observations. *Estuarine, Coastal and Shelf Science* 56, 305–311.
- Torréon, J.P., Bouvy, M., Arfi, R., 1994. Diel fluctuations of bacterial abundance and productivity in a shallow eutrophic tropical lagoon. *Archiv für Hydrobiologie* 131, 79–92.
- Troussellier, M., Courties, C., Lebaron, P., Servais, P., 1999. Flow cytometric discrimination of bacterial populations in seawater based on SYTO 13 staining of nucleic acids. *Microbial Ecology* 29, 319–330.
- Troussellier, M., Courties, C., Vaquer, A., 1993. Recent applications of flow cytometry in aquatic microbial ecology. *Biology of the Cell* 78, 111–121.
- Troussellier, M., Got, P., Bouvy, M., M'Bou, M., Arfi, R., Lebihan, F., Monfort, P., Corbin, D., Bernard, C., 2004. Water quality and health status of the Senegal River estuary. *Marine Pollution Bulletin* 48, 852–862.

- Troussellier, M., Got, P., M'Boup, M., Corbin, D., Giuliano, L., Capello, S., Bouvy, M., 2005. Daily bacterioplankton dynamics in a sub-Saharan estuary (Senegal River; West Africa): a mesocosm study. *Aquatic Microbial Ecology* 40, 13–24.
- Turner, J.T., Tester, P.A., 1992. Zooplankton feeding ecology – bacterivory by metazoan microzooplankton. *Journal of Experimental Marine Biology and Ecology* 160, 149–167.
- Uye, S.I., 1982. Length–weight relationships of important zooplankton from the Inland Sea of Japan. *Journal of the Oceanographical Society of Japan* 38, 149–158.
- Vincent, D., Hartmann, H.J., 2001. Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. *Hydrobiologia* 443, 193–204.
- Winkler, G., Dodson, J.J., Bertrand, N., Thivierge, D., Vincent, W.F., 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. *Marine Ecology Progress Series* 251, 59–73.
- Yentsch, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Research* 10, 221–231.