

Grazing impact on chromophoric dissolved organic matter (CDOM) by the larvacean *Oikopleura dioica*

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ABSTRACT: Experiments were conducted to determine if the pelagic larvacean *Oikopleura dioica* could graze on chromophoric dissolved organic material (CDOM) and if so, could such grazing affect the optical characteristics of the water column. Although *O. dioica* was found to graze on CDOM, it also contributes to the CDOM pool. *O. dioica* cleared large, >10 kDa CDOM, with clearance rates ranging from 0.5 ml d⁻¹ for humic material to 8.9 ml d⁻¹ for proteins suggesting a differential cycling of dissolved organic material by this larvacean. Its excretion of CDOM was in the <5 kDa size range. The clearance of large-sized CDOM (>10 kDa) and the excretion of small-sized CDOM (<5 kDa) resulted in an alteration of the molecular size distribution of the CDOM in natural seawater. Thus the relationship between grazing removal and excretion inputs will affect the cycling and molecular size distribution of CDOM and influence water color.

KEY WORDS: *Oikopleura dioica* · CDOM · Grazing · Water column · Water color

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INTRODUCTION

Oikopleuridae are pelagic tunicates that are non-selective filter-feeders, feeding on particles ranging from 0.3 to >30 µm (Flood 1978, Flood et al. 1992, Bedo et al. 1993). Oikopleurids live in transparent, gelatinous houses that serve as elaborate particle traps through which they pump water and from which they suck particle-enriched water into their pharynx (e.g. Alldredge 1977, Flood 1991). Flood (1991) estimated that the food-concentrating chamber of the house allowed the oikopleurid to feed on approximately 1000× concentrated suspension of particles. Later, Morris & Deibel (1993) measured concentration factors ranging from 74 to 1089, with higher values associated with smaller individuals. This concentrating mechanism of feeding could possibly lead to the ingestion of dissolved material either through the formation of larger colloids during concentration or through direct interception of the dissolved material with the filter apparatus.

Little is known about the relationship between oikopleurids and dissolved organic matter (DOM), but it has been suggested that they could be important in the flux of DOM and in the size distribution of DOM (Gorsky & Fenaux 1998). Because of the small size range of particles that they consume, they have been considered important regulators of bacterioplankton (Alldredge 1977, Fernandez & Acuña 2003) and thus the cycling of DOM by bacteria. In addition, the ability of oikopleurids to filter colloidal material has been demonstrated (Flood et al. 1992), and since much of the terrestrial DOM is in the high molecular weight range (Cauwet 2002) they could potentially directly consume this material and repackage it into biomass or rapidly sinking fecal pellets. Nothing is currently known about the excretion of DOM by oikopleurids.

DOM is a complex pool comprised of many different types of compounds. One significant component of DOM is chromophoric dissolved organic matter (CDOM). CDOM is important because it has the unique characteristic of absorbing ultraviolet and visible light,

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thereby affecting the optical characteristics of the water column (Kalle 1966, Arrigo & Brown 1996). The fluorescent characteristics of CDOM allow detection of small amounts of material, permitting CDOM to be used as a tracer (Zimmerman & Rommets 1974, de Souza Sierra et al. 1997). In addition changes in its fluorescent spectral characteristics can also be used to identify source materials (Coble 1996, McKnight et al. 2001). The majority of the CDOM is derived from terrestrial sources, so highest concentrations are found in coastal waters and decrease with increasing distance from the shore (Blough et al. 1993). Physical mixing, photo-oxidation and bacterial remineralization (Cabaniss & Shuman 1987, Skoog et al. 1996, De Souza Sierra et al. 1997, Moran & Zepp 1997, Moran et al. 2000) have been the only avenues explored to explain the cycling and removal of CDOM. We hypothesized that the pelagic larvacean *Oikopleura dioica* could clear CDOM from the water by means of its unique filtration system. Since these larvaceans can occur at high concentrations in neritic waters (Dagg 1995), if they can graze on CDOM they might affect the water color.

The objective of this study was to examine the grazing effects of *Oikopleura dioica* on colored dissolved organic matter (CDOM) to determine (1) if they can filter CDOM from the water, and (2) if their grazing changes the fluorescent characteristics or molecular weight distribution of the CDOM. The fluorescent characteristics of CDOM make this a good tracer in grazing experiments and allow for experiments with naturally occurring CDOM. In addition, fluorescent excitation–emission matrices allow for the detection of changes in fluorescent pools that can suggest different source materials.

MATERIALS AND METHODS

Laboratory experiments were conducted with the cultured appendicularian *Oikopleura dioica* at the University of Oviedo in northern Spain. Appendicularian cultures were started in spring 2001 with individuals collected from El Musel harbor in Gijón. The appendicularians were cultured in 5 l glass jars filled with 30 μm filtered seawater mixed with a Plexiglas spiral paddle rotating at 10 rpm (Fenaux and Gorsky 1979, 1985) in a temperature-controlled room at 15°C. The appendicularians were sorted according to size and transferred into freshly filtered (30 μm) seawater every 3 d using a wide-bore pipette. Only large adult (avg. trunk length 710 μm) *O. dioica* were used in the grazing experiments.

Grazing on 30 μm filtered seawater. Grazing experiments were conducted to examine the impact of *Oikopleura dioica* on CDOM composition of natural seawater. Adult *O. dioica* were preconditioned for 4 h in

30 μm filtered seawater collected from El Musel Harbor. We filled 3 treatment and control, 100 ml acid-washed, amber glass bottles with 90 ml of 30 μm filtered seawater. The appendicularians were then rinsed with 0.2 μm -filtered seawater and 50 *O. dioica* were added to each of the 3 treatment bottles and an equivalent volume (10 ml) of the 0.2 μm rinse-water was added to the control bottles. The bottles were rotated (0.5 rpm) on a plankton wheel in the dark for 1 h at 15°C in a temperature-controlled room. At the end of incubation, samples were taken for absorption and fluorescence of CDOM and bacterial abundance (see following subsections).

Samples were also taken to examine the molecular weight distribution of the colored dissolved fraction. Water from the treatment and control bottles was first filtered through a 0.2 μm polycarbonate filter and the filtrate was fractionated into <30 and <5 kDa using acid-cleaned, Centricon Plus-20 tubes. Milli-Q water was used as a blank and the fluorescence in the blank was subtracted from the samples to correct for any addition arising from absorption onto the tube walls. A total of 15 ml of sample water was spun for 15 min at 4000 $\times g$ at 15°C on Jouan MR1812 centrifuge with a fixed rotor. The retention efficiency (RE) of the tubes was determined by using fluorescently stained dextrans from molecular probes with reported molecular weights of 5000 and 30 000 Da. The RE of the 30 kDa tubes was 97% and that of the 5 kDa tubes was 88%. The filtrate and retentate were stored in frozen amber glass vials for later analysis of fluorescence and absorption.

Tracer experiment. Tracer experiments were conducted to determine if *Oikopleura dioica* was able to filter and ingest dissolved organic material and, if so, in what size range. Mixtures of fluorescent dextrans from molecular probes (<http://probes.invitrogen.com/>) were used in 2 separate experiments. The first treatment mixture (Tracer 1/Expt 1) consisted of dextrans with molecular weights of 3 and 40 kDa at a concentration of 2.5 μM in 0.2 μm filtered seawater. The second treatment mixture (Tracer 2/Expt 2) consisted of 10 and 70 kDa dextrans at a concentration of 2.5 μM in 0.2 μm filtered seawater. The tracer solutions were filtered through a 0.2 μm polycarbonate filter prior to the experiment to remove any aggregates.

The experimental design consisted of six, 40 ml amber vials filled with the tracer solution and 20 *Oikopleura dioica* in houses, 6 vials filled with the tracer solution and 20 empty *O. dioica* houses, and 6 vials filled only with tracer solution. The 2 control sets were designed to measure passive absorption onto the *O. dioica* houses and vial walls. Each vial was incubated for 5 min on a rotating plankton wheel. Since the object of the experiment was to determine if oikopleurids could filter and ingest the dissolved tracers

and to calculate the clearance rates of each tracer, experiments were restricted to 5 min to prevent excretion of the tracer. Previous work had shown that 5 min incubations allow significant uptake, but are shorter than the gut-passage time of the appendicularian, and thus do not change its functional ingestion rates or behavior (Acuña & Kiefer 2000). The appendicularians and houses or houses alone were transferred 3 times, using a wide-bore pipette, into 0.2 µm filtered seawater to rinse any extra label off the outside of the house. Appendicularians and houses were then immediately frozen in liquid nitrogen until they could be examined by fluorescent microscopy. Samples from the rinse-water and from the vials were taken for spectrofluorometric analysis.

Clearance rates were calculated for each of the treatments (3, 10, 40 and 70 kDa) using the methods of Acuña & Kiefer (2000) (see Table 2). Briefly, the change in fluorescence in the treatment bottles (corrected for any loss in the control bottles) compared to the initial fluorescence in the water was divided by the number of individuals per vial and incubation period. This value was then converted into standard units of ml ind.⁻¹ d⁻¹.

Natural CDOM experiment. Grazing experiments on different size fractions of naturally occurring CDOM were conducted to determine if *Oikopleura dioica* could remove CDOM and thereby influence the optical characteristics of the water. Water was collected from a local estuary in northern Spain and filtered through a 0.2 µm Supor Cup Filter (GelmanScience SuporCap 100) that was acid-washed and rinsed with 20 l of Milli-Q water prior to collecting the CDOM water. The water was size-fractionated, the 4 size fractions comprising material >30, <30, >5 and <5 kDa. The concentrates were prepared by adding 10 ml to 30 and 5 kDa Centricon Plus-20 centrifuge tubes and spinning at 4000 × *g* for 10 min on a Jouan MR1812 Centrifuge with a fixed rotor. The <30 and <5 kDa filtrates were collected and placed in combusted 40 ml scintillation vials for use as experimental water. The <30 and <5 kDa filtrates were not diluted, and were used directly in the incubation vials. The retentate was collected by inverting the tubes and spinning them for 3 min at 1000 × *g*. The process was repeated until 10 ml of retentate had been collected in both the 30 and 5 kDa tubes. We added 10 ml aliquots of CDOM retentate (>30 and >5 kDa) to 120 ml of photo-oxidized 0.2 µm filtered seawater to prepare 2 treatments with CDOM fractions of different size.

Twelve 20 ml scintillation vials were used with 6 vials containing appendicularians in houses and 6 control vials with only CDOM-enriched seawater. Similar to the tracer experiment, incubations lasted only 5 min and the vials were rotated on a plankton wheel. The oikopleurids were picked out of the vial and trans-

ferred into 2 sequential vials with 0.2 µm-filtered seawater for rinsing, and were then frozen in liquid nitrogen. This protocol was followed for each of the 4 size fractions of CDOM. Water samples for fluorescent spectrophotometry were taken.

Clearance rates of the humic and protein fractions were calculated wherever possible, as described previously for the tracer experiment. (For average clearance rate of all humic components [Peaks A, C and M combined] see Table 2).

Water analysis. Samples for CDOM absorption and fluorescence were collected by filtering 70 to 200 ml of water through a 0.2 µm Nuclepore filter. The filtrate was stored frozen in amber vials for analysis in the laboratory. All samples were analyzed within 2 mo of collection and no significant changes in absorption of fluorescence were observed within that time frame (J. Urban-Rich unpubl. data). CDOM absorption was measured on a Carey 50 spectrophotometer using a 10 cm cuvette, and corrected with a Milli-Q blank. Absorption coefficients at 355 nm were calculated as described by Green & Blough (1994). This value was chosen as it is the most commonly used and thus allowed us to compare our results with literature values. Spectral slopes (*S*) were determined by linear, least-squares regression based on the exponential decay relationship of:

$$a(\lambda) = a(\lambda_0)e^{-S(\lambda-\lambda_0)}$$

where $a(\lambda)$ = absorption at a desired wavelength (355 nm is the usual value for CDOM), λ_0 = 280 nm (the initial wavelength), and S is the fitted parameter for the exponential decay of $a(\lambda)$ with increasing λ . Thus, S describes the rate of decrease in CDOM absorption with increasing wavelength. S varies with the source of CDOM, and changes in S can reflect biological or chemical modifications of CDOM.

CDOM fluorescent excitation–emission matrices were measured on a SPEX FluoroMax-3. Excitation (ex) scans from 250 to 550 nm at 5 nm intervals and emission (em) scans from 265 to 710 nm at 2 nm intervals and 2 s integration created 61 individual excitation–emission scans. Slit widths were 5.0 nm for excitation and 2.0 nm for emission. The instrument was corrected as per the manufacturer's instructions. Data were normalized to the water Raman Peak at ex/em = 350/397 nm and converted into quinine sulfate units (QSU) using a correction curve generated with a quinine sulfate standard in 0.05 M sulfuric acid (Coble et al. 1993).

Fluorescent excitation–emission matrix scans (EEMS) can give information on different humic and protein components of the CDOM pool. Regions within EEMS have been defined by Coble (1996) as humic-like or protein-like (Table 1). In order to compare our data

Table 1. Major fluorescent components of seawater defined by Coble (1996) compared with those found in present study (Figs. 2, 3 & 7). Coble's maximum excitation/emission (ex, em) values taken as standard

Peak	Coble (1996)		Present study		Description
	ex _{max}	em _{max}	ex _{max}	em _{max}	
B	275	310	275	315	Tyrosine-like, protein-like
T	275	340	275	340	Tryptophan-like, protein-like
A	260	380–460	250	440	Humic-like
M	312	380–420	325	418–440	Marine humic-like
C	350	420–480	350	450	Humic-like

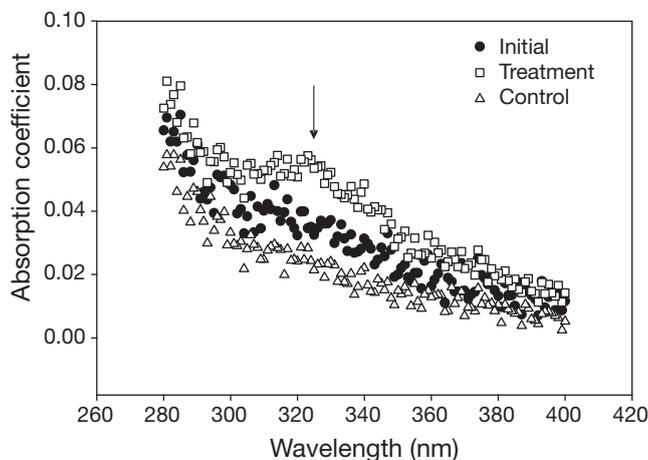


Fig. 1. *Oikopleura dioica*. Average CDOM absorption in grazing experiment using 30 µm filtered seawater. See 'Materials and methods' for explanations of treatments. Arrow indicates region where there was significant increase in absorption in treatment bottles

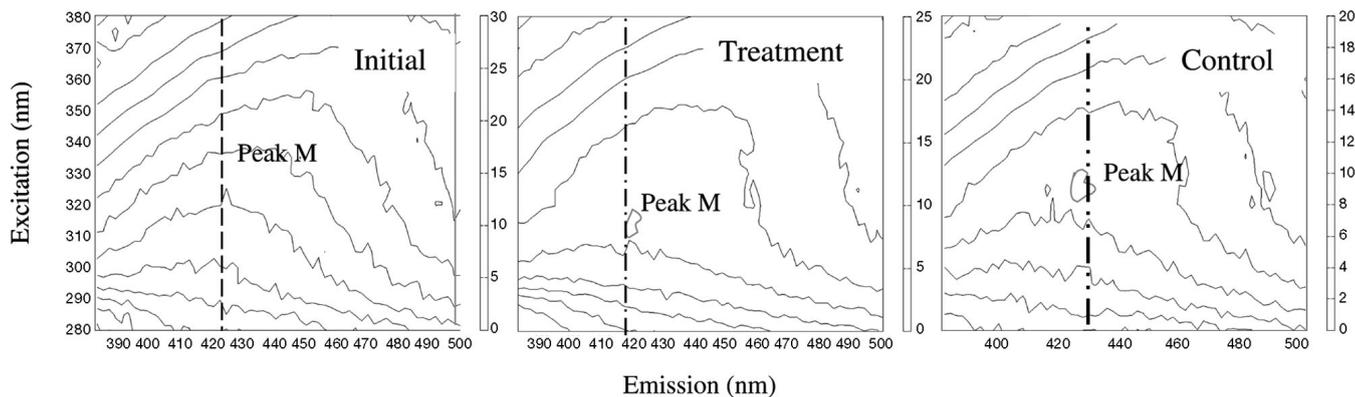


Fig. 2. *Oikopleura dioica*. Excitation (ex)–emission (em) (3D) scans from the grazing experiment using 30 µm filtered seawater. Scale bar on right of each graph is in normalized quinine sulfate units (QSU). Peak M: humic Peak M region (maximum ex/em in treatments was at 320/420 nm). Peak M values increased by 1.3 in treatment bottles (2.1 QSU) compared to initial values (1.5 QSU) and control bottles (1.50 QSU); however, maximum peak region shifted to blue in treatment bottles (maximum intensity represented by dotted/dashed lines) and total peak region broadened

with other published data, we have used these same notations for the peak regions, although slight changes in excitation and emission maximums were observed in our study. Changes in the region of maximum peak intensity for Humic Peaks M and C and the fluorescent proteins were monitored in each experiment.

We preserved 5 to 25 ml of water from each sample and control bottle with 0.1% glutaraldehyde for bacteria counts. The water was gently filtered onto a black Poretics 0.2 µm membrane filter and stained with acridine orange (Hobbie et al. 1977). Slides were frozen until counted in the laboratory with epifluorescent microscopy.

RESULTS

Grazing on natural seawater

Grazing experiments conducted with the pelagic larvacean *Oikopleura dioica* revealed it to be a source of CDOM. Significant increases in CDOM absorption at 355 nm (a_{355}) were detected in the treatment (T) bottles compared to the initial (I) and control (C) bottles ($I_{\text{abs}} = 0.43 \pm 0.03 \text{ m}^{-1}$, $T_{\text{abs}} = 0.56 \pm 0.05 \text{ m}^{-1}$, $C_{\text{abs}} = 0.28 \pm 0.09 \text{ m}^{-1}$, $p < 0.05$). An examination of all the absorption data revealed a peak around 320 nm (Fig. 1) which caused S to decrease in the treatment bottles (for I, $s = 6.5 \times 10^3$, for C = 6.8×10^3 , for T = $6.0 \times 10^3 \text{ nm}^{-1}$). An examination of the EEMs revealed a strong increase in the region of Peak M (ex/em 320/420 nm) fluorescence (Fig. 2) that corresponded with the hump in the absorption data (Fig. 1).

Analysis of the molecular weight distribution of the fluorescent material in the grazing experiment indicated that the humic material (average ex/em 320/420

and 280/420 nm) added by *Oikopleura dioica* was <30 kDa (Fig. 3). In addition, the added protein material was primarily in the 5 to 30 kDa range. However, there was a loss of protein material and humic material in the >30 kDa range.

Bacterial numbers decreased slightly in the treatment bottles and increased in the controls (Fig. 4). The increase in bacterial numbers in the control bottles may explain the slight decrease in CDOM absorption in the control bottles compared to initial bottles as the bacteria may have degraded some of the CDOM. No significant changes in chlorophyll concentrations occurred in the treatment or control bottles during the 1 h incubation.

Tracer experiment

The tracer experiments were run to determine if *Oikopleura dioica* could ingest DOM. Epifluorescent micrographs of *O. dioica* clearly show the presence of the fluorescent dextrans in the guts and within its fecal pellets, illustrating the ability of *O. dioica* to filter and ingest DOM (Fig. 5). On average, 1% of the removed tracer adhered to the empty oikopleurid houses and to the vial walls; however for the treatment vials, we corrected for this loss in our calculations. Significant removal of the tracer was detected for the 3, 10 and 40 kDa dextrans, but not for the 70 kDa fraction (Fig. 6), with most removal occurring with the 10 and

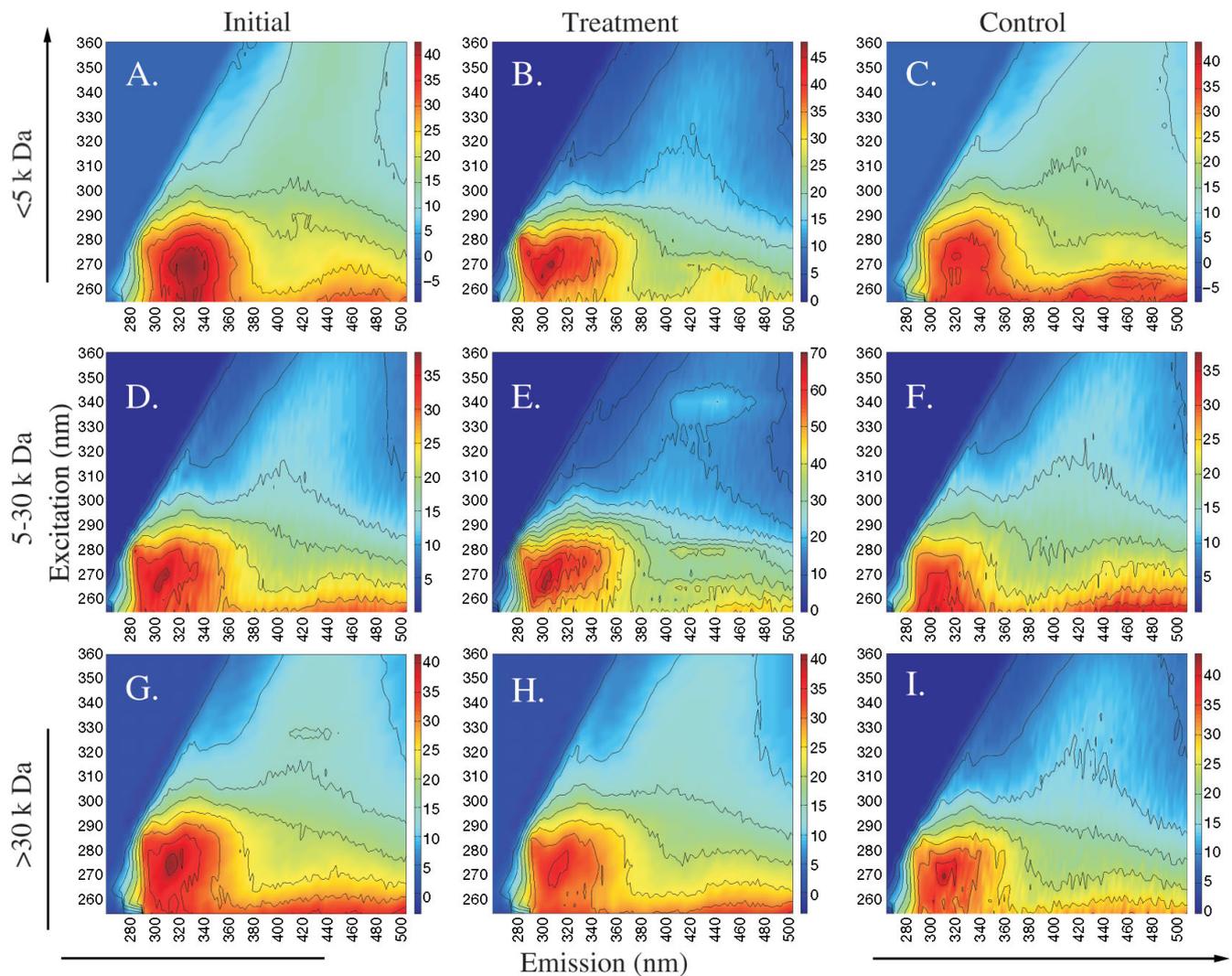


Fig. 3. *Oikopleura dioica*. Excitation–emission scans of molecular weight fractions from grazing experiment using 30 µm filtered seawater. Scale bar on right of each graph is in normalized quinine sulfate units (QSU); x-axis is emission range from 270 to 500 nm in 20 nm intervals; y-axis is excitation range from 250 to 360 nm in 10 nm intervals. In filtrate of 5 kDa, Humic Peak M (ex/em 320/420 nm) and protein material (ex/em 275/310 and 275/350 nm) increased in treatment bottles (B); in filtrate between 5 and 30 kDa, Humic Peak M (ex/em 320/440 nm), region of 280/420 nm, Humic Peak C (ex/em 340/450 nm) and protein region (ex/em 275/300–350 nm) increased in treatment bottles (E); in filtrate of >30 kDa, Humic Peaks C and M decreased in treatment bottles (H), as did protein region of 275/350 nm and area of 280/400 nm

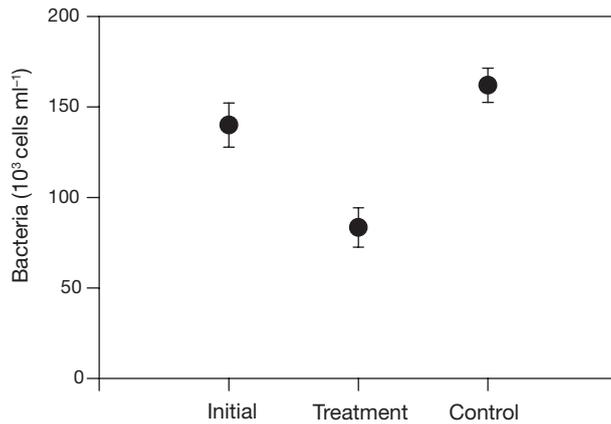


Fig. 4. Bacterial cell abundance during grazing experiment with *Oikopleura dioica*. Decreases in bacterial numbers were observed in treatment bottles, while slight increases in control bottles were seen



Fig. 5. *Oikopleura dioica*. Fluorescent dextran (40 kDa) visible in pharynx (arrowed), gut and fecal pellets after 5 min incubation

40 kDa dextrans ($p < 0.001$; Wilcoxon paired-sample T -test). Estimated clearance rates ranged from 28.4 and 28.9 $ml\ d^{-1}$ for the 40 and 3 kDa dextrans to 55.1 $ml\ d^{-1}$ for the 10 kDa dextrans for individuals with an average trunk length of 710 μm (Table 2).

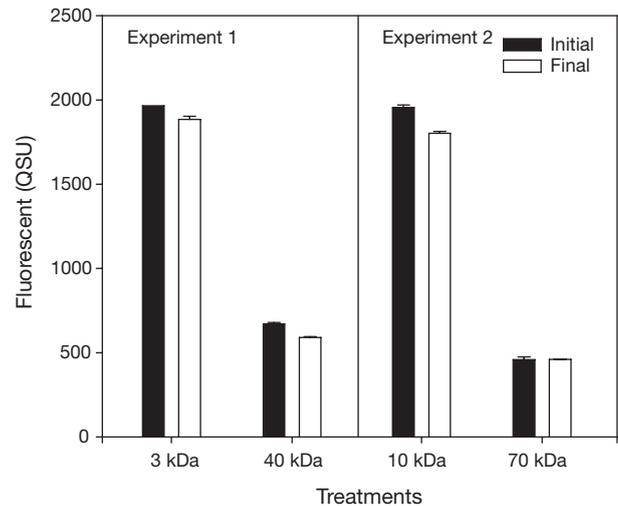


Fig. 6. *Oikopleura dioica*. Fluorescent dextrans were added to 0.2 μm filtered seawater and incubated with appendicularians for 5 min to determine if it could clear and ingest DOM. Significant removal of the 3, 10 and 40 kDa tracers occurred ($p < 0.05$, $p < 0.001$ and $p < 0.05$, respectively; Wilcoxon paired-sample t -test). Treatments corrected for any loss observed in control bottles (loss in controls was negligible, and was not significant in any dextran treatment)

Grazing on natural CDOM

CDOM is a large pool comprised of many different compounds with different structures, functions, and sizes. Results of the grazing experiment with CDOM collected from a local estuary indicate that *Oikopleura dioica* can remove material from some size fractions. Significant removal of humic and protein material occurred in the >30 kDa treatment (Student t -test, $p < 0.05$), highly variable results were recorded in the 5 to 30 kDa treatments, and significant inputs occurred in the humic and protein material in the <5 kDa treatment (Fig. 7; $p < 0.05$). Clearance rates for the >30 kDa fraction ranged from 0.5 to 8.5 $ml\ d^{-1}$ for individuals with an average trunk length of 700 μm , with higher clearance rates on the protein material and lower clearance rates on the humic material.

Table 2. *Oikopleura dioica*. Estimated (mean \pm SE) clearance rates ($ml\ ind.^{-1}\ d^{-1}$) of fluorescent dextran tracers and natural humic and protein material in >30 kDa fraction. Average rates determined from 5 min grazing experiments ($n = 6$) in laboratory using individuals with average trunk length (TL) of 700 to 710 μm . nd: not calculated as no significant clearance detected

Expt	TL (μm)	Dextran con.				Material in >30 kDa fraction	
		3 kDa	10 kDa	40 kDa	70 kDa	Humic	Protein
Tracer 1	710 \pm 10	28.9 \pm 3.1		28.4 \pm 1.8			
Tracer 2	710 \pm 5		55.1 \pm 2.7		nd		
>30 kDa natural CDOM	700 \pm 8					0.5 \pm 0.02	8.5 \pm 1.3

DISCUSSION

Source and sink of CDOM

Relatively little is known about biological sources or sinks of chromophoric dissolved organic material (CDOM). Bacteria and phytoplankton have been pro-

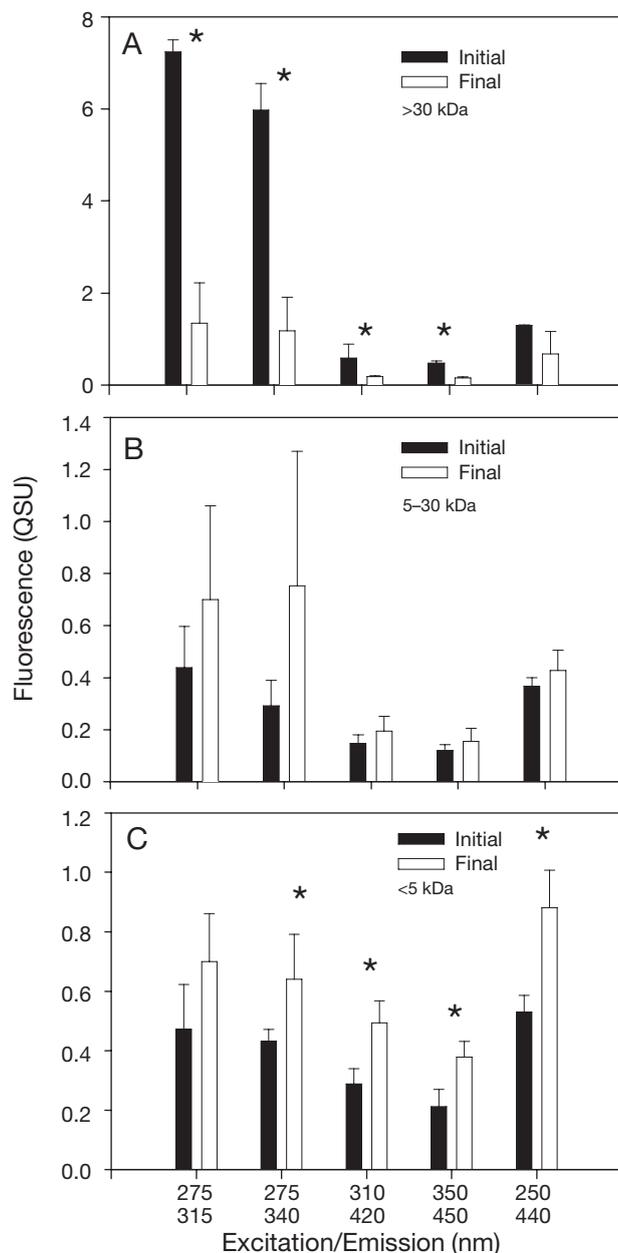


Fig. 7. *Oikopleura dioica*. Appendicularian was incubated for 5 min with different molecular size fractions of naturally occurring CDOM to determine if it could clear CDOM. (A) In >30 kDa fraction, significant (* $p < 0.05$; Student's t -test) removal of humic and protein fractions occurred; (B) 5 to 30 kDa fraction, no significant differences were found between initial and final water; (C) in <5 kDa fraction, significant (* $p < 0.05$) input occurred in humic and protein fractions

posed as potential sources of CDOM (Carder et al. 1989, Nelson et al. 1998), and recently zooplankton have been shown to be sources of CDOM (Steinberg et al. 2004, Urban-Rich et al. 2004). However, bacteria have been considered to be the only biological organisms that can utilize and degrade CDOM (Moran et al. 2000). The results of our study clearly show that *Oikopleura dioica* is not only a source of CDOM, but can also be a means of removing CDOM from seawater (Figs. 3 & 7).

The importance of oikopleurids as a source or removal mechanism of CDOM may vary with season and location depending upon the food-web structure. *Oikopleura dioica* is a widely distributed coastal species reported from a diversity of environments, including many coastal regions of the North Atlantic Ocean, the Mediterranean Sea, the South Atlantic Ocean, the Gulf of Mexico and the Pacific Ocean (reviews in Fenaux 1998, Fernandez & Acuna 2003). *O. dioica* generally occurs seasonally, and during blooms when it is still actively feeding, it has been reported at concentrations ranging from 20 to 53 ind. l^{-1} (Dagg 1995, Fernandez & Acuna 2003). During nonbloom conditions, larvaceans are generally found at concentrations of 0.1 to 0.5 ind. l^{-1} (Fenaux 1998). During periods of maximum abundance, which can last for a day to several weeks they have the potential to significantly affect the optical characteristics of the water in many marine environments through their clearance and release of CDOM.

DOM cycling by oikopleurids

The results of this study indicate that *Oikopleura dioica* can influence the size distribution of DOM, and thus potentially DOM cycling along with optical water characteristics, by modifying CDOM size distribution. *O. dioica* can clear and ingest DOM in the 10 to 40 kDa range, but excretes material in the <30 kDa range, primarily in the <5 kDa region (Fig. 3). The efficiency of DOM ingestion and the mechanisms by which it is ingested are unknown. Larvaceans feed by pumping water through an elaborate gelatinous house consisting of several filters (Deibel et al. 1985, Deibel & Powell 1987). During this process, matter is concentrated to levels up to 1000 \times higher than ambient levels. It is possible that the DOM coagulates and thus increases to a size at which it is able to be retained by the larvaceans, filter (filter pore-size = 0.2 μ m). Another possibility is that the DOM is cleared by direct interception and adhesion to the filter mesh. However, the lack of significant tracer on the empty houses suggests that adhesion to the house (and presumably subsequent adhesion to the filter apparatus) is not an overly important path of DOM removal. Another possibility is diffusion-

deposition which is governed by Brownian movement. As smaller particles move more than large particles, they would be more likely to connect with the filter or to randomly coagulate with another particle, thus becoming large enough to be filtered by the larvacean. Again, the lack of significant amounts of tracer on the empty houses suggests that diffusion–deposition alone is not important. However, diffusion–deposition combined with the pumping of water by the larvacean could prove important. This process could not be determined during this study.

Marine humic matter is characteristically of low molecular weight and low aromaticity (Hedges 1992). Therefore, the finding that *Oikopleura dioica* excretes marine humic material of low molecular weight is not surprising. It is not clear if all DOM released by *O. dioica* is of small molecular weight, or if this applies to the CDOM fraction only. In the few studies that have examined the size distribution of DOM released by grazing zooplankton or flagellates, large, high molecular weight DOC or colloidal material has been the dominant form of DOM (Tranvik 1994, Strom et al. 1997). In most copepod grazing experiments, increased bacterial numbers and activity are recorded, and are attributed to the release of DOC by the grazing copepods, however in our grazing study with *O. dioica* bacterial numbers declined, most probably due to consumption by the larvaceans. Thus, the turnover and fate of DOM and its role in the microbial loop may differ as a function of zooplankton community-structure.

The clearance rates measured in this study for the fluorescent dextran tracers were in the same range as measured for *Oikopleura dioica* on particles (Fenaux & Malara 1990, Bedo et al. 1993); however, the clearance rates on natural CDOM were 3 to 100 times lower. This difference in clearance rates could be due to variations in concentrations of the material or in the intrinsic properties of the DOM. Humic material may not coagulate into larger aggregates as easily as carbohydrates. Carbohydrates and proteins are known to be adhesive, which could increase their aggregation potential. The almost 20 times greater clearance rates of the protein compared to the humic material suggests that properties of the DOM influence the retention efficiency and thus clearance rate of DOM size classes by oikopleurids. Thus, grazing by *O. dioica* leads to a differential cycling of DOM pools, based on both the size of the molecules as well as its chemical composition.

To our knowledge, the relationship between oikopleurids and dissolved organic matter (DOM) has not been investigated earlier. In a parallel study, it was shown that *Oikopleura dioica* excretes CDOM (J. Urban-Rich et al. unpubl. data). Results from our grazing experiments support the excretion results, and indicate that *O. dioica* excretes primarily small molec-

ular weight CDOM <5 kDa, while consuming the larger >10 kDa fraction. This interesting interaction between ingestion and excretion has important implications for total DOM cycling as well as for CDOM cycling. Terrestrially derived humic and fulvic acids tend to be of high molecular weight, with a higher degree of aromaticity than marine humic and fulvic acids. *O. dioica* would seem to be able to clear and ingest terrestrially derived CDOM, and either assimilate or repackage it into fecal pellets. While most of the DOM is of low molecular weight, much of the recalcitrant DOM in the oceans is of high molecular weight, HMW (Carlson 2002), and in estuaries and coastal environments HMW DOM is also added to by rivers (Cauwet 2002). Thus *O. dioica* may play an important role in DOM cycling, especially in coastal environments. Since oikopleurids can form short and direct trophic links between their food and higher trophic-level organisms predators, including commercial fishes (Nedreaas 1987, Shimamoto & Watanabe 1994) and other gelatinous zooplankton (Larson 1991), they could transfer DOM up the food web.

Implications of oikopleurid grazing on CDOM

CDOM is a significant but variable portion of the total DOM pool that has the capability of absorbing light in both the ultraviolet and visible regions, thus influencing the amount of photosynthetically available radiation that reaches the phytoplankton (Arrigo & Brown 1996). Therefore, processes that remove CDOM promote water clarity and biological production, while processes that add CDOM reduce the water clarity and decrease primary production. Oikopleurids appear to be one of the unique biological organisms that contribute to both processes. We estimated the grazing impact of *Oikopleura dioica* on CDOM in the Bay of Biscay as follows: assuming an abundance of 15 *O. dioica* l⁻¹ (Fernandez & Acuna 2003), a measured clearance rate of 0.5 ml d⁻¹, and a fluorescent CDOM concentration of 40 QSU l⁻¹, *O. dioica* would clear ~18% CDOM d⁻¹, assuming that all CDOM at ex/em 355/450 nm was >30 kDa. However, if only a portion of the CDOM were in the large size range, then clearance rates for this fraction could be much higher than 18%. This estimate suggests that under certain conditions (i.e. in areas in which CDOM is comprised of the large size fraction) oikopleurids could play an important role by clearing the water. The measured clearance rate in our experiments was substantially below laboratory and field clearance rates on particles, which range from 22 to 210 ml d⁻¹ (Bedo et al. 1993, López-Urrutia et al. 2003). This reflects the reduced effectiveness of *O. dioica* in clearing DOM compared to clearing parti-

cles. However it can still remove a substantial portion of the DOM, and more importantly, affect the size distribution of the DOM. In our grazing experiment on natural seawater, CDOM absorption at 355 nm (Fig. 1) increased, suggesting that excretion exceeded grazing in this instance. However, the size distribution of the CDOM also changed, which could affect the turnover of the CDOM pool and thus the water color.

This study has shown that *Oikopleura dioica* can clear CDOM from seawater. Grazing plus excretion by this oikopleurid changes the size distribution of the CDOM; this could affect its role in the water column in relation to determining ocean color and/or in the complex of trace metals and pollutants. In addition, grazing by *O. dioica* results in differential cycling of the DOM pool based on both the molecular size of the material and on its chemical composition. The importance of this larvacean in DOM cycling needs to be investigated. The present experiments suggest that oikopleurids can alter the molecular size distribution of DOM, thus influencing organic matter cycles as well as water color.

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LITERATURE CITED

- Acuña JL, Kiefer M (2000) Functional response of the appendicularian *Oikopleura dioica*. *Limnol Oceanogr* 45:608–618
- Allredge AL (1977) House morphology and mechanisms of feeding in the Oikopleuridae (Tunicata, Appendicularia). *J Zool* 181:175–188
- Arrigo KR, Brown CW (1996) Impact of chromophoric dissolved organic matter on UV inhibition of primary productivity in the sea. *Mar Ecol Prog Ser* 140:207–216
- Bedo AW, Acuña JL, Robins D, Harris RP (1993) Grazing in the micronic and sub-micronic particle size range: the case of *Oikopleura dioica* (Appendicularia). *Bull Mar Sci* 53:2–14
- Blough NV, Zafirov CC, Bonilla J (1993) Optical absorption spectra of waters from the Orinoco river outflow: terrestrial input of colored organic matter to the Caribbean. *J Geophys Res* 98:2271–2278
- Carder KL, Steward RG, Harvey GR, Ortner PB (1989) Marine humic and fulvic acids: their affects on remote sensing of ocean chlorophyll. *Limnol Oceanogr* 34:68–81
- Carlson CA (2002) Production and removal processes. In: Hansell DA, Carlson CA (eds) *Biogeochemistry of marine dissolved organic matter*. Academic Press, San Diego, CA p 91–152
- Cauwet G (2002) DOM in the coastal zone. In: Hansell DA, Carlson CA (eds) *Biogeochemistry of marine dissolved organic matter*. Academic Press, San Diego, CA, p 579–611
- Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. *Mar Chem* 51:325–346
- Coble PG, Schultz CA, Mopper K (1993) Fluorescence contouring analysis of DOC intercalibration experiment samples: a comparison of techniques. *Mar Chem* 41:173–178
- Dagg MJ (1995) Copepod grazing and the fate of phytoplankton in the northern Gulf of Mexico. *Cont Shelf Res* 15:1303–1317
- Deibel D, Powell CBL (1987) Ultrastructure of the pharyngeal filter of the appendicularian *Oikopleura vanhoeffeni*: implications for particle size selection and fluid mechanics. *Mar Ecol Prog Ser* 35:243–250
- Deibel D, Dickson ML, Powell CBL (1985) Ultrastructure of the mucous feeding filter of the house of the appendicularian *Oikopleura vanhoeffeni*. *Mar Ecol Prog Ser* 27:79–86
- De Souza Sierra MM, Donard OFX, Lamotte M (1997) Spectral identification and behavior of dissolved organic fluorescent material during estuarine mixing processes. *Mar Chem* 58:51–58
- Fenaux R (1998) The classification of Appendicularia. In: Bone Q (ed) *The biology of pelagic tunicates*. Oxford University Press, New York, p 295–308
- Fenaux R, Gorsky G (1985) Nouvelle technique d'élevage des appendiculaires. *Rapp P-V Réun Comm Int Mer Méditerr* 29:291–292
- Fenaux R, Malara G (1990) Taux de filtration de l'appendiculaire *Oikopleura dioica* Fol 1872. *Rapp P-V Réun Comm Int Explor Sci Mer Méditerr* 32:20
- Fernandez D, Acuña JL (2003) Enhancement of marine phytoplankton blooms by appendicularian grazers. *Limnol Oceanogr* 48:587–593
- Flood PR (1991) Architecture of, and water circulation and flow rate in the house of the planktonic tunicate *Oikopleura labradoriensis*. *Mar Biol* 111:95–111
- Flood PR, Deibel D, Morris C (1992) Filtration of colloidal melanin from sea water by planktonic tunicates. *Nature* 355:630–632
- Gorsky G, Fenaux R (1998) The role of appendicularians in marine food webs. Bone Q (ed) *The biology of pelagic tunicates*. Oxford Press, New York, p 161–170
- Green SA, Blough NV (1994) Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnol Oceanogr* 39:1903–1916
- Hedges J (1992) Global biochemical cycles: progress and problems. *Mar Chem* 39:67–93
- Hobbie JD, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* 33:1225–1228
- Larson RJ (1991) Diet prey selection and daily ration of *Stomolophus meleagris*, a filter feeding scyphomedusa from the NE Gulf of Mexico. *Estuar Coast Shelf Sci* 32:511–525
- López-Urrutia A, Acuña JL, Irigoien X, Harris R (2003) Food limitation and growth in temperate epipelagic appendicularians (Tunicata). *Mar Ecol Prog Ser* 252:143–157
- McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr* 46:38–48
- Moran MA, Sheldon WM, Zepp RG (2000) Carbon loss and optical property changes during long term photochemical and biological degradation of estuarine dissolved organic matter. *Limnol Oceanogr* 45:1254–1264
- Morris CC, Deibel D (1993) Flow rate and particle concentration within the house of the pelagic tunicate *Oikopleura vanhoeffeni*. *Mar Biol* 115:445–452
- Nedreaas K (1987) Food and feeding habits of young saithe, *Pollachius virens*, on the coast of western Norway. *Fiskeridir Skrift Ser Fisk* 18:263–301

- Nelson NB, Seigel DA, Michaels AF (1998) Seasonal dynamics of colored dissolved organic material in the Sargasso Sea. *Deep-Sea Res I* 45:931–957
- Shimamoto N, Watanabe J (1994) Seasonal changes in feeding habit of red sea bream *Pagrus major* in the eastern Set Inland sea, Japan. *Bull Jpn Soc Sci Fish* 60:65–71
- Skoog A, Wedborg M, Fogelqvist E (1996) Photobleaching of fluorescence and the organic carbon concentration in a coastal environment. *Mar Chem* 55:333–345
- Steinberg DK, Nelson NB, Carlson CA, Prusak, A (2004) Production of chromophoric dissolved organic matter (CDOM) in the open ocean by zooplankton and the colonial cyanobacterium *Trichodesmium* spp. *Mar Ecol Prog Ser* 267:45–56
- Strom SL, Benner R, Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol Oceanogr* 42:1364–1374
- Tranvik L (1994) Colloidal and dissolved organic matter excreted by a mixotrophic flagellate during bacterivory and autotrophy. *Appl Environ Microbiol* 60:1884–1888
- Urban-Rich J, McCarty JT, Shailer M (2004) Effects of food concentration and diet on chromophoric dissolved organic matter accumulation and fluorescent composition during grazing experiments with the copepod *Calanus finmarchicus*. *ICES J Mar Sci* 61:542–551
- Zimmerman JTF, Rommets JW (1974) Natural fluorescence as a tracer in the Dutch Wadden Sea and the adjacent North Sea. *Neth J Sea Res* 8:117–125

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