

Larvaceans and copepods excrete fluorescent dissolved organic matter (FDOM)

Juanita Urban-Rich^{a,*}, James T. McCarty^a, Diego Fernández^b, Jose Luis Acuña^b

^a University of Massachusetts, Boston, Environmental, Earth and Ocean Science, 100 Morrissey Boulevard, Boston, MA 02125, United States

^b University of Oviedo, Departamento Biología de Organismos y Sistemas, C/Catedrático Rodrigo Uría (S/N), 33071 Oviedo, Asturias, Spain

Received 17 May 2005; received in revised form 11 October 2005; accepted 11 November 2005

Abstract

Chromophoric dissolved organic matter (CDOM) can play an important role in regulating biological production in coastal environments. Fluorescent dissolved organic matter (FDOM) is a subset of the larger CDOM pool that can be used to look at sources of CDOM. Experiments were conducted with copepods and the pelagic larvacean, *Oikopleura dioica* to determine if these two types of zooplankton excrete FDOM. Shipboard bottle experiments were conducted with copepods in the Gulf of Mexico, in the Mississippi River Plume in April 2001 and laboratory experiments with the pelagic larvacean, *O. dioica* were conducted in Oviedo, Spain in June 2001. Both copepods and *O. dioica* were found to excrete FDOM. Excitation/emission matrices revealed that both animals contributed to fluorescent protein pools (ex/em 275/315–350 nm) and to humic-like material (maximum ex/em 300/420 nm). The humic-like material excreted by the copepods and larvaceans was shifted toward shorter wavelengths, providing a possible unique fluorescent signature for zooplankton. Using a ratio of the humic-like fluorescence excreted by the animals (ex/em 300/420) to the humic-like fluorescence maximum in controls (ex/em 320/420 nm) showed the zooplankton could contribute nearly 50% of the Peak M fluorescence. Molecular weight analysis of the CDOM in an *O. dioica* excretion study showed that *O. dioica* excreted CDOM was primarily <30 kDa. Both copepods and larvaceans are sources of marine FDOM and CDOM and can potentially influence the amount and type of CDOM present in coastal waters.

© 2005 Elsevier B.V. All rights reserved.

Keywords: CDOM; Copepod; FDOM; *Oikopleura*; Zooplankton

1. Introduction

Chromophoric dissolved organic matter (CDOM) comprises a variable but significant fraction of the dissolved organic matter (DOM) pool and is capable of influencing the optical characteristics of the water column. Understanding the nature, sources and turnover of

CDOM is critical for modeling the optical characteristics of the water in coastal environments. CDOM absorbs light in both the ultraviolet and visible wavelengths thereby influencing the amount of photosynthetically available radiation. In many coastal environments absorption by CDOM often exceeds that of particles (DeGrandpre et al., 1996), therefore it can help to regulate primary production. While the majority of CDOM in coastal regions is derived from terrestrial sources (Obermosterer and Herndl, 2000), marine sediments, phytoplankton and bacteria have been considered as possible autochthonous sources of CDOM (Carder

* Corresponding author. Tel.: +1 617 287 7485; fax: +1 617 287 7474.

E-mail address: juanita.urban-rich@umb.edu (J. Urban-Rich).

et al., 1989; Chen et al., 1993; Hayase and Shinozuka, 1995; Skoog et al., 1996; Nelson et al., 1998). Other biological sources of CDOM could be viral lysis or zooplankton grazers. The turnover of terrestrial derived CDOM versus marine CDOM may vary, thus the source of CDOM may affect its ecological significance. In a recent study, Tranvik and Kokalj (1998) found that photooxidation of terrestrial CDOM created biological labile material that was quickly used by bacteria, while photooxidation of marine CDOM created material with decreased biodegradability.

Within the larger CDOM pool exists a smaller fluorescence pool (FDOM). Due to the increased sensitivity of the fluorescence methods the FDOM can be used to look at sources of DOM and potentially at the cycling of certain DOM pools (Coble et al., 1993; McKnight et al., 2001). Known fluorescence DOM signatures are Peak C (ex/em 350/460 nm) believed to represent terrestrially derived CDOM, Peak M (ex/em 320/420 nm) representative of marine humic-like material, Peaks T and B (ex/em 275/310 and 340 nm) which are protein-like materials and Peak A (ex/em 260/460 nm) which is considered to be bacterial derived (Coble, 1996). These peak regions represent a mixture of fluorescent compounds and slight changes in the excitation and emission maximum can reflect changes in the relative proportion of different compounds or changes in their oxidation state. These changes might reflect changes in the source of the marine humic-like material.

Previous studies have shown that grazing copepods contribute to dissolved organic material and to free amino acids (Copping and Lorenzen, 1980; Roman et al., 1988), therefore it is logical to assume grazers could contribute to FDOM as well. However, nothing is known about the role of zooplankton in FDOM formation or cycling. The objectives of this study were to (1) determine if copepods and the pelagic larvacean, *Oikopleura dioica* excrete FDOM, (2) examine the fluorescence characteristics of the DOM produced by copepods and *O. dioica* relative to the known fluorescence peak regions.

Copepods and *O. dioica* were chosen for this study, as they are both common neretic zooplankters with contrasting feeding mechanisms. Copepods are the most numerous multicellular organisms in the ocean and are present year-around at concentrations generally ranging from 0.2 to 5 per liter (Roff et al., 1988; Mauchlin, 1998), though swarms greater than 100 copepods per liter have been reported (Mauchlin, 1998 and references within). They feed by filtering particles >10 µm, which they grind with their mandibles. In contrast, *O. dioica* is generally present sea-

sonally (Acuna, 1994; Gorsky et al., 1988; Lazarus and Dowler, 1979; Esnal et al., 1985; Dagg, 1995; Dagg et al., 1996; Buck and Newton, 1995; Nakamura, 1998; Kitalong, 1986) and when it occurs it forms swarms that can have concentrations of grazing oikopleurids as high as 30 animals per liter (Dagg et al., 1996). In addition, *O. dioica* is a filter feeder that consumes particles ranging in size from 0.2 to 30 µm (Bedo et al., 1993) and they have no hard mouthparts for grinding food rather they feed using a mucous house filtration system (Deibel and Powell, 1987; Flood, 1991).

2. Methods

2.1. Copepod excretion experiments

Experiments were conducted in April 2001 in the Gulf of Mexico to examine copepod inputs to FDOM. Copepods were collected aboard the *R.V. Pelican* with vertical net tows in the upper 10 m, using a 0.5-m diameter net with 183 µm mesh and a closed cod end. Water for the bottle experiments was collected from CTD casts immediately prior to the net tow. Upon retrieval of the net, the cod end was diluted in 20 l of surface seawater. Actively swimming, undamaged copepods with full guts, were picked out and rinsed with 0.2 µm filtered seawater before being placed into acid washed, glass jars that were blackened with electrical tape. The dominant copepods used in the experiments were *Acartia tonsa*, *Labidocera* sp. and *Temora turbinata*.

The aim of the excretion experiments was to determine if copepods release FDOM and what type of FDOM accumulates. Five copepods were placed into each 0.5 l treatment bottle. Treatment and control bottles were filled with 0.2 µm filtered seawater and incubated in running seawater in a darkened incubator on deck, water temperature ranged from 20 to 23 °C. Bottles were wrapped with electrical tape to prevent photooxidation of the DOM. A set of treatment and control bottles were sampled at 0 and 6 h. Experiments were conducted within the Mississippi River Plume and outside of the plume in order to vary the amount of background terrestrial CDOM to help determine the relative importance of copepod derived FDOM to water color. Samples were taken for DOM fluorescence along with bacterial abundance.

2.2. *O. dioica* FDOM excretion experiments

Laboratory experiments were conducted with cultured larvaceans, *O. dioica*, at the University of Oviedo

in northern Spain. Excretion experiments were conducted to examine *O. dioica* inputs to FDOM. Large (average trunk length=713 μm) and medium (average trunk length=350 μm) sized *O. dioica* were preconditioned for 4 h in 0.2 μm photooxidized seawater with added *Tetraselmis suecica* or *Chlorella* sp. at a cell concentration of 100 $\mu\text{g C l}^{-1}$. The animals were then rinsed twice by transferring the oikopleurids within their house using a calibrated wide bore pipette into two, sequential beakers filled with 0.2- μm filtered seawater. Fifty large or eighty medium-sized *O. dioica* were added to 3100 ml acid washed, amber glass bottles. The treatment and control bottles were filled with the 0.2 μm filtered seawater and an equal volume (10 ml) of the final rinse water. The bottles were rotated on a plankton wheel, in the dark for 1 h at 15 °C in a temperature-controlled room. At the end of 1 h, the larvaceans were still in their houses and pumping water, indicating the filtered seawater had not unduly stressed them. Samples for DOM fluorescence were taken along with samples for bacterial abundance.

In one larvacean excretion experiment, samples were taken to examine the molecular weight distribution of the dissolved fraction. The 0.2- μm filtered water 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 due to the tubes. A total of 15 ml of sample water was spun for 15 min at 4000 $\times g$ at 15 °C on a Jouan MR1812 Centrifuge with a fixed rotor. The retention efficiency (RE) of the tubes was determined by using fluorescent stained dextrans from molecular probes with reported molecular weights of 5 and 30 kDa. The RE of the 30 kDa tubes was 97% and for the 5 kDa tubes RE was 88%.

2.3. Water analysis

Samples for DOM fluorescence were collected by filtering 70–200 ml of water through a 0.2 μm Nucleopore filter. The filtrate was stored frozen in amber vials for analysis in the laboratory. Excitation/emission matrices (EEMs) were measured on a SPEX Fluoromax-3 (Urban-Rich et al., 2004). Briefly, excitation scans from 250 to 550 nm at 5 nm intervals and emission 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 was normalized to the water Raman Peak at ex/em=275/303 nm and converted into normalized quinine sulfate units (QSU) using a correction curve generated with a quinine sulfate standard in 0.05 M sulfuric acid (Coble et al., 1993; Hoge et al., 1993). The EEMs were corrected for Raman and Rayleigh scatter peaks with MATLAB, using an algorithm that was developed by Zepp et al. (2004), to excise the scatter peaks and replace them with values developed using a three dimensional interpolation. The net input of FDOM was calculated for each experiment by:

$$\text{Net input} = (\text{Treatment}_{\text{EEM}} - \text{Initial}_{\text{EEM}}) - (\text{Control}_{\text{EEM}} - \text{Initial}_{\text{EEM}}).$$

Five to twenty five milliliters of water from each sample and control bottle before and after the incubation were preserved with 0.1% glutaraldehyde for bacteria counts (Table 1). 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 they were counted in the laboratory with epifluorescent microscopy.

Table 1
Bacterial abundance ($\times 10^2$ cells $\text{ml}^{-1} \pm \text{S.E.}$) in the copepod and larvacean excretion experiments

Experiment	Initial	Treatment	Control
Copepod experiment: within the Mississippi River Plume, 5 animals/jar	6.4 \pm 1.8 <i>n</i> =2	7.2 \pm 1.2 <i>n</i> =3	7.1 \pm 0.5 <i>n</i> =2
Copepod experiment: outside of the Mississippi River Plume, 5 animals/jar	2.3 \pm 0.3 <i>n</i> =2	3.0 \pm 0.3 <i>n</i> =3	2.9 \pm 0.2 <i>n</i> =2
<i>O. dioica</i> : large (<i>Chlorella</i> sp.), 50 animals/jar	16.1 \pm 5.1 <i>n</i> =3	11.1 \pm 4.0 <i>n</i> =3	18.3 \pm 2.2 <i>n</i> =3
<i>O. dioica</i> : large (<i>Tetraselmis suecica</i>), 50 animals/jar	12.3 \pm 2.1 <i>n</i> =3	6.0 \pm 1.3* <i>n</i> =3	17.4 \pm 2.5 <i>n</i> =3
<i>O. dioica</i> : medium (<i>Tetraselmis suecica</i>), 80 animals/jar	13.6 \pm 1.7 <i>n</i> =3	4.2 \pm 2.0* <i>n</i> =3	21.1 \pm 7.1 <i>n</i> =3

* Significantly different from initial concentrations (*t*-test, *p*<0.01).

3. Results

3.1. Fluorescence characteristics of copepod excreted DOM

Excretion experiments were conducted both within and outside of the Mississippi River Plume in April 2001. When the copepods were transferred into the treatment bottles or when rinse water was added to the control bottles, bacteria were unavoidably added as well. However, in both excretion experiments there were no significant changes in the bacterial numbers in the treatment or control bottles in the 12 h incubation ($p=0.74$) thus the changes in DOM fluorescence are believed to be due to the copepods not to bacterial activity (Table 1).

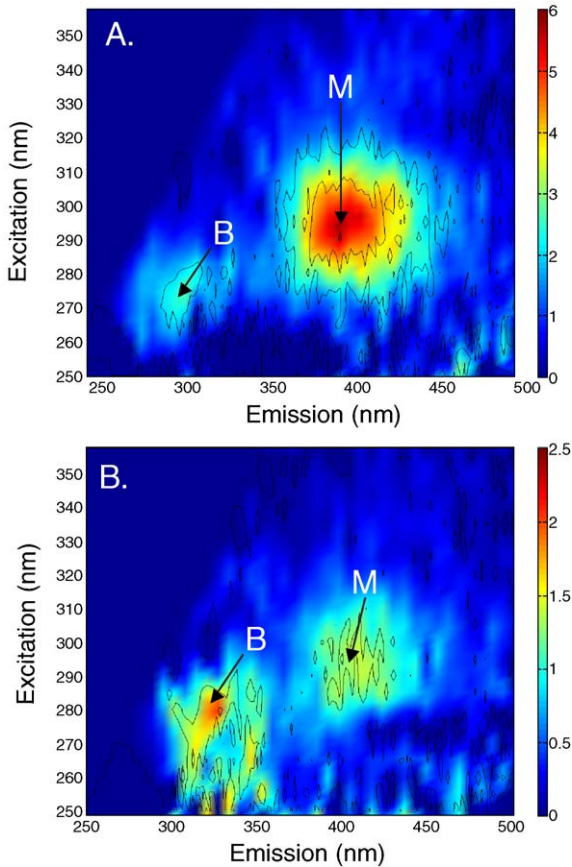


Fig. 1. Net input of FDOM during copepod excretion experiments in the Gulf of Mexico in April 2001. Fluorescence converted to normalized quinine sulfate units. (A) Net input results from experiment conducted within the Mississippi River Plume. (B) Net input results from experiment conducted outside of the Mississippi River Plume. M points to the humic-like material (ex/em 295/405 nm), B points to the fluorescent protein-like material (ex/em 275–280/315 nm).

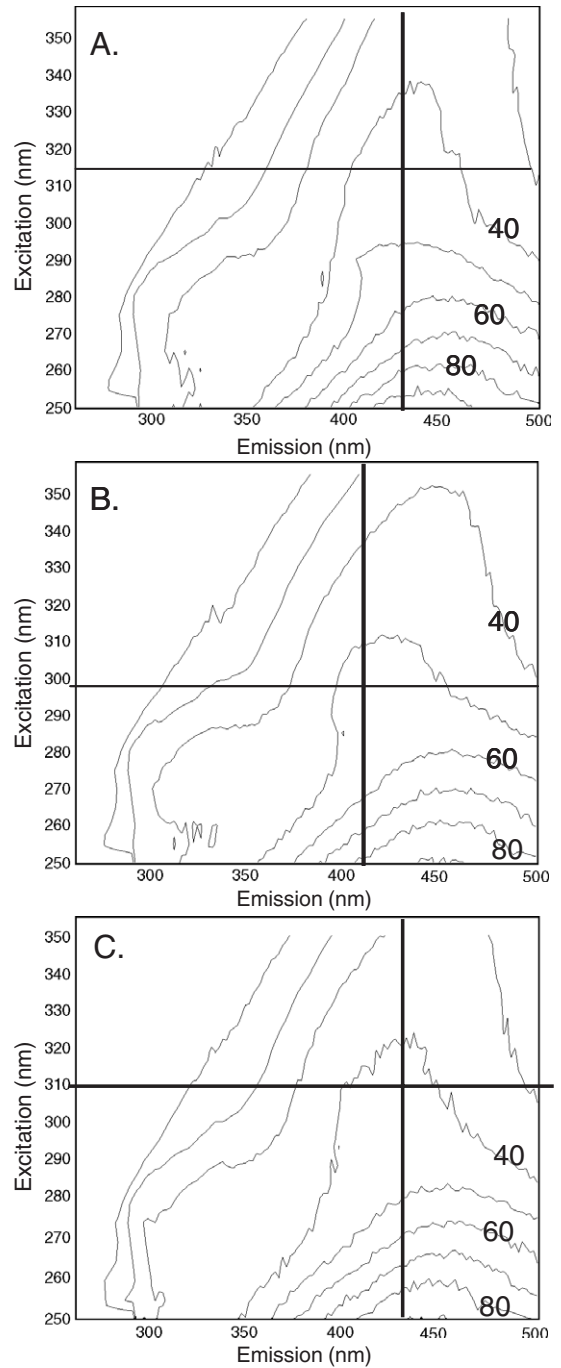


Fig. 2. Fluorescent excitation/emission matrices from the copepod excretion experiment within the Mississippi River Plume in the Gulf of Mexico in April 2001, fluorescence in NQSU. (A) Initial 0.2 μm filtered seawater. (B) Treatment water after 6 h incubation. (C) Control water after 6 h incubation. The solid lines show the location of the excitation/emission maximum. There was a shift in the excitation/emission maximum of the humic-like material to lower wavelengths in the treatment samples compared to the initial and control bottles.

Excitation/emission matrices (EEMs) can give information on the source and general types of FDOM present. EEMs from the excretion experiments showed a net input of both fluorescent protein-like (ex/em 275/320 nm) and humic-like materials (ex/em 295/420 nm; Fig. 1). The net input reflects the increase in material in the treatment bottles relative to the initial and control bottles. However it does not necessarily equal the total amount of fluorescent CDOM released by the copepods, rather it reflects the interactions between input and turnover. The net input of humic-like material in both excretion experiments had an excitation maximum from 290 to 305 nm, this excitation is at or below the lower end of the excitation range for the marine humic-like material found in Peak M (excitation maximum 320 nm, Coble, 1996). The net input of this humic-like material by the copepods resulted in a blue

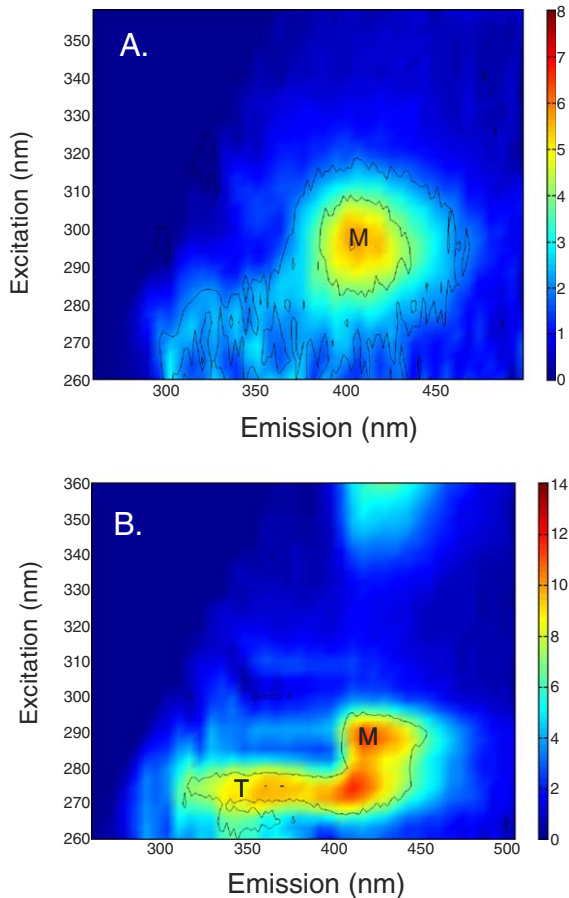


Fig. 3. Net inputs of humic-like material (M ex/em 295/410 nm) and protein-like material (T ex/em 275/340 nm) in the *O. dioica* excretion experiments. *O. dioica* had fed on *T. suecica* prior to the start of the experiment. Fluorescence reported in NQSU. (A) 50 large (700 μ m) oikopleurids were incubated for 1 h. (B) 80 medium (350 μ m) sized animals were incubated for 1 h.

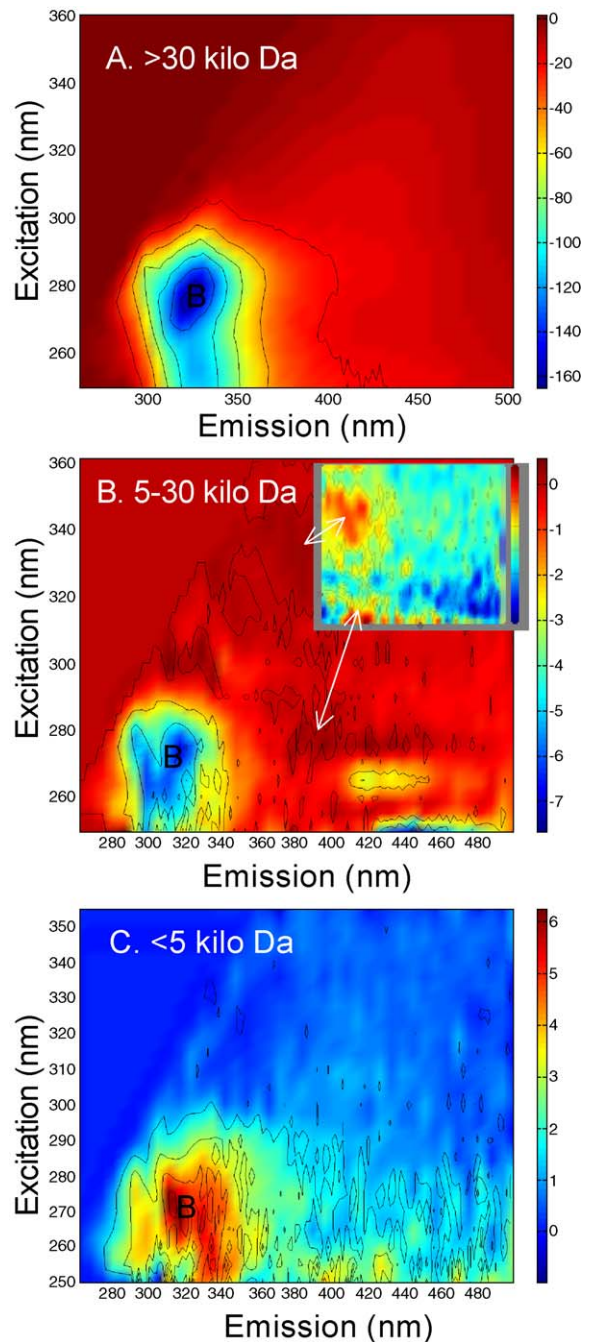


Fig. 4. Net inputs for three molecular weight size fractions of water collected from *O. dioica* excretion experiment. Water less than 0.2 μ m was size fractionated using Centricon Plus 20C molecular weight centrifuge tubes. All samples were corrected with Milli-Q blanks processed through the tubes. (A) >30 kDa fraction, there was a net loss of fluorescent protein-like material (B) within this size fraction. (B) 5–30 kDa fraction, there was a net loss of protein-like fluorescent material (B) but a net input of humic-like material. Insert shows the net input of fluorescent humic-like material (ex/em 280/390 nm and 330/380 nm). The scale on the insert graph goes to 0.5 NQSU. The arrows show where the insert connects to the larger figure. (C) <5 kDa fraction, there was a net input of protein-like fluorescence (B).

shift in the shape and excitation/emission maximum of the Peak M, humic-like material in the treatment bottles compared to initial and control bottles (Fig. 2). The net input of humic-like material in the treatment bottles resulted in an increase of marine (Peak M)/terrestrial (Peak C) fluorescent humic material of 48% outside of the plume and 16% within the plume.

3.2. Fluorescence characteristics of *O. dioica* excreted DOM

Excretion experiments conducted with the pelagic larvacean, *O. dioica* revealed that these animals are a source of FDOM (Fig. 3). The fluorescence to absorption ratio at 355 nm (ex/em 355/450nm: 355nm) increased in the treatment bottles by 14% and 86% in the medium (trunk length of 350 μm) and large (trunk length of 713 μm) *O. dioica* experiments. These results indicate that the oikopleura were excreting FDOM material.

The net input of FDOM appears to be primarily humic-like material with an excitation/emission maximum of 290/400–420 nm. This material is released by both medium and large oikopleurids (Fig. 3). There is a small net input of fluorescent protein-like material. Diet appears to affect the amount and type of FDOM excreted by the larvaceans. When the animals had guts filled with the prasinophyte, *T. suecica*, they excreted fluorescent humic-like material (Fig. 3) however when they had guts filled with the chlorophyte, *Chlorella* sp., only a small amount of colored material (ex/em 275/390 nm) accumulated in the bottles and there was a net loss of fluorescent protein-like material. Bacterial numbers during the excretion experiments decreased in the treatment bottles compared to the initial and control bottles (Table 1); indicating that the oikopleurids were feeding on the bacteria and thus the net inputs are believed to reflect excreted FDOM from *O. dioica*.

An examination of the molecular weight distribution of the FDOM revealed a net input of protein-like material (ex/em 275/320 nm) in the <5 kDa fraction and a net input of humic-like material in the 5–30 kDa fraction and a net loss of all colored material, especially protein in the >30 kDa fraction (Fig. 4).

4. Discussion

4.1. Copepods and larvaceans as a source of FDOM

Very little is known about in situ, biological sources of CDOM and FDOM. Both phytoplankton and bacteria have been proposed as possible sources of

CDOM. However many studies have failed to find strong correlations between CDOM and chlorophyll *a* (DeGrandpre et al., 1996; Hoge et al., 1998; Rochelle-Newall et al., 1999; Rochelle-Newall and Fisher, 2002a) suggesting that phytoplankton are not a direct source of CDOM. In a recent laboratory experiment with phytoplankton cultures, Rochelle-Newall and Fisher (2002b) found little evidence for the production of CDOM by phytoplankton. Instead they suggested that bacteria converted the non-colored dissolved material released by the phytoplankton into CDOM. Previous studies have also suggested that bacteria may be a source of CDOM (Hayase et al., 1988; Hayase and Shinozuka, 1995; Tranvik, 1993; Nelson et al., 1998). Other in situ biological sources of CDOM and FDOM could be zooplankton, viruses, flagellates, or seagrasses. Recent work by Steinberg et al. (2004) in the Sargasso Sea shows that zooplankton, protozoa, polychaete worms and cyanobacteria can be sources of CDOM. Results from these studies indicate that copepods and the pelagic larvacean, *O. dioica* are also sources of FDOM (Figs. 1 and 3).

The connections between zooplankton and dissolved organic material are just beginning to be unraveled. Recent studies with grazing copepods have found that 14–37% of the ingested carbon could be lost to DOC prior to ingestion (Strom et al., 1997) and 50% of fecal pellet carbon can be lost to DOC (Urban-Rich, 1999) suggesting these animals can play a vital role in the DOC cycle. In addition, Steinberg et al. (2000, 2002) found vertically migrating zooplankton can contribute 0–39% of carbon flux in the North Atlantic at the Bermuda Atlantic Time-series Study station through excretion of DOC. Thus copepods have the potential to influence microbial activity through the input of DOC, the flux of carbon through the active production of DOM by migratory zooplankton and the passive sinking of POC and DOM in fecal pellets, and the optical characteristics of the water through the release of CDOM. In contrast to copepods, little is known about the excretion of organic material by larvaceans. Previous work has found that *O. dioica* is capable of filtering and ingesting submicron particles down to 0.2 μm (Flood et al., 1992; Bedo et al., 1993). These are the first results indicating that *O. dioica* is a source of DOM. Results from this study indicate that fluorescent protein-like and humic-like material is excreted by copepods and *O. dioica*.

4.2. Consequences of zooplankton excretion of FDOM

Dissolved organic carbon (DOC) in the ocean is the largest reservoir of aquatic organic carbon and thus

plays a critical role in the global carbon budget. CDOM and FDOM is a significant and variable fraction of the DOC that has the unique properties of influencing the optical characteristics of the water column and the chemical speciation and transport of trace metals through complexation reactions (Blough and Del Vecchio, 2002; Nelson and Seigel, 2002). Thus the finding that zooplankton are a direct source of FDOM means these animals can potentially influence carbon cycling, optical characteristics of the water column and the transport and fate of trace metals. Recent work conducted in the Sargasso Sea has found that vertically migrating zooplankton also excrete CDOM, thus zooplankton also have the potential to enhance the vertical flux of CDOM (Steinberg et al., 2004).

Copepods can contribute to DOM through several avenues, sloppy feeding, excretion and fecal pellet dissolution. Results from these studies show that copepods excrete FDOM. More work is needed to determine the turnover and potential ecological role of copepod and larvacean produced FDOM. However, it is clear that zooplankton are a source of CDOM and FDOM (Figs. 1–5, Steinberg et al., 2004). Zooplankton can influence the chemical composition of CDOM pools by producing material that changes the fluorescent properties of the water (Figs. 1–5).

Excitation/emission matrices have been used to differentiate the source of DOM and to trace water masses (Del Castillo et al., 1999, 2000; McKnight et al., 2001). Fluorescence is a useful tool as it is more sensitive than absorption and can be used to measure inputs from various sources (Chen and Bada, 1992, 1994). Net inputs of FDOM were observed in both the copepod and larvacean excretion experiments. The finding in the *O. dioica* experiment that the excreted fluorescent compounds were of low to mid molecular weight (<5 or 5–30 kDa) agrees with previous findings that have characterized marine fulvic and humic acids as being primarily of low molecular weight (Nissenbaum and Kaplan, 1972; Malcolm, 1990; Hedges, 1992). Nothing is currently known about the chemical composition, molecular size or aromatic content of the copepod produced FDOM, however the similarity of the excitation/emission maximum for the humic-like material in the copepod and larvacean experiments suggests they are of similar aromatic content.

Marine fluorescent material and microbial derived humic-like material generally have a lower excitation/emission maximum (Peak M) in comparison to terrestrial derived material (Peak C; Coble et al., 1990,

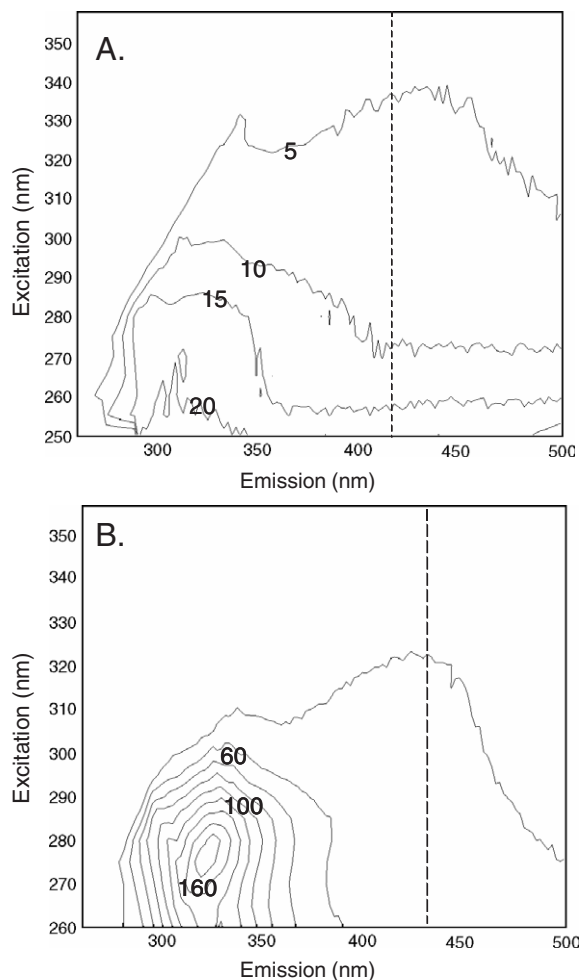


Fig. 5. Excitation/emission matrices of the >30 kDa fraction collected from an *O. dioica* excretion experiment. There was a net loss of humic-like material in this fraction due to the large input in the controls, however there was an input of humic-like material in the treatment bottles that had a lower wavelength emission maximum. (A) Treatment bottles, emission maximum 420 nm. (B) Control bottles, emission maximum 440 nm.

1998). The interesting finding in these excretion experiments was the humic-like material that accumulated in both the copepod and larvacean experiments had excitation/emission maximums (290–305/390–430 nm) at the lower range of reported Peak M material (Zepp et al., 2004; Coble, 1996) similar to that found during grazing studies with copepods (Urban-Rich et al., 2004). The shift to lower wavelength humic-like material may reflect a unique zooplankton signal. This is most clearly seen in the *O. dioica* excretion experiment with molecular weight analysis. In the >30 kDa fraction, the control bottles had an increase in humic-like material relative to the initial bottle with an excitation/emission maximum of 315/420 nm while the treatment

Table 2

Percent composition of the marine humic-like (Peak M) material that is due to excreting zooplankton

Experiment	Initial, mean \pm S.E.	Treatment, mean \pm S.E.	Control, mean \pm S.E.
Copepod experiment: within the Mississippi River Plume, 5 animals/jar	18 \pm 2 <i>n</i> = 2	27 \pm 2* <i>n</i> = 3	16 \pm 1 <i>n</i> = 2
Copepod experiment: outside of the Mississippi River Plume, 5 animals/jar	19 \pm 1 <i>n</i> = 2	28 \pm 3* <i>n</i> = 3	19 \pm 2 <i>n</i> = 2
<i>O. dioica</i> : large (<i>Chlorella</i> sp.), 50 animals/jar	20 \pm 2 <i>n</i> = 3	26 \pm 2 <i>n</i> = 3	21 \pm 2 <i>n</i> = 3
<i>O. dioica</i> : large (<i>Tetraselmis suecica</i>), 50 animals/jar	19 \pm 2 <i>n</i> = 3	38 \pm 3* <i>n</i> = 3	20 \pm 2 <i>n</i> = 3
<i>O. dioica</i> : medium (<i>Tetraselmis suecica</i>), 80 animals/jar	20 \pm 3 <i>n</i> = 3	49 \pm 4* <i>n</i> = 3	21 \pm 2 <i>n</i> = 3

This was determined using a ratio of the zooplankton excreted humics (ex/em 290–300/420 nm) to bacterial produced or background marine humics (ex/em 315–320/420 nm). %Comp = Zoop_{290–300/420}/Bact_{315–320/420} * 100.

* Significant changes from initial and control water ($p < 0.001$).

bottles had an increase of humic-like material relative to the initial bottles with an excitation/emission maximum of 300/400 nm (Fig. 5). While there was no net input of humics in this molecular size class, the shape and maximum of the Peak M humic-like material was different between the treatments and controls. The control bottles had an increase in bacterial numbers throughout the incubation, suggesting they may have been the source of the humic-like material in the control bottles while the bacterial numbers decreased in the treatment bottles, presumably due to grazing by *O. dioica*.

Calculating a ratio of bacterial-derived marine humics (ex/em 315–320/420 nm): zooplankton-derived marine humics (ex/em 290–300/420 nm) for every excretion experiment gave an average value of 0.80 for the controls while the treatment bottles ranged from 0.51 to 0.84 (Table 2), thus zooplankton contributed 16–49% of the humic-like material in the Peak M region. In every experiment, except the *O. dioica* fed *Chlorella* sp., the treatment bottles had significantly more zooplankton-derived humics present (Table 2). It may be possible to use this relationship to determine the relative importance of zooplankton and bacteria as sources of marine humics.

In summary, zooplankton can influence the optical characteristics of coastal waters through the material they excrete. The type of humic-like material that is excreted and that accumulates is blue-shifted compared to the traditional Peak M excitation/emission maximum and to the humic-like material in the control bottles. Zooplankton can affect the type of ultraviolet radiation absorbed in the water. On average it appears that zooplankton derived marine fluorescent humic-like material comprises 20% of the Peak M pool, however it could contribute up to 50% in treatment bottles which suggests that in patches or during high zooplankton abun-

dance, zooplankton could be a dominant source of marine humic-like material.

Acknowledgements

We would like to thank Dr. Bob Chen for allowing us to participate in the Mississippi River Cruise. We would like to thank Mark Shailer, Rachel Ruppel and Prassede Vella for their help in the laboratory with sample and data analysis. This work was supported by an Office of Naval Research Grant (ONR-N00014-01-1-0247). [SS]

References

- Acuna, J.L., 1994. Summer vertical distribution of appendicularians in the central Cantabrian Sea (Bay of Biscay). J. Mar. Biol. Assoc. U.K. 74, 585–601.
- Bedo, A.W., Acuña, J.-L., Robins, D., Harris, R.P., 1993. Grazing in the micronic and sub-micronic particle size range: the case of *Oikopleura dioica* (Appendicularia). Bull. Mar. Sci. 53, 2–14.
- Blough, N.V., Del Vecchio, R., 2002. Chapter 10: chromophoric DOM in the coastal environment. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 509–546.
- Buck, K.R., Newton, J., 1995. Fecal pellet flux in Dabob Bay during a diatom bloom: contribution of microzooplankton. Limnol. Oceanogr. 40, 306–315.
- Carder, K.L., Steward, R.G., Harvey, G.R., Ortner, P.B., 1989. Marine humic and fulvic acids: their effects on remote sensing of ocean chlorophyll. Limnol. Oceanogr. 34, 68–81.
- Chen, R.F., Bada, J.L., 1992. The fluorescence of dissolved organic matter in seawater. Mar. Chem. 37, 191–221.
- Chen, R.F., Bada, J.L., 1994. The fluorescence of dissolved organic matter in porewaters of marine sediments. Mar. Chem. 45, 31–42.
- Chen, R.F., Bada, J.L., Suzuki, Y., 1993. The relationship between dissolved organic carbon (DOC) and fluorescence in anoxic marine porewaters: implications for estimating benthic DOC fluxes. Geochim. Cosmochim. Acta 57, 2149–2153.

- Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. *Mar. Chem.* 51, 325–346.
- Coble, P.G., Green, S.A., Blough, N.V., Gagosian, R.B., 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature* 348, 432–435.
- Coble, P.G., Schultz, C.A., Mopper, K., 1993. Fluorescence contouring analysis of DOC intercalibration experiment samples: a comparison of techniques. *Mar. Chem.* 41, 173–178.
- Coble, P.G., Del Castillo, C.E., Avril, B., 1998. Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon. *Deep-Sea Res. II* 45, 2195–2223.
- Copping, A.E., Lorenzen, C.J., 1980. Carbon budget of a marine phytoplankton–herbivore system with carbon-14 as a tracer. *Limnol. Oceanogr.* 25, 873–882.
- Dagg, M.J., 1995. Copepod grazing and the fate of phytoplankton in the northern Gulf of Mexico. *Cont. Shelf Res.* 15, 1303–1317.
- Dagg, M.J., Green, E.P., McKee, B.A., Ortner, P.B., 1996. Biological removal of fine-grained lithogenic particles from a large river plume. *J. Mar. Res.* 54, 149–160.
- DeGrandpre, M.D., Vodacek, A., Nelson, R.K., Bruce, E.J., Blough, N.V., 1996. Seasonal seawater optical properties of the U.S. Middle Atlantic Bight. *J. Geophys. Res.* 101, 22727–22736.
- Deibel, D., Powell, C.B.L., 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.
- Del Castillo, C.E., Coble, P.E., Morell, J.M., Lopez, J.M., Corredor, J.E., 1999. Analysis of the optical properties of the Orinoco River plume by absorption and fluorescence spectroscopy. *Mar. Chem.* 66, 35–51.
- Del Castillo, C.E., Gilbes, F., Coble, P.G., Muller-Karger, F.E., 2000. On the dispersal of riverine colored dissolved organic matter over the West Florida Shelf. *Limnol. Oceanogr.* 45, 1425–1432.
- Esnal, G.B., Sankarankutty, C., Castrol, R.J., 1985. Diurnal and seasonal fluctuations of *Oikopleura dioica* in the mouth of the River Patengi (North Brazil). *Physic-A* 43, 65–71.
- Flood, P.R., 1991. Architecture of, and water circulation and flow rate in the house of the planktonic tunicate *Oikopleura labradoriensis*. *Mar. Biol.* 111, 95–111.
- Flood, P.R., Deibel, D., Morris, C., 1992. Filtration of colloidal melanin from seawater by planktonic tunicates. *Nature* 355, 630–632.
- Gorsky, G., Dallot, S., Sardou, J., Fenaux, R., Carre, C., Palazzoli, I., 1988. C and N composition of some Mediterranean zooplankton and micronekton species. *J. Exp. Mar. Biol. Ecol.* 124, 133–144.
- Hayase, K., Shinozuka, N., 1995. Vertical distribution of fluorescent organic matter along with AOU and nutrients in the equatorial Pacific. *Mar. Chem.* 48, 283–290.
- Hayase, K., Tsubota, H., Sunada, I., Goda, S., Yamazaki, H., 1988. Vertical distribution of fluorescent organic matter in the North Pacific. *Mar. Chem.* 25, 373–381.
- Hedges, J., 1992. Global biochemical cycles: progress and problems. *Mar. Chem.* 39, 67–93.
- Hobbie, J.D., Daley, R.J., Jasper, S., 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33, 1225–1228.
- Hoge, F.E., Vodacek, A., Blough, N.V., 1993. Inherent optical properties of the ocean: retrieval of the absorption coefficient of chromophoric dissolved organic matter from fluorescence measurements. *Limnol. Oceanogr.* 38, 1394–1402.
- Hoge, F.E., Wright, C.W., Swift, R.N., Uungel, J.K., Berry, R.E., Mitchell, R., 1998. Fluorescence signatures of an iron enriched phytoplankton community in the eastern equatorial Pacific Ocean. *Deep-Sea Res., Part 2, Top. Stud. Oceanogr.* 45, 1082–1083.
- Kitalong, A.E., 1986. A preliminary study of emergence patterns of microfauna in Kaneohe Bay, Hawaii. In: Jokiel, P.L., Richmond, R.H., Rogers, R.A. (Eds.), *Coral Reef Population Biology*. Univ. of Hawaii, pp. 414–423.
- Lazarus, B.I., Dowler, D., 1979. Pelagic Tunicata of the west and southwest coasts of South Africa, 1964–65. *Fish. Bull. Div. Sea Fish. S. Afr.*, 93–119.
- Malcolm, R., 1990. The uniqueness of humic substances in each of soil, stream, and marine environments. *Anal. Chim. Acta* 232, 19–30.
- Mauchlin, J., 1998. In: Blaxter, J.H.S., Southward, A.J., Tyler, P.A. (Eds.), *Advances in Marine Biology, The Biology of Calanoid Copepods*. Academic Press, New York.
- McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T., Andersen, D.T., 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* 46, 38–48.
- Nakamura, Y., 1998. Blooms of tunicates *Oikopleura* spp. and *Doliolletta gegenvauri* in the Seto Inland Sea, Japan, during summer.
- Nelson, N.B., Seigel, D.A., 2002. Chromophoric DOM in the open ocean. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 547–578.
- Nelson, N.B., Seigel, D.A., Michaels, A.F., 1998. Seasonal dynamics of colored dissolved organic material in the Sargasso Sea. *Deep-Sea Res., Part 1, Oceanogr. Res. Pap.* 45, 931–957.
- Nissenbaum, A., Kaplan, I.R., 1972. Chemical and isotopic evidence for the in situ origin of marine humic substances. *Limnol. Oceanogr.* 17, 570–582.
- Obermosterer, I., Herndl, G.H., 2000. Differences in the optical and biological reactivity of the humic and nonhumic dissolved organic carbon component in two contrasting coastal marine environments. *Limnol. Oceanogr.* 45, 1120–1129.
- Rochelle-Newall, E.J., Fisher, T.R., 2000a. Chromophoric dissolved organic matter and dissolved organic carbon in Chesapeake Bay. *Mar. Chem.* 77, 23–41.
- Rochelle-Newall, E.J., Fisher, T.R., 2002b. Production of chromophoric dissolved organic matter fluorescence in marine and estuarine environments: an investigation into the role of phytoplankton. *Mar. Chem.* 77, 7–21.
- Rochelle-Newall, E.J., Fisher, T.R., Fan, C., Glibert, P.M., 1999. Dynamics of chromophoric dissolved organic matter and dissolved organic carbon in experimental mesocosms. *Int. J. Remote Sens.* 20, 627–641.
- Roman, M.R., et al., 1988. Production, consumption and nutrient cycling in a laboratory mesocosm. *Mar. Ecol., Prog. Ser.* 42, 39–52.
- Roff, J.C., Middlebrook, K., Evans, F., 1988. Long-term variability in North Sea zooplankton off the Northumberland coast: productivity of small copepods and analysis of trophic interactions. *J. Mar. Biol. Assoc. U.K.* 68, 143–164.
- Skoog, A., Hall, P.O.J., Hulth, S., Paxeus, N., Van Der Loeff, M.R., Westerlund, S., 1996. Early diagenetic production and sediment–water exchange of fluorescent dissolved organic matter in a coastal environment. *Geochim. Cosmochim. Acta* 60, 3619–3629.
- Steinberg, D.K., Carlson, C.A., Bates, N.R., Goldthwait, S.A., Madin, L.P., Michaels, A.F., 2000. Zooplankton vertical migration and the

- active transport of dissolved organic and inorganic carbon in the Sargasso Sea. *Deep-Sea Res. I* 47, 137–158.
- Steinberg, D.K., Godthwait, S.A., Hansell, D.A., 2002. Zooplankton vertical migration and the active transport of dissolved organic and inorganic nitrogen in the Sargasso Sea. *Deep Sea Res.* 49, 1445–1461.
- Steinberg, D.K., Nelson, N.B., Carlson, C.A., 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, S.L., Benner, R., Dagg, M.J., 1997. Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol. Oceanogr.* 42, 1364–1374.
- Tranvik, L.J., 1993. Microbial transformations of labile organic matter into humic-like matter in seawater. *Microb. Ecol.* 12, 177–183.
- Tranvik, L.J., Kokalj, S., 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. *Aquat. Microb. Ecol.* 14, 301–307.
- Urban-Rich, J., 1999. Release of dissolved organic carbon from copepod fecal pellets in the Greenland Sea. *J. Exp. Mar. Biol. Ecol.* 232, 107–124.
- Urban-Rich, J., McCarty, J.T., Shailer, M., 2004. Effects of food concentration and diet on CDOM accumulation and fluorescent composition during grazing experiments with the copepod, *Calanus finmarchicus*. *ICES J. Mar. Sci.* 61, 542–551.
- Zepp, R., Sheldon, W.M., Moran, M.A., 2004. Dissolved organic fluorophores in southeastern US coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation–emission matrices. *Mar. Chem.* 89, 15–36.