SHORT NOTE

Production of dissolved organic carbon by *Oithona nana* (Copepoda: Cyclopoida) grazing on two species of dinoflagellates

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Abstract

Production of dissolved organic carbon (DOC) by sloppy feeding copepods may represent an important source of DOC in marine foodwebs. By using the $^{14}$C labeling technique, we quantify for the first time the production of DOC by the small cyclopoid copepod *Oithona nana* on two species of dinoflagellates; *Oxyrrhis marina* and *Karlodinium* sp.. We found significant production of DOC when *O. nana* grazed on *O. marina*, corresponding to 6-15 % of the carbon ingested. When grazing the smaller *Karlodinium* sp., no DOC was produced. In additional experiments we compared *O. nana* feeding rates on the dinoflagellate species *Prorocentrum micans*, *Akashiwo sanguinea*, *Karlodinium* sp. and *O. marina*. Clearance rates varied with prey size, with highest and lowest clearance rates on *O. marina* and *Karlodinium* sp., respectively. Our study indicates that even though *O. nana* feed efficiently on dinoflagellates, some of the carbon cleared can be lost as DOC. However, the DOC production by *O. nana* was lower than rates reported for calanoid copepods. We hypothesize that this is a result of the ambush feeding behavior of *O. nana*, which is considered a more specialized feeding mode than for instance suspension feeding. Due to high abundances and global distribution, we suggest that *Oithona* can represent an important source of DOC in marine ecosystems. This would particularly be the case during autumn and winter, where they may contribute to maintaining the microbial loop activities during periods of low primary production.

Introduction

Dissolved organic carbon (DOC) is an important source of energy in the ocean, and one of the largest actively cycling reservoirs of organic carbon on earth (Kirchman et al. 1991). Phytoplankton are considered the main source of DOC in the ocean, providing a substrate for heterotrophic bacteria (Azam et al. 1983). However, only about half of the carbon requirements of bacteria can be met directly from extracellular release from phytoplankton (Baines and Pace 1991), indicating that other sources of DOC in the ocean must be important for the microbial food web as well. Production of labile DOC as a byproduct by animal feeding was first proposed as a potentially important source in the 1970’s (Lampert 1978; Eppley et al. 1981), and
more recent studies have confirmed this relationship. Production of DOC by sloppy feeding copepods has been quantified for selected calanoid copepod species including *Acartia tonsa* (Møller 2007; Saba et al. 2009; Saba et al. 2011), *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* (Møller et al. 2003), *Centropages typicus* and *Temora longicornis* (Møller 2007). Common for these calanoid copepods are the suspension feeding modes, in contrast to the ambush feeding mode (Paffenbøger et al. 1982; Kiørboe 2011b). For example, a suspension feeding behavior, or the capability of switching between a suspension feeding and an ambush feeding mode has been described for multiple calanoid copepod species (Kiørboe et al. 1996; Saage et al. 2009; Kiørboe 2011a). For copepods with a strict ambush feeding behavior, such as the cyclopoid copepod *Oithona* spp. (Svensen and Kiørboe 2000), DOC production from sloppy feeding has not been quantified. However, based on determination of *O. davisae* mouth opening (~ 10 µm x 20 µm) compared to prey size, Saiz et al. (2014) suggested that DOC production by sloppy feeding could explain high ingestion rates.

*Oithona* spp. is one of the most numerous copepods worldwide (Gallienne and Robins 2001), although their role in the carbon-cycle is not yet fully understood. A tight coupling to the microbial foodweb has been suggested to contribute to their successful strategy (Svensen et al. 2011), but the nature of these links remain unclear. In this study we investigated feeding rates of *O. nana* on four differently sized dinoflagellate species, and hypothesize that DOC is a by-product of feeding also for an ambush feeding copepod. We test this hypothesis by a direct measure of the production of DOC from *O. nana* feeding on two species of dinoflagellates by using the 14C labeling technique. Our results are relevant for 1) providing new knowledge on potential sources of DOC in the marine ecosystem and 2) increase the understanding of the link between *Oithona* and the microbial foodweb.

Materials and methods

Collection of *Oithona*

Copepods were collected from Scripps Pier, or with a boat from a nearby locality in the California Current. A plankton net (General Oceanics) with 120 µm mesh and a
non-filtering cod-end was used. The content of the cod-end was gently poured into a larger container filled with surface water and immediately brought to a temperature-controlled room. The animals were kept in 10 L containers at 17-18 °C and with light aeration. Female *Oithona nana* were sorted out for the experiments within 1-2 days after collection. The copepods used for experiments were acclimatized to the experimental food type and concentration for approximately 24 h before each experiment. Copepods were collected prior to each experiment to ensure availability of fresh *O. nana*. Prosome length was measured for 50 females, and ash free dry weight was calculated from a length-weight regression for *O. nana* (Hopcroft et al. 1998) and converted to carbon assuming a 48 % carbon content (Kiørboe 2013).

**Dinoflagellate cultures**

Dinoflagellates were used as prey for *Oithona nana* in four grazing experiments and five DOC production experiments. Cultures of dinoflagellates were grown in a modified f/2 medium prepared in filtered, autoclaved seawater (FSW) according to recipe of Guillard and Ryther (1962) but without adding Silicate. Four species were grown: the autotrophic *Prorocentrum micans* (CCMP694) and *Akashiwo sanguinea* (CCMP3265), the mixotrophic *Karlodinium* sp. (unknown strain) and the heterotrophic *Oxyrrhis marina* (CCMP1739). The autotrophs and mixotroph were grown in a 12 h light:dark cycle at 18 °C, while the heterotrophic *O. marina* was kept in the dark. *O. marina* was fed daily with the small flagellate *Dunaliella tertiolecta* (CCMP1320), except the day before the grazing experiments. *D. tertiolecta* was grown under the same conditions as the autotrophic dinoflagellates. All species were sized by measuring length and width of 30 random cells in a light microscope (Olympus AX70) at 20x magnification. Sizes were then converted to carbon according to the carbon to volume relationship for protist plankton given by Menden-Deuer and Lessard (2000).

To obtain autotrophic dinoflagellates labeled with ^14^C, cultures were incubated for 10-14 days with NaH^14^CO_3 at 300-500 µCi L\(^{-1}\). It should be mentioned that *P. micans* and *A. sanguinea* grew poorly when incubated with ^14^C, and therefore these species could not be used for DOC production experiments. The heterotrophic species *O. marina* was labeled with ^14^C by feeding it with ^14^C labeled *Dunaliella tertiolecta*. 
The *D. tertiolecta* had been incubated with NaH$^{14}$CO$_3$ at 600 µCi L$^{-1}$ for 4-5 days to allow 4-5 doublings. In order to reduce the amount of excess $^{14}$C in the *D. tertiolecta* culture prior to feeding, 20 mL of the culture was reduced to 2-3 mL concentrated culture by centrifugation for 6 min at 2500 rpm. Viability of cells was visually confirmed after centrifugation. The concentrated suspension of *D. tertiolecta* was then fed to *O. marina*. This was repeated daily for 4-6 days, after which the $^{14}$C labeled *O. marina* was used as prey for *O. nana* in the DOC production experiments. In order to make sure that *D. tertiolecta* were not included in the DOC production experiments, the culture of *O. marina* was not fed the day before the experiment. Visual inspection confirmed that *D. tertiolecta* were not present in the incubation water of the DOC production experiments.

**Grazing experiments**

Grazing experiments were conducted to compare the feeding rates of *Oithona nana* on four dinoflagellate species at food concentrations in the range 384-795 µg C L$^{-1}$ (Table 1). The dinoflagellates were grown as described above, and fed to the copepods when growing in an exponential phase. *O. nana* females were sorted under a dissecting microscope (Wild Heerbrugg) and were acclimatized to the prey for approximately 24 h prior to the grazing experiments. The copepods were then individually sorted and distributed to 3 beakers containing approximately 10 mL incubation water which were then gently poured into the experimental treatment bottles with total volume 172 mL. Each grazing experiment consisted of 8 bottles; 3 with copepods, 3 controls without copepods and 2 time-zero ($T_0$) bottles for the initial concentration of prey. The $T_0$ bottles were terminated at experimental start by adding 1% Lugol’s solution. The concentration of *O. nana* in the experiments was in the range 25-35 individuals bottle$^{-1}$. The bottles were sealed with parafilm to prevent air bubbles and incubated on a slowly rotating plankton wheel (1 rpm) at 17 °C and in the dark. After 24 h incubation, the content of each bottle was preserved with 1% Lugol’s solution. From each bottle 3 sub-samples à 1 mL was counted in a Sedgewick-Rafter counting chamber under a light microscope (Olympus AX70) at 20x magnification. Grazing by *O.nana* on four species of dinoflagellates was
estimated by calculating clearance rates (CR, mL female d\(^{-1}\)) and ingestion rates (I, ng C female d\(^{-1}\)) according to Frost (1972), modified by Kiørboe et al. (1982).

**DOC production by Oithona nana**

We conducted five experiments to study the production of DOC by grazing *Oithona nana* (Table 2). Due to expected low grazing rates by *O. nana*, and hence a likelihood of operating close to detection limits regarding DOC production, incubation times (20 h) were relatively long compared to previous experiments for calanoid copepods (Møller et al. 2003). Therefore, no attempt was made to distinguish between different sources of DOC related to the feeding process of *O. nana*. What is referred to as “sloppy feeding” must be regarded as the total DOC production related to *O. nana* grazing on dinoflagellates, including potential leakage from faecal pellets. Experiment I-III was performed with *O. marina* as prey, while in experiment IV and V we used *Karlodinium* sp. as prey. The \(^{14}\)C labeled culture of *O. marina* was enumerated by counting a sub-sample and then diluted with 0.2 µm-filtered seawater to obtain the desired experimental concentration. The \(^{14}\)C labeled culture of *Karlodinium* sp. was centrifuged (< 5000 rpm for 5 min) in Falcon tubes to concentrate the cells and remove access water. The cells were then transferred to a new falcon tube filled up with FSW, and centrifuged again. Cell viability after centrifugation was confirmed by microscope. Finally, the cells were transferred to a clean vial and diluted with FSW until desired experimental cell concentration. The aim was to provide the copepods with food in non-limiting concentrations, similar to the grazing experiments. The initial prey concentrations in the DOC production experiments ranged from 403 to 679 µg C L\(^{-1}\) (Table 2). 20 mL incubation water (containing the labeled dinoflagellates in 0.2 µm FSW) was filled in each of 12 vials, where half contained 7-10 female *O. nana* and the other half served as controls without copepods. Three of the control vials were used as initial (T\(_0\)) bottles and were terminated immediately, according to the procedure described below. One mL FSW was added together with the copepods to the treatment bottles, and the same volume of FSW was also added to the controls (but without the copepods). The bottles were incubated in the dark for 20 h and at 18°C. The vials were not rotated during the incubation. This may have resulted in an un-homogenous distribution of dinoflagellates in the vials, and must be
regarded as a potential source of error. However, as the experiments were conducted in 20 ml scintillation vials, it was not feasible to close the lids without capturing an air-bubble. It was therefore decided that leaving the vials static would be the gentlest treatment for the organisms. Given the high concentration of organisms in the incubation vials, we assumed that encounter rates would not be negatively affected. Visual inspection also confirmed that the copepods and dinoflagellates were relatively evenly distributed in the experimental vials during incubation. The experiment was repeated 3 times for *O. marina* and twice for *Karlodinium* sp. (Table 2).

The experiments were terminated according to the following procedure: The copepods were removed from each vial by pouring the content through a small sieve with 150 µm mesh. For Exp. I, II and III, the content of each vial was first gently filtered onto a 3 µm Millipore filter to retain the particulate organic carbon (PO\(^{14}\)C). The resulting filtrate was then filtered onto a 0.2 µm polycarbonate filter (Costar) to retain the bacteria size fraction. This was done as an attempt to estimate the relative increase of bacteria biomass during incubation. The isotopic activity (DPM’s) on the 0.2 µm filter were used as a proxy for bacteria biomass. By comparing the DPM’s in the 0.2 µm filter at experiment start (T\(_0\)) and after 20 h (in controls and treatments) we found a 38 % and 36 % increase of DPM’s in the 0.2 µm fraction in the control and treatment bottles, respectively (data not shown). This indicates that the biomass increase in the 0.2 µm fraction was comparable in the treatments and controls, and likely unaffected by sloppy feeding during our 20 h incubations. To retain PO\(^{14}\)C in Exp. IV-V, the content of each vial was filtered directly on a GF/F filter without filtering first through a 3 µm filter. From the final filtrate of all five experiments, 3 replicate sub-samples of 3 mL were sampled for DO\(^{14}\)C. The filters and the filtrate samples were placed in individual scintillation vials and 300 µL 20 % HCl was added. Samples were left for aeration for 24 h to remove inorganic \(^{14}\)C, after which 15 mL scintillation cocktail (Ultima Gold) was added. \(^{14}\)C isotopic activity was quantified using a Liquid scintillation counter (Beckman LS 6000IC). The isotopic activity of the dinoflagellate samples (DPM) was converted to carbon (µg C) by dividing the isotopic activity of the incubation water at T\(_0\) (containing a dilution of the dinoflagellate culture) with the carbon-content of the same sample (DPM µgC\(^{-1}\)).
For quantification of DO\textsuperscript{14}C production by the copepods, we followed the procedures described by Møller et al. (2003) and Møller (2007). The average PO\textsuperscript{14}C (µg C) concentrations in the control- (PO\textsuperscript{14}C\textsubscript{d-average}) and Oithona bottles (PO\textsuperscript{14}C\textsubscript{O-average}) were calculated according to Frost (1972), modified by Kiørboe et al. (1982):

\[
PO^{14}\text{C}_{\text{average}} = \frac{PO^{14}\text{C}_{\text{end}} - PO^{14}\text{C}_{\text{start}}}{\ln (PO^{14}\text{C}_{\text{end}}) - \ln (PO^{14}\text{C}_{\text{start}})}
\]

The amount of DO\textsuperscript{14}C excreted by the dinoflagellates will be a function of cell concentration. Hence, the DO\textsuperscript{14}C production rate by dinoflagellates was calculated per PO\textsuperscript{14}C per time (DO\textsuperscript{14}C\textsubscript{d}, µgC µgC\textsuperscript{-1} h\textsuperscript{-1}) from the total DO\textsuperscript{14}C production (DO\textsuperscript{14}C\textsubscript{T}, µgC mL\textsuperscript{-1} h\textsuperscript{-1}) in the control bottles as

\[
DO^{14}\text{C}_{\text{d}} = \frac{DO^{14}\text{C}_{\text{T}}}{PO^{14}\text{C}_{\text{d-average}}}
\]

Production of DO\textsuperscript{14}C by *Oithona* (DO\textsuperscript{14}C\textsubscript{O}, µgC mL\textsuperscript{-1} h\textsuperscript{-1}) was calculated based on the assumption that the DO\textsuperscript{14}C production by the dinoflagellates per biomass (DO\textsuperscript{14}C\textsubscript{d}) was similar in the control bottles and the bottles with copepods. The DO\textsuperscript{14}C production by the dinoflagellates (DO\textsuperscript{14}C\textsubscript{d}) was therefore multiplied by the average PO\textsuperscript{14}C concentration in the copepod bottle (PO\textsuperscript{14}C\textsubscript{O-average}) to find the DO\textsuperscript{14}C production per dinoflagellate biomass. The DO\textsuperscript{14}C produced by sloppy feeding *Oithona* was determined by subtracting the DO\textsuperscript{14}C produced per dinoflagellate biomass from the total DO\textsuperscript{14}C production (DO\textsuperscript{14}C\textsubscript{T}).

\[
DO^{14}\text{C}_{\text{O}} = DO^{14}\text{C}_{\text{T}} - DO^{14}\text{C}_{\text{d}} \times PO^{14}\text{C}_{\text{O-average}}
\]

The production of DO\textsuperscript{14}C by *Oithona* was then compared to carbon (PO\textsuperscript{14}C) ingested. Ingestion of PO\textsuperscript{14}C was calculated as specified for the grazing experiments described above, but based on the removal of \textsuperscript{14}C labeled POC.

**Statistical analyses**
A regression analysis (SPSS, version 22) was used to analyze the correlation between ingestion rates by *Oithona nana* and production rates of DOC, when feeding on *Oxyrrhis marina* and *Karlodinium* sp..

**Results and discussion**

We quantify for the first time the production of DOC by sloppy feeding *Oithona nana*. A total of five experiments were conducted (Table 2), three with *O. marina* as prey (Exp. I, II and III) and two with *Karlodinium* sp. (Exp. IV and V). There was a statistical significant correlation ($r^2 = 0.224$, $p< 0.05$, $n=21$) between ingestion rates and DOC production rates for *O. nana* feeding on *O. marina* (Fig. 1A). The range of average DOC production for each of the three experiments was 5.6 – 18.2 ng C *Oithona* $^1$ d$^{-1}$, implying that 6-15 % of the carbon ingested was released as DOC due to sloppy feeding (Table 2). However, no statistically significant correlation between *O. nana* ingestion rates and DOC production was found in Exp. IV and V with *Karlodinium* sp. as prey ($r^2 = 0.131$, $p= 0.25$, $n= 14$, Fig. 1B). The average DOC production in Exp. IV and V was negative (Table 2), implying that DOC was not produced when *O. nana* fed on *Karlodinium* sp.. It should be commented upon that the prey concentrations in the DOC production experiments (ranging from 403 to 679 µg C L$^{-1}$) were high compared to *in situ* concentrations and could have had negative effects on the copepods (and hence the grazing rates), representing a possible source of error. The main reason for utilizing such high concentrations was to secure non-limiting food concentrations throughout the experiment, and thus rule out the potential error that variable access to food caused a variable DOC production.

Furthermore, previously published studies reported no negative effects for *O. davisae* feeding on *O. marina* at comparable concentrations (Saiz et al. 2014). We therefore believe that the high prey concentrations were not harmful for the copepods, and did not negatively influence the outcome of the experiments.

The rates of DOC production found in our study are significantly lower than those reported for calanoid copepods. A DOC production of 50 % of the carbon cleared has been reported for large sized *Calanus* spp. (Møller et al. 2003). For smaller sized calanoid copepods like *Acartia tonsa*, DOC production ranges from 27-
when feeding on *Ditylum brightweili* and 10-19 % when grazing *Heterocapsa rotundata* (Møller 2007). The large variability of DOC production from sloppy feeding copepods depends on the relative size difference between the copepod and the prey, rather than prey quantity and quality (Møller 2007). When the prey is small compared to the predator, little or no DOC is produced. Møller (2007) found that when the size ratio (ESD:ESD) between the copepod and the prey was more that 41, no DOC production by sloppy feeding could be measured for *A. tonsa*, *Centropages typicus* and *Temora longicornis*. In our study, the size-ratio between *O. nana* (ESD 139 µm) and the prey were well below this threshold; 7.3 for *O. marina* (ESD 19 µm) and 12.1 for *Karlodinium* sp. (ESD 11 µm). Therefore a significant DOC production of 30-40 % of the carbon removed from suspension could be expected. For example, a DOC production of 27-36 % and 10-19 % of POC removed from suspension was found for *A. tonsa* when the copepod to prey size-ratio was 8.4 and 21.8, respectively (Møller 2007). However, as pointed out by Møller (2007), it is the dimension of the mouth opening rather than the prosome length of the predator that is of importance for sloppy feeding. We did not measure directly the mouth opening of *O. nana*, but for the slightly smaller *O. davisae* (female prosome length ~300 µm) the mouth opening of an adult female is ~10 µm x 20 µm (Saiz et al. 2014). It is therefore reasonable to assume that the DOC production from sloppy feeding *Oithona* sp. will increase as a function of prey size, especially when the prey size exceeds the mouth opening of the copepod. When the prey is smaller than the mouth opening it could be swallowed whole, resulting in no DOC leakage from breaking cells. This could explain the lack of detectable DOC production found for *Karlodinium* sp., while this was not the case for the larger prey *O. marina*. We were unable to investigate DOC production by sloppy feeding on the large autotrophic dinoflagellates *P. micans* (ESD 34 µm) and *A. sanguinea* (ESD 42 µm), as they were unable to divide in the ^14^C labeled medium (CS, personal observation). Reduced growth of dinoflagellates, including *P. micans* and *A. sanguinea*, exposed to ^14^C over several days has been reported (Skovgaard and Menden-Deuer 2003). The reasons are not clear, but reduced growth due to damaged DNA in the nucleus has been suggested (Skovgaard and Menden-Deuer 2003).

We propose that the relatively low DOC production measured for *O. nana* compared to rates reported for calanoid copepods of similar size and comparable predator:prey size rations, is a consequence of feeding behavior. Copepods that are...
highly specialized for one type of prey could have a feeding behavior that is
optimized, resulting in lower losses of carbon due to sloppy feeding (Møller 2007).

Most (if not all) calanoid copepods feed by creating a feeding current, and some
species can also switch between a suspension and ambush feeding mode (Kiørboe
2011b). In contrast to this flexibility in feeding behavior among calanoids, Oithona
sp. is a strict ambush feeder that is dependent on a hydromechanical signal from a
motile prey (Svensen and Kiørboe 2000; Paffenhöfer and Mazzocchi 2002). A
suspension feeding copepod is both more efficient and can consume a broader range
of prey types than the strict ambush feeder (Kiørboe 2011b). This is also supported by
generally higher feeding rates for calanoid copepods than for Oithona (Saiz and
Calbet 2007; Saiz et al. 2014). To conclude, the ambush feeder is associated with
lower feeding rates and a higher degree of prey specialization compared to suspension
feeders, and we propose that these are the main reasons for the lower DOC production
rates obtained for O. nana in our experiments, compared to rates reported for calanoid
copepods.

We conducted four grazing experiments to compare feeding rates of O. nana
on differently sized dinoflagellates (Table 1). The experimental prey concentrations,
384-795 µg C L⁻¹, were high compared to previous experiments using O. nana and
ambient food concentrations (Calbet et al. 2000; Atienza et al. 2006), and satiated
concentrations of 100-140 µg C L⁻¹ have been reported for O. nana feeding on P.
micans (Lampitt and Gamble 1982). We therefore assume that food was not a limiting
factor in our experiments. The lowest clearance rate (0.37 ± 0.11 mL ind⁻¹ d⁻¹) was
found for the smallest prey species, Karlodinium sp., while the highest rate (0.94 ±
0.29 mL ind⁻¹ d⁻¹) was obtained with O. marina as prey (Fig 2). Comparable clearance
rates were obtained for the two larger prey, P. micans (0.82 ± 0.25 mL ind⁻¹ d⁻¹) and
A. sanguinea (0.70 ± 0.25 mL ind⁻¹ d⁻¹). Ingestion rates for P. micans and A.
sanguinea were 269 ± 76 ng C ind⁻¹ d⁻¹ and 404 ± 181 ng C ind⁻¹ d⁻¹, respectively (Fig.
2). To the best of our knowledge, few grazing experiments have been reported
specifically for O. nana with dinoflagellates offered as prey, making direct
comparisons difficult. However, Lampitt and Gamble (1982) reported maximum
clearance rates of 0.29 mL animal d⁻¹ for O. nana feeding on P. micans but at lower
temperature (10 ºC) and lower food concentration (maximum concentration about 140
µg C L⁻¹). The rates obtained in our experiments are however within the range of
reported rates obtained for *O. davisae* at comparable experimental conditions, with maximum clearance rates of ~1 to 4 mL female\(^{-1}\) d\(^{-1}\) when feeding on *O. marina*, *P. micans* and *A. sanguinea* (Saiz et al. 2014). In our experiment the daily rations of *O. nana* females increased with prey size and corresponded to 13-61 % of body C d\(^{-1}\) (Table 1). Daily rations needed to cover basic metabolic activity (based on respiration measurements) of *O. similis* was found to be about 14 % body C d\(^{-1}\) at 20 °C (Castellani et al. 2005). The daily ration of 13 % body C d\(^{-1}\) for *Karlodinium* sp. found in our experiments could cover the minimum requirement to cover basic metabolism, but is most likely not sufficient to sustain growth and reproduction of *O. nana*. For that reason the larger dinoflagellates *O. marina*, *P. micans* and *A. sanguinea* appear more suitable as prey.

*Oithona* spp. are abundant in nearly all marine habitats, even though the abundance is often underestimated (Gallienne and Robins 2001; Svensen et al. 2011). Given their high abundances, lack of diapause and a reproductive strategy that is apparently decoupled from the spring bloom, *Oithona* may have a strong link to the microbial food webs. Production of DOC by sloppy feeding *Oithona* could represent one such link, although an attempt to quantify it will be hampered with uncertainty. Estimating the potential contribution of DOC produced by sloppy feeding *Oithona* to *in situ* systems depends on several variables, including (but probably not limited to) copepod abundance/biomass, ingestion rates and the fraction of DOC lost due to sloppy feeding. The abundance and biomass of *O. nana* is highly variable with season and locality, but is generally reported to be high when sampled with small mesh size or water bottles. For example, reported maximum abundance (and biomass) of *O. nana* in July at the southern coast of England was 48 200 ind. m\(^{-3}\) (10 mg C m\(^{-3}\)) (Williams and Muxagata 2006), 27 000 ind. m\(^{-3}\) (4.1 mg C m\(^{-3}\)) in the North West Mediterranean coastal waters in summer (Atienza et al. 2006) and 10 100 ind. m\(^{-3}\) (2.2 mg C m\(^{-3}\)) in coastal waters off Argentina in December (Temperoni et al. 2011). Reported ingestion rates of *O. nana* are variable as well. However, minimum carbon requirement based on respiration rates is reported to be as low as about 1.8 % of body carbon d\(^{-1}\) at low temperatures (Castellani et al. 2005; Atienza et al. 2006). Relatively high daily rations of 61 % body carbon d\(^{-1}\) was found for *O. nana* grazing on *A. sanguinea* in our experiments, and we assume a range of daily ratios between 2 % and 60 %. As for the fraction of DOC produced from sloppy feeding, our finding of 6-15
% of the carbon ingested represents presently the only estimate available for *O. nana*.

Based on the assumptions above, and being aware of its shortcomings, the estimated 

*in situ* contribution of DOC from a population of *O. nana* could be in the range 0.002 

to 0.9 mg C m$^{-3}$ d$^{-1}$ when prey is abundant.

Phytoplankton are the main producers of DOC in the oceans (Lasternas and 

Agustí 2014), and as much as 50 % of daily primary production can be released as 

DOC (Karl et al. 1998). However, DOC production is also a function of nutrient 

concentrations and phytoplankton cell health (Lasternas and Agustí 2014). Reported 

rates of DOC production by phytoplankton in oligotrophic, intermediate and 

upwelling systems are 13.9 mg C m$^{-3}$ d$^{-1}$, 15.3 mg C m$^{-3}$ d$^{-1}$ and 9.84 mg C m$^{-3}$ d$^{-1}$, 

respectively (Lasternas and Agustí 2014). Compared to DOC production rates from 

phytoplankton, the potential contribution from sloppy feeding *Oithona* is probably 

modest during bloom and post bloom situations. However, during winter when 

primary production is very low (or zero), the production of DOC from non- 

hibernating small copepods, like *Oithona* spp., could represent an important source of 

carbon to sustain the microbial loop.
Acknowledgements

We thank the reviewers for constructive comments, David Checkley for technical support, Michael Latz for providing some of the culture species, Lindsey Ekern for assistance with the liquid scintillation counter and Phil Zerofski for help collecting zooplankton.

Funding

This work was funded by a Fulbright Arctic Chair Award to CS, and the CarbonBridge project (Bridging marine productivity regimes: How Atlantic advective inflow affects productivity, carbon cycling and export in a melting Arctic Ocean), project number 226415, funded by Polar Program under the Research Council of Norway.

Compliance with ethical standards

Conflict of interest: The authors declare they have no conflict of interest.

Ethical approval: All international, national and institutional guidelines for the care and use of animals (copepods only) were followed. This article does not contain any studies with human participants performed by any of the authors.
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Fig 1 DOC production (ng C ind$^{-1}$ d$^{-1}$) as a function of ingestion (ng C ind$^{-1}$ d$^{-1}$) for *O. nana* feeding on *O. marina* (A) and *Karlodinium* sp. (B). Note different scales on axes.

Fig 2 *O. nana* grazing experiments, showing clearance rate (mL cop$^{-1}$ d$^{-1}$) and ingestion rate (ng C cop$^{-1}$ d$^{-1}$) as a function of prey size. K= *Karlodinium*, Om= *Oxyrrhis marina*, Pm= *Prorocentrum micans* and As = *Akashiwo sanguinea*. 
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<th>Species</th>
<th>Strain</th>
<th>T</th>
<th>ESD (µm)</th>
<th>Cells mL⁻¹</th>
<th>µg C L⁻¹</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>CCMP694</td>
<td>A</td>
<td>34 ± 3</td>
<td>174 ± 14</td>
<td>457 ± 37</td>
<td>45</td>
</tr>
<tr>
<td><em>Akashiwo sanguinea</em></td>
<td>CCMP3265</td>
<td>A</td>
<td>42 ± 6</td>
<td>177 ± 10</td>
<td>795 ± 47</td>
<td>61</td>
</tr>
<tr>
<td><em>Karlodinium sp.</em></td>
<td>unknown</td>
<td>M</td>
<td>11 ± 2</td>
<td>2014 ± 46</td>
<td>384 ± 8.7</td>
<td>13</td>
</tr>
<tr>
<td><em>Oxyrrhis marina</em></td>
<td>CCMP1739</td>
<td>H</td>
<td>19 ± 2</td>
<td>619 ± 36</td>
<td>384 ± 22</td>
<td>45</td>
</tr>
</tbody>
</table>

* The average ± SD prosome length of individual *O. nana* females was 460 ± 28 µm and the carbon-content was 0.84 ± 0.17 µg C female⁻¹ (N= 50 individuals).
Table 2 DOC production experiments by *O. nana* feeding on two species of dinoflagellates: *Oxyrrhis marina* (Om) in exp. I-III and *Karlodinium* sp. (K) in exp. IV-V. Prey concentrations at experiment start ($T_0$) are given as cells mL$^{-1}$ and µg C L$^{-1}$. Incubation time was 20 h. *Oithona* ingestion rate (I, ng C ind$^{-1}$ d$^{-1}$) and DOC production rate (ng C ind$^{-1}$ d$^{-1}$) is given as mean values ± SD for each experiment (N= 6 experimental bottles). DOC/I gives the fraction of DOC produced (DOC) as a function of carbon ingested (I) (mean values ± SD for each experiment (N= 6 experimental bottles). *Oithona* (n) is the number of copepods per experimental bottle.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Prey</th>
<th>Prey cells mL$^{-1}$</th>
<th>Prey µg C L$^{-1}$</th>
<th>Oithona (n)</th>
<th>DOC</th>
<th>DOC/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Om</td>
<td>767</td>
<td>475</td>
<td>10</td>
<td>67 ± 52</td>
<td>5.6 ± 7.0</td>
</tr>
<tr>
<td>II</td>
<td>Om</td>
<td>1095</td>
<td>679</td>
<td>10</td>
<td>165 ± 75</td>
<td>18.7 ± 25.6</td>
</tr>
<tr>
<td>III</td>
<td>Om</td>
<td>1052</td>
<td>652</td>
<td>10</td>
<td>223 ± 74</td>
<td>14.0 ± 8.5</td>
</tr>
<tr>
<td>IV</td>
<td>K</td>
<td>2113</td>
<td>403</td>
<td>6</td>
<td>228 ± 151</td>
<td>-33.0 ± 10.3</td>
</tr>
<tr>
<td>V</td>
<td>K</td>
<td>3520</td>
<td>671</td>
<td>7</td>
<td>22 ± 121</td>
<td>-0.3 ± 11.3</td>
</tr>
</tbody>
</table>
A

**Oxyrrhis marina**

\[ y = 0.61 + 0.08 X \]

\[ R^2 = 0.224 \]

\[ p < 0.05 \]

B

**Karldoninum sp.**

\[ y = -11.2 + -0.04 X \]

\[ R^2 = 0.131 \]

\[ p > 0.05 \]
Clearance rate

Ingestion rate

Fig 2