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 (Paffenhöfer 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 ^{14}C 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 $\mu Ci\ L^{-1}$. 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

157 estimated by calculating clearance rates (CR, mL female d^{-1}) and ingestion rates (I, ng
158 C female d^{-1}) according to Frost (1972), modified by Kiørboe et al. (1982).
159
160 **DOC production by *Oithona nana***
161
162 We conducted five experiments to study the production of DOC by grazing *Oithona*
163 *nana* (Table 2). Due to expected low grazing rates by *O. nana*, and hence a likelihood
164 of operating close to detection limits regarding DOC production, incubation times (20
165 h) were relatively long compared to previous experiments for calanoid copepods
166 (Møller et al. 2003). Therefore, no attempt was made to distinguish between different
167 sources of DOC related to the feeding process of *O. nana*. What is referred to as
168 "sloppy feeding" must be regarded as the total DOC production related to *O. nana*
169 grazing on dinoflagellates, including potential leakage from faecal pellets. Experiment
170 I-III was performed with *O. marina* as prey, while in experiment IV and V we used
171 *Karlodinium* sp. as prey. The ^{14}C labeled culture of *O. marina* was enumerated by
172 counting a sub-sample and then diluted with 0.2 μm-filtered seawater to obtain the
173 desired experimental concentration. The ^{14}C labeled culture of *Karlodinium* sp. was
174 centrifuged (< 5000 rpm for 5 min) in Falcon tubes to concentrate the cells and
175 remove access water. The cells were then transferred to a new falcon tube filled up
176 with FSW, and centrifuged again. Cell viability after centrifugation was confirmed by
177 microscope. Finally, the cells were transferred to a clean vial and diluted with FSW
178 until desired experimental cell concentration. The aim was to provide the copepods
179 with food in non-limiting concentrations, similar to the grazing experiments. The
180 initial prey concentrations in the DOC production experiments ranged from 403 to
181 679 μg C L^{-1} (Table 2). 20 mL incubation water (containing the labeled
182 dinoflagellates in 0.2 μm FSW) was filled in each of 12 vials, where half contained 7-
183 10 female *O. nana* and the other half served as controls without copepods. Three of
184 the control vials were used as initial (T_0) bottles and were terminated immediately,
185 according to the procedure described below. One mL FSW was added together with
186 the copepods to the treatment bottles, and the same volume of FSW was also added to
187 the controls (but without the copepods). The bottles were incubated in the dark for 20
188 h and at 18 °C. The vials were not rotated during the incubation. This may have
189 resulted in an un-homogenous distribution of dinoflagellates in the vials, and must be

190 regarded as a potential source of error. However, as the experiments were conducted
191 in 20 ml scintillation vials, it was not feasible to close the lids without capturing an
192 air-bubble. It was therefore decided that leaving the vials static would be the gentlest
193 treatment for the organisms. Given the high concentration of organisms in the
194 incubation vials, we assumed that encounter rates would not be negatively affected.
195 Visual inspection also confirmed that the copepods and dinoflagellates were relatively
196 evenly distributed in the experimental vials during incubation. The experiment was
197 repeated 3 times for *O. marina* and twice for *Karlodinium* sp. (Table 2).
198 The experiments were terminated according to the following
199 procedure: The copepods were removed from each vial by pouring the content
200 through a small sieve with 150 μm mesh. For Exp. I, II and III, the content of each
201 vial was first gently filtered onto a 3 μm Millipore filter to retain the particulate
202 organic carbon ($PO^{14}C$). The resulting filtrate was then filtered onto a 0.2 μm
203 polycarbonate filter (Costar) to retain the bacteria size fraction. This was done as an
204 attempt to estimate the relative increase of bacteria biomass during incubation. The
205 isotopic activity (DPM´s) on the 0.2 μm filter were used as a proxy for bacteria
206 biomass. By comparing the DPM´s in the 0.2 μm filter at experiment start (T_0) and
207 after 20 h (in controls and treatments) we found a 38 % and 36 % increase of DPM´s
208 in the 0.2 μm fraction in the control and treatment bottles, respectively (data not
209 shown). This indicates that the biomass increase in the 0.2 μm fraction was
210 comparable in the treatments and controls, and likely unaffected by sloppy feeding
211 during our 20 h incubations. To retain $PO^{14}C$ in Exp. IV-V, the content of each vial
212 was filtered directly on a GF/F filter without filtering first through a 3 μm filter. From
213 the final filtrate of all five experiments, 3 replicate sub-samples of 3 mL were
214 sampled for $DO^{14}C$. The filters and the filtrate samples were placed in individual
215 scintillation vials and 300 μL 20 % HCl was added. Samples were left for aeration for
216 24 h to remove inorganic ^{14}C, after which 15 mL scintillation cocktail (Ultima Gold)
217 was added.
218 ^{14}C isotopic activity was quantified using a Liquid scintillation counter
219 (Beckman LS 6000IC). The isotopic activity of the dinoflagellate samples (DPM) was
220 converted to carbon (μg C) by dividing the isotopic activity of the incubation water at
221 T_0 (containing a dilution of the dinoflagellate culture) with the carbon-content of the
222 same sample (DPM μgC^{-1}).

For quantification of $DO^{14}C$ production by the copepods, we followed the procedures described by Møller et al. (2003) and Møller (2007). The average $PO^{14}C$ (μg C) concentrations in the control- ($PO^{14}C_{d\text{-}average}$) and *Oithona* bottles ($PO^{14}C_{O\text{-}average}$) were calculated according to Frost (1972), modified by Kiørboe et al. (1982):

$$PO^{14}C_{average} = \frac{PO^{14}C_{end} - PO^{14}C_{start}}{\ln(PO^{14}C_{end}) - \ln(PO^{14}C_{start})}$$

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

$$DO^{14}C_d = \frac{DO^{14}C_T}{PO^{14}C_{d-average}}$$

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

$$DO^{14}C_O = DO^{14}C_T - DO^{14}C_d x\ PO^{14}C_{o-average}$$

The production of $DO^{14}C$ by *Oithona* was then compared to carbon ($PO^{14}C$) ingested. Ingestion of $PO^{14}C$ was calculated as specified for the grazing experiments described above, but based on the removal of ^{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 ($r2 = 0.224$, $p < 0.05$, $n=21$) 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 ($r2 = 0.131$, $p= 0.25$, $n= 14$, 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-

36 % when feeding on *Ditylum brightwelli* 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

353 reported rates obtained for *O. davisae* at comparable experimental conditions, with
354 maximum clearance rates of ~1 to 4 mL female^{-1} d^{-1} when feeding on *O. marina*, *P.*
355 *micans* and *A. sanguinea* (Saiz et al. 2014). In our experiment the daily rations of *O.*
356 *nana* females increased with prey size and corresponded to 13-61 % of body C d^{-1}
357 (Table 1). Daily rations needed to cover basic metabolic activity (based on respiration
358 measurements) of *O. similis* was found to be about 14 % body C d^{-1} at 20 °C
359 (Castellani et al. 2005). The daily ration of 13 % body C d^{-1} for *Karlodinium* sp. found
360 in our experiments could cover the minimum requirement to cover basic metabolism,
361 but is most likely not sufficient to sustain growth and reproduction of *O. nana*. For
362 that reason the larger dinoflagellates *O. marina*, *P. micans* and *A. sanguinea* appear
363 more suitable as prey.
364 *Oithona* spp. are abundant in nearly all marine habitats, even though the
365 abundance is often underestimated (Gallienne and Robins 2001; Svensen et al. 2011).
366 Given their high abundances, lack of diapause and a reproductive strategy that is
367 apparently decoupled from the spring bloom, *Oithona* may have a strong link to the
368 microbial food webs. Production of DOC by sloppy feeding *Oithona* could represent
369 one such link, although an attempt to quantify it will be hampered with uncertainty.
370 Estimating the potential contribution of DOC produced by sloppy feeding *Oithona* to
371 *in situ* systems depends on several variables, including (but probably not limited to)
372 copepod abundance/biomass, ingestion rates and the fraction of DOC lost due to
373 sloppy feeding. The abundance and biomass of *O. nana* is highly variable with season
374 and locality, but is generally reported to be high when sampled with small mesh size
375 or water bottles. For example, reported maximum abundance (and biomass) of *O.*
376 *nana* in July at the southern coast of England was 48 200 ind. m^{-3} (10 mg C m^{-3})
377 (Williams and Muxagata 2006), 27 000 ind. m^{-3} (4.1 mg C m^{-3}) in the North West
378 Mediterranean coastal waters in summer (Atienza et al. 2006) and 10 100 ind. m^{-3} (2.2
379 mg C m^{-3}) in coastal waters off Argentina in December (Temperoni et al. 2011).
380 Reported ingestion rates of *O. nana* are variable as well. However, minimum carbon
381 requirement based on respiration rates is reported to be as low as about 1.8 % of body
382 carbon d^{-1} at low temperatures (Castellani et al. 2005; Atienza et al. 2006). Relatively
383 high daily rations of 61 % body carbon d^{-1} was found for *O. nana* grazing on *A.*
384 *sanguinea* in our experiments, and we assume a range of daily ratios between 2 % and
385 60 %. As for the fraction of DOC produced from sloppy feeding, our finding of 6-15

386 % of the carbon ingested represents presently the only estimate available for *O. nana*.
387 Based on the assumptions above, and being aware of its shortcomings, the estimated
388 *in situ* contribution of DOC from a population of *O. nana* could be in the range 0.002
389 to 0.9 mg C m^{-3} d^{-1} when prey is abundant.
390 Phytoplankton are the main producers of DOC in the oceans (Lasternas and
391 Agusti 2014), and as much as 50 % of daily primary production can be released as
392 DOC (Karl et al. 1998). However, DOC production is also a function of nutrient
393 concentrations and phytoplankton cell health (Lasternas and Agusti 2014). Reported
394 rates of DOC production by phytoplankton in oligotrophic, intermediate and
395 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},
396 respectively (Lasternas and Agusti 2014). Compared to DOC production rates from
397 phytoplankton, the potential contribution from sloppy feeding *Oithona* is probably
398 modest during bloom and post bloom situations. However, during winter when
399 primary production is very low (or zero), the production of DOC from non-
400 hibernating small copepods, like *Oithona* spp., could represent an important source of
401 carbon to sustain the microbial loop.
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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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Figure captions

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*.

554 **Tables**
555

556 **Table 1** *Oithona nana** grazing experiments on four dinoflagellate species as prey.
557 Strain, size (mean equivalent spherical diameter, ESD ± SD for N= 30 cells) and initial
558 concentration of prey (cells mL^{-1} and $\mu g\ C\ L^{-1}$, average ± SD, N= 6) at experiment start
559 (T_0) is given. "T" indicates trophic mode of the dinoflagellate; A is autotrophic, M is
560 mixotropic and H is heterotrophic. DR is the daily ration (% C ingested body $C^{-1}\ d^{-1}$)
561 obtained for *O. nana* females for each prey item.

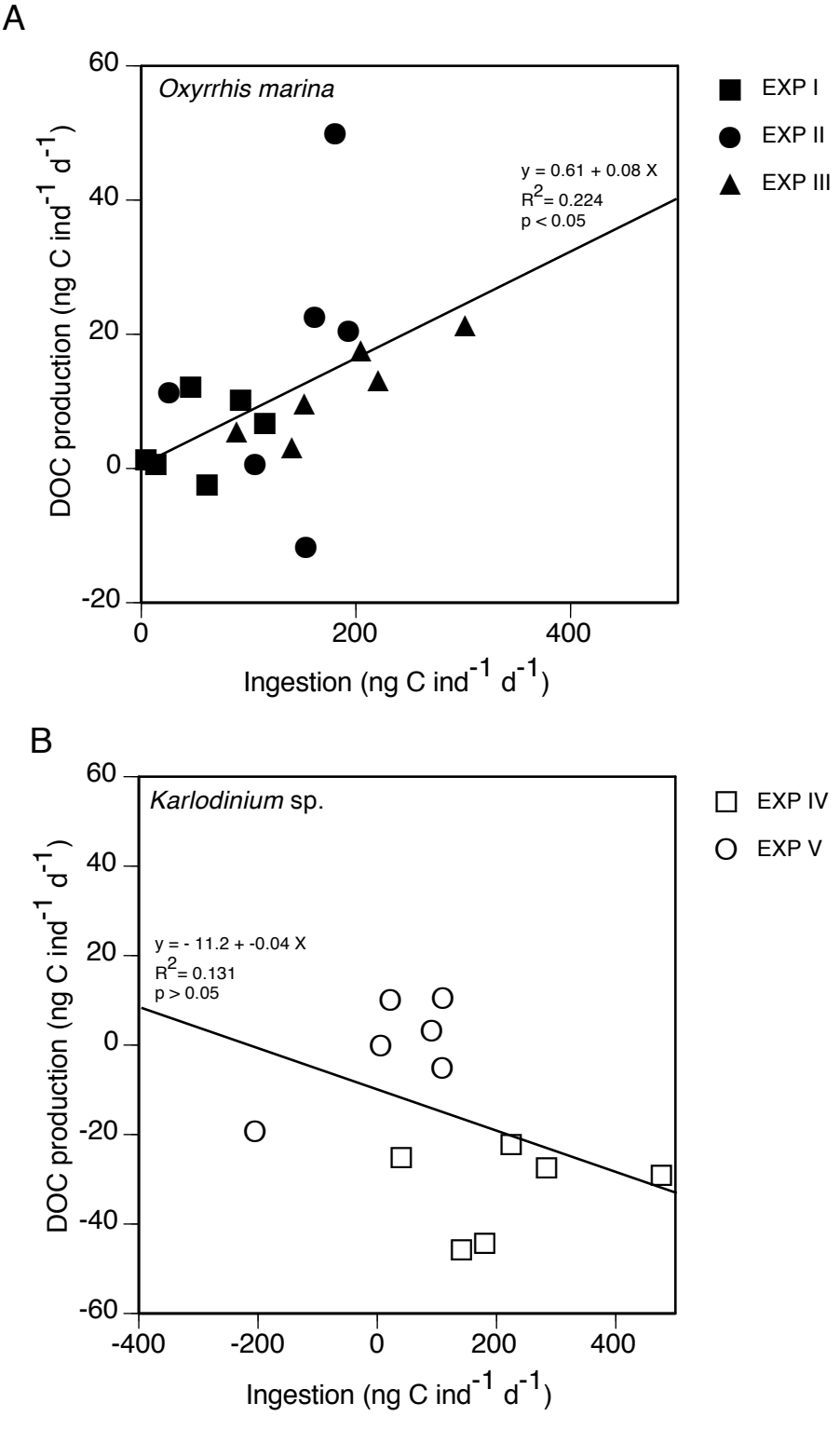
Species	Strain	T	ESD (μm)	Cells mL^{-1}	$\mu g\ C\ L^{-1}$	DR (%)
Prorocentrum micans	CCMP694	A	34 ± 3	174 ± 14	457± 37	45
Akashiwo sanguinea	CCMP3265	A	42 ± 6	177 ± 10	795 ± 47	61
Karlodinium sp.	unknown	M	11 ± 2	2014 ± 46	384 ± 8.7	13
Oxyrrhis marina	CCMP1739	H	19 ± 2	619 ± 36	384 ± 22	45

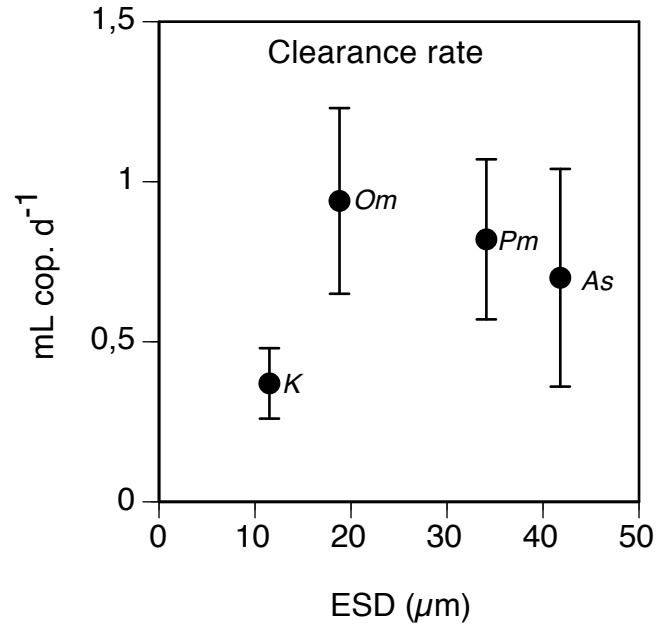
562 * The average ± SD prosome length of individual *O. nana* females was 460 ± 28 μm and the carbon-content was 0.84 ±
563 0.17 μg C female-1 (N= 50 individuals).
564
565
566

567 **Table 2** DOC production experiments by *O. nana* feeding on two species of
568 dinoflagellates: *Oxyrrhis marina* (Om) in exp. I-III and *Karlodinium* sp. (K) in exp.
569 IV-V. Prey concentrations at experiment start (T_0) are given as cells mL^{-1} and $\mu g\ C\ L^{-1}$.
570 Incubation time was 20 h. *Oithona* ingestion rate (I, ng C $ind^{-1}\ d^{-1}$) and DOC
571 production rate (ng C $ind^{-1}\ d^{-1}$) is given as mean values ± SD for each experiment (N=
572 6 experimental bottles). DOC/I gives the fraction of DOC produced (DOC) as a
573 function of carbon ingested (I) (mean values ± SD for each experiment (N= 6
574 experimental bottles) . *Oithona* (n) is the number of copepods per experimental bottle.

Exp.	Prey	cells mL^{-1}	$\mu g\ C\ L^{-1}$	*Oithona* (n)	I	DOC	DOC/I
I	Om	767	475	10	67 ± 52	5.6 ± 7.0	0.12 ± 0.13
II	Om	1095	679	10	165 ± 75	18.7 ± 25.6	0.15 ±0.19
III	Om	1052	652	10	223 ±74	14.0 ± 8.5	0.06 ± 0.02
IV	K	2113	403	6	228 ±151	-33.0 ± 10.3	-0.24 ± 0.19
V	K	3520	671	7	22 ±121	-0.3 ± 11.3	-0.09 ± 0.19

575


A
Oxyrrhis marina
y = 0.61 + 0.08 X
R2= 0.224
p < 0.05
EXP I
EXP II
EXP III
DOC production (ng C ind-1 d-1)
Ingestion (ng C ind-1 d-1)
60
40
20
0
-20
0
200
400
B
Karlodinium sp.
y = - 11.2 + -0.04 X
R2= 0.131
p > 0.05
EXP IV
EXP V
DOC production (ng C ind-1 d-1)
Ingestion (ng C ind-1 d-1)
60
40
20
0
-20
-40
-60
-400
-200
0
200
400


Clearance rate
mL cop. d-1
1,5
1
0,5
0
0
10
20
30
40
50
ESD (μm)
K
Om
Pm
As

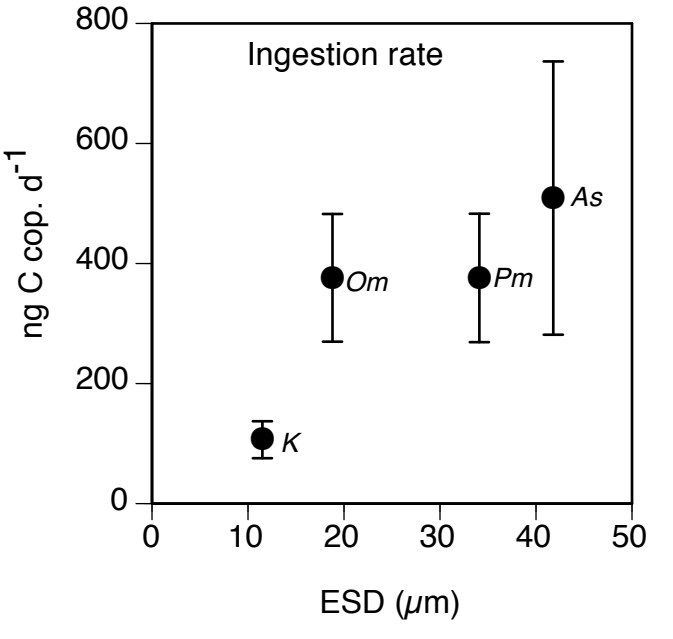

Ingestion rate
ng C cop. d-1
800
600
400
200
0
0
10
20
30
40
50
ESD (μm)
K
Om
Pm
As

Fig 2