Diel feeding rhythms in marine microzooplankton: effects of prey concentration, prey condition, and grazer nutritional history

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Abstract

In this study we aim at disentangling the causes and consequences of diel feeding rhythms in marine microzooplankton. We focused on the diel feeding activity of two heterotrophic dinoflagellate species, *Gyrodinium dominans* (one laboratory strain) and *Oxyrrhis marina* (laboratory cultivated and wild strains). We observed higher ingestion during the day in both dinoflagellate species. Feeding rhythms appeared to be independent of circadian changes in prey biochemical composition. Grazers fed with prey under stationary phase, with equivalent stoichiometric composition between day and night, showed 5 (*G. dominans*) and 10 (*O. marina*) times higher ingestion rates during the day. Previous grazer feeding history (starved vs well-fed) did not affect the feeding rhythm. However, prey concentration altered the rhythm; food limiting conditions reduced the amplitude of the rhythms. Our results establish a resource dependence of diel periodicity in microzooplankton grazing, which can have unanticipated consequences for standard field dilution grazing experiments.

20 **Introduction**

21 Light is a major driver of life in our planet, and as such it regulates the production and
22 distribution of phototrophic organisms; for instance, the vertical distribution and
23 seasonal production peaks of plankton in aquatic ecosystems are dependent on light
24 availability (Sverdrup 1953; Margalef 1978). It is known that light also drives the
25 feeding rhythms of mesozooplankton both in marine and freshwater systems (e.g. Duval
26 and Geen 1976; Mackas and Bohrer 1976). In most occasions, feeding rhythms in
27 mesozooplankton are linked to daily patterns of vertical migration (surface during night,
28 deeper during daytime; Gauld 1938; Stearns 1983; Saiz and Alcaraz 1990; Saiz et al.
29 1992; Putzeys and Hernández-León 2005), typically triggered by the presence of visual
30 predators (i.e. fish) in the upper layers of the oceans (Bollens and Frost 1991; Bollens
31 1996). Nocturnal feeding has also been observed, however, in the absence of vertical
32 migration in some copepod species (Peruyeva 1977; Boyd et al. 1980; Calbet et al.
33 1999).

34 In the case of microzooplankton, a key group in the transfer of energy from primary
35 producers to upper trophic levels in the marine pelagic environment (Calbet and Landry
36 2004; Calbet and Saiz 2005; Schmoker et al. 2013), less is known about their diel
37 feeding rhythms and the underpinning factors modulating them. This functional group is
38 taxonomically diverse and overall encompasses organisms with limited migratory
39 capacity. The few evidences available on microzooplankton diel feeding behaviour
40 indicate, contrarily to mesozooplankton, that ingestion rates are higher during the day
41 (Strom 2001; Jakobsen and Strom 2004; Tarangkoon and Hansen 2011).

42 Several hypotheses have been suggested to explain this particular behaviour. Jakobsen
43 and Strom (2004) advocated the presence of an endogenous circadian cycle, light-
44 modulated, for feeding and growth of many protozoans. On the other hand, Strom
45 (2001) proposed that light may enhance digestion by generating reactive oxygen species
46 in the protozoan food vacuole, while promoting ingested material break down and
47 increasing assimilation and gross growth efficiencies. By this process, digestion would
48 not limit (or limit less) the incorporation of new items into the food vacuoles, enhancing
49 the feeding rates. Alternatively, Ng and Liu (2015) suggested that diel variations on
50 phytoplankton stoichiometry (i.e. higher C:N ratio during day) can potentially influence
51 the feeding behaviour of grazers. A diel periodicity of the C:N ratio has been detected in

various phytoplankton groups (Stramski and Reynolds 1993; Clark et al. 2002; Jauzein et al. 2011), and it is suggested to be regulated by a circadian clock (Edmunds 1988). Bonded to this diel variation of the stoichiometric composition of prey are the changes of size result of synchronous division (Sweeney and Hastings 1958; Edmunds 1965; Eppley and Coatsworth 1966; Paasche 1968; Bruce 1970). Most of the hypotheses above, however, are questioned by the facts that i) the presence of diel feeding rhythms seem to be preserved under either continuous light or darkness, and ii) diel feeding rhythms appear even when protists are fed on "inert", dead cells (Sweeney and Hastings 1958; Chisholm and Brand 1981; Jakobsen and Strom 2004). We believe the presence of reverse diel feeding rhythms in microzooplankton, though endogenous, might have evolved as an adaptation to avoid being predated. Because feeding typically implies swimming, grazers become more conspicuous and increase encounter rates when feeding (Broglia et al. 2001); therefore, to reduce the probability of being predated protozoans should display diel feeding rhythms reverse to that of their major grazers, i.e. the copepods (Saiz and Calbet 2011). Until now, however, no experimental evidence confirms this plausible hypothesis, although in copepods such behavioural mechanisms have been reported (Saiz et al. 1993; Heuschele et al. 2014).

Here we studied the diel feeding activity of two heterotrophic dinoflagellate species, *Gyrodinium dominans* (a laboratory cultivated strain kept for many generations) and *Oxyrrhis marina* (both a laboratory cultivated and a wild strain), and examined the effect of several factors on their feeding behaviour. Considered cosmopolitan species, they inhabit different environments with contrasting biological, physical and chemical properties; this has lead to different adaptive ecological and physiological strategies (Calbet et al. 2013). We determined: (1) the presence of diel feeding rhythms in our target grazers and then checked whether nutritional properties of prey between day and night may explain the presence of rhythms; (2) whether the growth phase of the prey can evoke changes on the grazers' feeding behavior; (3) the diel response of well-fed vs starved grazers (i.e. 48h unfed) to prey; in this case, we expected rhythms to be influenced by the grazer previous feeding history, with well-fed grazers showing higher amplitude diel feeding rhythms than starved ones; (4) the effects of prey concentration on the amplitude on the diel feeding rhythms of microzooplankton. One could expect the diel activity being influenced by prey availability. Under non-limiting food conditions, microzooplankton may "opt" to feed less during the night period, when

potential predators may have a larger impact, and therefore the differences between day and night feeding would become higher; under food limitation, the grazers might be forced to search for food both during the day and during the night to cover their metabolic demands, as it occurs in more complex organisms, such as copepods (Huntley and Brooks 1982; Calbet et al. 1999).

Materials and methods

Culture of the dinoflagellate predators and the algal prey

Laboratory cultures of the heterotrophic dinoflagellates *Gyrodinium dominans* (GYR-BCN), and *Oxyrrhis marina* (OXY-BCN and OXY-BCN-2016, a new strain recently isolated) were used to study diel cycles in feeding and growth under different conditions. All strains were isolated by A. Calbet off Barcelona coast (NW Mediterranean, 41° 23' 0 N) in 2011, 1996 and 2016 respectively, and then kept in the laboratory at the Institut de Ciències del Mar in Barcelona.

For these experiments, the grazer cultures were grown in round flasks with metal-enriched autoclaved seawater (1mL metal stock solution per litre; Guillard 1975) at 19±1°C, 38 PSU under a 10L:14D light-darkness cycle. Grazer stocks were daily fed with a culture of the cryptophyte *Rhodomonas salina* grown on f/2 medium (Guillard 1975) in 5 L Pyrex culture flasks provided with air and diluted daily to ensure exponential growing conditions. The grazer cultures were maintained in these conditions several weeks (>4) prior to conducting the feeding experiments.

Prey and grazer diel changes in size and biochemical composition

We assessed the morphological (size) and biochemical (C:N:P) changes during the different growing phases (i.e. exponential and stationary phases) of *R. salina* by following the development of a triplicated culture of *R. salina* since inoculation until the beginning of the decay phase. The cultures were sampled before the light and dark periods started to determine cell size and concentration with the Coulter counter. Concurrently, we also analysed the elemental composition (carbon, nitrogen and phosphorus) of *R. salina* during the exponential and stationary phases of growth. For C

and N analysis, 5 mL aliquots of the *R. salina* culture were filtered onto 25-mm diameter pre-combusted GF/F filters (450 °C, 6h), dried at 60°C during 48 h and kept in a desiccator until analysis with an elemental analyser FlashEA1112 (ThermoFinnigan). For P analysis, 2 mL aliquots were used and immediately frozen at -80 °C after filtration; later, samples were digested with NaOH-K₂S₂O₈, and then analysed as inorganic P with an AA3HR autoanalyser (Seal Analytical). Following the same procedure, we analysed the biochemical composition of OXY-BCN and GYR-BCN before the light and dark periods after 2-days of starvation, when no prey was present in the suspension. For these samples we filtered from 20 to 50 ml, depending on the grazer concentration.

Experimental set-up

1. General set-up. The general procedure for the experiments was as follows. At each experiment, grazer and prey stock concentrations were determined with a Beckman Coulter Multisizer III particle counter (100-µm aperture tube) within 1-2 h before the beginning of, respectively, the light period (9:00 h) and the night period (19:00 h). Then, the desired predator-prey suspensions were prepared and distributed at intervals by filling one-third of experimental (both grazer and prey) and control (only prey) bottles (72 mL polyethylene culture flasks; 3-4 replicates). Extra bottles at each prey-predator mixture were also prepared for determination of initial concentrations. Once set, the bottles were incubated on a plankton wheel (0.2 r.p.m.). About 1 h before the end of the respective light and night periods, the corresponding incubations were terminated and grazer and prey concentrations determined. During the light period the bottles were exposed to fluorescent lamps providing an irradiation that ranged between 80 and 290 µE m⁻² s⁻¹ through a complete rotation of the wheel. Grazer and prey concentrations (in cells and in biovolume) at the beginning and at the end of the incubations were determined with the Beckman Coulter Multisizer III particle counter.

2. Effect of prey growth condition on diel feeding rhythms. We designed a series of experiments to explore whether the diel differences in feeding and growth rates of the grazers were affected by the prey growth phase (i.e. exponential vs. stationary), and at its turn, by its biochemical composition. For this experiments we used the strains GYR-

BCN and OXY-BCN, previously starved for 48 h. All the experimental procedures are as described in the *General set-up* section. Based on the results obtained (see Results), we decided to use *R. salina* ($7-8 \times 10^4$ cells ml^{-1}) in stationary phase for the rest of the experiments in order to minimize the day-night cells size variation.

3. Effect of the grazer feeding history on diel feeding rhythms. We investigated whether the previous feeding history of the grazer affected the amplitude of the diel feeding rhythm. Hence, we compared the grazing and growth rates of GYR-BCN and OXY-BCN fed ad libitum with *R. salina* with those of 48 h-starved grazers (i.e. unfed for two days). The experiments were conducted following the general procedures described above.

4. Effect of food concentration on diel feeding rhythms. We evaluated the effect of different prey concentration on the feeding behaviour of GYR-BCN, OXY-BCN and OXY-BCN-2016. According to the results of the previous experiments we establish the experimental protocol to use prey on stationary phase and to starve the grazers for 48 h prior to the experiments. Prey concentrations were chosen in order to encompass three different scenarios of the functional feeding response (based on data from Calbet et al. 2013): limiting, intermediate and saturated food conditions (Table 1). The prey:predator ratios at the beginning of the incubations were about 35:1 in *G. dominans* and 100:1 for *O. marina*. All the experiments were conducted in triplicate and followed the general procedures described above.

Calculation of feeding rates

The calculation of feeding rates followed the exponential equations of Frost (1977). The grazing coefficient g (h^{-1}) was estimated as:

$$g = \mu - k$$

where μ (h^{-1}) is the intrinsic prey growth in the control bottles (only prey), and k is the apparent prey growth determined in the experimental bottles (with predators). Clearance rate F ($\mu\text{l grazer}^{-1} \text{h}^{-1}$) was estimated as:

$$F = \frac{g}{\hat{C}_{\text{grazer}}}$$

where $\hat{C}_{\text{grazer}}$ (cells mL^{-1}) is the average grazer concentration in the incubation, estimated as:

$$\hat{C}_{grazer} = \frac{C_{0,grazer} - C_{1,grazer}}{\ln(C_{1,grazer}/C_{0,grazer})}$$

where $C_{0,grazer}$ and $C_{1,grazer}$ are, respectively, the grazer initial and final concentrations in the incubation and t (h) is the incubation time.

Ingestion rates I (cells grazer⁻¹ h⁻¹) were estimated as:

$$I = F \times \hat{C}_{prey}$$

where $\hat{C}_{prey}$ (cells mL⁻¹) is the average prey concentration estimated according to the equations in Frost (1977).

Feeding rates were converted into prey biovolume consumption by multiplying cell ingestion rates by the (geometric) mean prey volume during the incubation.

Results

Prey and grazer diel changes in size and biochemical composition

Rhodomonas salina entered in exponential growth ($\mu = 0.38$ d⁻¹) after a short (1 day) lag phase and remained exponentially growing for four days (Fig. 1a). Stationary phase reached densities of 1.6×10^6 cells mL⁻¹. Cells divided mostly during the night, which produced important differences in cell size between day and night. These differences were evident only during the exponential phase (Fig. 1b); cells were about 33% larger in volume during the day hours than during the night hours.

We present in Table 2 the stoichiometric composition of *R. salina* under exponential (days 12-13, both day and night periods, in Fig. 1) and stationary (from day 16, day period, to day 18, night period, in Fig. 1) phases. All elemental ratios were significantly higher during day time in the exponential phase of growth (C:N, 20% higher; C:P, 64%; N:P, 42%), whereas no difference between day and night composition were detected in stationary phase (Table 2). When comparing between exponential and stationary phases, the C:N and C:P were more than double during stationary phase respect exponential, whereas for N:P values differences between growth phases were of much lower magnitude.

The stoichiometric ratios of *O. marina* and *G. dominans* did not overall differ significantly between day and night (Table 3); only the C:N ratios of *G. dominans* differed between day and night, but the magnitude of variation was rather small (4% higher during the day; Table 3).

Effect of prey growth conditions on diel feeding rhythms

We compared the diel feeding response of GYR-BCN and OXY-BCN when feeding on *R. salina* in the exponential and stationary growth phases. The feeding rates obtained are presented in terms of cell (cells ind⁻¹ hour⁻¹) and volume ingested (μm³ grazer⁻¹ hour⁻¹) in Fig. 2. In both species prey on stationary phase induced feeding rhythms of higher amplitude than when on exponential phase, being the rates about 5 (GYRO-BCN) and 10 (OXY-BCN) times higher during the day ($p < 0.001$, t-test). In contrast, the results obtained with prey growing at exponential rates did not result in such a clear outcome. Whereas for GYR-BCN ingestion rates were higher during the day ($p < 0.001$ for cells and volume, t-test), OXY-BCN showed an opposite pattern, with higher cell-based ingestion rates during the night ($p < 0.05$, t-test), and no statistically significant differences between day and night when on a volume basis.

Effect of the feeding history on diel feeding rhythms

Diel ingestion rates of previously fed and starved GYR-BCN and OXY-BCN are presented in terms of cells and volume ingested in Fig. 3. In all cases ingestion rates were significantly higher during the light period ($p < 0.05$; t-test). For GYR-BCN ingestion rates were about 55% (fed grazers) and 44% (starved grazers) higher during the day, whereas for OXY-BCN the increase was 13% and 34% for fed and starved grazers, respectively (Fig. 3).

Effect of food concentration on diel feeding rhythms

Food availability clearly modified the feeding rhythms of the 3 grazers studied (Fig. 4a, b). Under saturating food conditions all grazers showed the largest differences between day and night ingestion rates (in terms of prey volume consumed); these differences diminished as food concentration decreased. At limiting food conditions the daily rhythms disappeared for OXY-BCN and it was poorly marked in the other strains. Out of the 3 strains studied, GYR-BCN showed the highest rhythm amplitude, followed by

OXY-BCN-2016 (Fig. 4). Except for GYR-BCN, the slopes of the relationship between food concentration and the ratio between day and night ingestion rates were significantly different from zero ($p < 0.05$; Fig. 4), indicating a significant effect of prey concentration; in the case of GYR-BCN, however, the removal of one outlier value made the regression turn out significant ($p < 0.05$). The effects of food concentration on the diel feeding rhythms of the three grazers were similar, as indicated by the lack of significant differences among slopes (Fig. 4, $p = 0.51$; ANCOVA test). However, the intercepts were significantly different between regression lines ($p < 0.01$), which support that the magnitude of the rhythm is species/strain specific. In all treatments the grazers showed negligible growth rate (data not shown).

Discussion

Corroborating previous evidence on protozoan diel behaviour (Christoffersen 1994; Liu et al. 1997; Dolan and Šimek 1999; Strom 2001; Binder and DuRand 2002; Jakobsen and Strom 2004) we found reverse circadian feeding rhythms (i.e. higher feeding rates during daytime) in two marine heterotrophic dinoflagellate species. The causes and consequences of this particular behaviour are discussed below.

Causes of microzooplankton diel feeding rhythms

Phytoplankton activity is affected by light, leading to a diel periodicity in cell division and cellular properties (including C:N ratio) (Prézelin 1992; Vaulot et al. 1995; Liu et al. 1997; Vaulot and Marie 1999; Binder and DuRand 2002; Ng and Liu 2015). Synchronized cell division during nighttime is confirmed for many phytoplankton groups and results in a cell size decrease during this period of the day (Prézelin 1992; Binder and DuRand 2002; Ng and Liu 2015). Likewise, because of photosynthetic carbon fixation during light hours, C:N and C:P ratios increase during the day (Stramski and Reynolds 1993; Clark et al. 2002; Jauzein et al. 2011; Ng and Liu 2015). Diel variations of both cellular properties and cell size in phytoplankton should have ecological implications and could affect the dynamic of diel trophic interactions (Ng and Liu 2015). Ng and Liu (2015) argued that the feeding behaviour of nanoflagellated grazers could be strongly induced by these diel stoichiometric variations of prey, as a

result of compensatory feeding response by increasing grazing rates on low quality prey (i.e. high C:N) during the daytime. However, the same authors found also a distinct diel grazing pattern when flagellates were feeding on fluorescently-labelled dead bacteria. Also, Strom (2001) found that under saturating food conditions, the ingestion of dead fluorescently-labelled algae was 2.2 times higher in the light; she suggested a light-aided digestion mechanism. These two latter evidences seem to contradict the role of diel changes in algae composition as triggers of their grazer's diel feeding activity. Moreover, Jakobsen and Strom (2004) detected that the diel variations in growth and ingestion rates during day and night persisted in 24-h continuous darkness (although the rhythm slowly eroded after a few days), challenging Ng and Liu (2015) and Strom (2001) hypotheses. It seems, then, that either particular rules apply to each species, or that there must be an alternative explanation for the presence of diel feeding rhythms in microzooplankton. In our experiments we also detected a diel well-marked difference in *R. salina* cell size and stoichiometric composition during exponential phase, but these differences faded away in early stationary phase. This fact allowed us to test the role of diel changes in size and elemental composition in causing the existence of feeding rhythms. We found that GYR-BCN and OXY-BCN showed higher ingestion rates during the day when fed on stationary phase prey, therefore corroborating that these two factors cannot explain the presence of feeding rhythms. Nevertheless, the smaller prey size during night under exponential growth may mask, in occasions, these feeding patterns, as we observed in OXY-BCN (Fig. 2); this effect disappeared under stationary phase conditions where prey size was similar. Our results, therefore, lead to the conclusion that other factors, not only related to prey characteristics, must play an important role in determining the diel feeding activity of microzooplankton.

Oceanic planktonic habitats are known to be often food limited (Conover 1968). When prey are limiting, feeding behaviour of a grazer may be compromised as it is linked to swimming and, therefore, a grazer must increase its search effort. Hence, it increases the encounter rate with their own predators and, at the same time, they become more conspicuous to them. In this situation, a balance between feeding to maintain minimum nutritional requirements and the risk of being predated is necessary (Huntley and Brooks 1982; Saiz et al. 1992; Calbet et al. 1999), in particular given the high preference for dinoflagellates and ciliates displayed by copepods, the major contributors to mesozooplankton (Saiz and Calbet 2011). Several adaptive strategies of

microzooplankton to famine have been proposed and demonstrated in laboratory studies. A reduction of metabolic rate in starving protozoans was suggested by Fenchel and Finlay (1983). Some species are also known to recur to resting cysts formation when prey concentration is low (Goodman 1987; Fenchel 1990). We suggest that microzooplankton may “opt” to diminish the feeding rhythm. As we initially hypothesised, the feeding rhythm of the studied microzooplanktonic grazers was of higher amplitude when food was not limiting. It would be expected that at low food concentration, starved grazers would feed in an arbitrarily manner, without following a light-darkness cycle to feed. As a consequence, this would lead to a major mobility by the protozoans, which implies being more detectable by their own predators. This means that the threat of predation may be an important component explaining microzooplankton diel feeding activity. Backing up this hypothesis we found a greater diel response in the recently isolated *O. marina* (OXY-BCN-2016) compared to the long term laboratory-cultivated one (OXY-BCN). It is expected, as it occurs in other planktonic organisms, that some of the natural behaviours (i.e. the risk of predation) might be partially lost after consecutive generations of cultivation in the laboratory (Tiselius et al. 1995; Calbet et al. 1999).

In our study, the magnitude of the feeding rhythm (Fig. 4) differed between species/strains, being GYR-BCN the species with the most marked rhythm. *G. dominans* is typically found in coastal and oceanic waters where vertical migrations of mesozooplankton are common and predation risk is higher at night (Saiz et al. 2014). On the other hand, *O. marina* inhabits intertidal pools and salt marshes (Begun et al. 2004), being infrequent in open waters (Lowe et al. 2010; Watts et al. 2011), and has life history traits that allow it to quickly exploit resources whenever conditions are favourable (Calbet et al. 2013).

We have demonstrated so far that, at least for the species of microzooplankton studied here, feeding rhythms appear to be independent of circadian changes in prey biochemical composition or previous grazer feeding history, but are modified by prey concentration. It may be argued, however, that decreased feeding rates during the night may be consequence of synchronized division of the grazer at night, constraining feeding while dividing. Even though we cannot disregard this hypothesis, our data do not seem to confirm it. Growth rates after the two-day starvation period were negligible

for both GYR-BCN and OXY-BCN in all the experiments. Therefore, it is unlikely that the arrangement of the cell organelles during division can explain the arrest of ingestion during night.

Consequences of microzooplankton diel feeding rhythms

Regardless of the ecological reasons behind the existence of diel feeding rhythms in microzooplankton, their consequences in natural ecosystems are important. Higher ingestions during day, on pre-dividing algal cells should have more impacts on phytoplankton populations than the same grazing activity on already divided (night) algae. Given the relationship between prey availability and intensity of the diel feeding activity we found, it can be hypothesized diel rhythms are more relevant in upwellings and productive systems than in oligotrophic ones. In all these systems microzooplankton appear as the major herbivore (Calbet and Landry 2004; Schmoker et al. 2013). Unfortunately, there are not field data on diel microzooplankton herbivory. The only field study we are aware of dealt with bacterivory in coastal South China Sea and Hong Kong waters and found evidences of a higher diurnal grazing activity on bacteria (Ng and Liu 2015).

A relevant outcome of our data is that it establishes a resource dependence of diel periodicity in microzooplankton grazing, which can have unanticipated consequences for the most common way to determine microzooplankton grazing rates in the field, the dilution grazing experiments (Landry and Hassett 1982). Along the dilution series diel feeding activity will be artificially modified since food availability is modified. Moreover, the consideration of predator-prey growth and grazing coupling is of important relevance when choosing the starting point of the microzooplankton grazing experiments, as for any other grazing experiment with organisms displaying diel feeding rhythms (e.g. copepods); especially under non steady-state situations. From what has been shown here, it is expected that the outcome of 24-h microzooplankton grazing experiments will not be the same if started during the day or during the night. Theoretically, when the experiments are initiated during the day, the grazing during this period should impact more the phytoplankton populations than when started during the night, when phytoplankton are dividing and microzooplankton are less active. Field data also back up the differences on diel concentrations of phytoplankton and uneven grazing over the daily cycle (Neveux et al. 2003), although the microzooplankton

grazing rhythms in that study did not clearly match defined day-night periods. The possible bias in dilution experiments should depend on the prey concentration in the water, being productive areas the most likely affected. We advise, therefore, to indicate in the manuscripts the actual time the experiments begin. We hope future modelling/experimental efforts would provide a way to correct for this artifact, which nowadays reminds as an incognita.

In summary, the coupling or uncoupling of microzooplankton grazing activity with the rhythms of activity of their predators and prey may have relevant consequences for the carbon flow, and biogeochemical cycles in general, in marine ecosystems, and certainly deserves more attention in future studies. The proper integration of protists laboratory data into models of planktonic ecosystem functioning (Jakobsen and Strom 2004) and the inadequate interpretation of this behaviour in field studies can lead to a biased approximation to the efficiency of matter and energy transfer in the trophic web and on the overall understanding of the functioning of the marine ecosystem.

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396 **Compliance with Ethical Standards**

397 This study was funded by project FERMI (CGL2014-59227-R; MINECO/FEDER, UE).
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400 **Conflict of Interest:**

401 Anna Arias, Enric Saiz and Albert Calbet declare that they have no conflict of interest.

402 **Ethical approval:**

403 This article does not contain any studies with animals performed by any of the authors.

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References

- Begun AA, Orlova TY, Selina MS (2004) A “Bloom” in the Water of Amursky Bay (Sea of Japan) Caused by the Dinoflagellate *Oxyrrhis marina* Dujardin, 1841. *Russ J Mar Biol* 30:51–55. doi: [10.1023/B:RUMB.0000020569.49887.7e](https://doi.org/10.1023/B:RUMB.0000020569.49887.7e)
- Binder BJ, DuRand MD (2002) Diel cycles in surface waters of the equatorial Pacific. *Deep Sea Res Part II Top Stud Oceanogr* 49:2601–2617. doi: [10.1016/S0967-0645\(02\)00050-4](https://doi.org/10.1016/S0967-0645(02)00050-4)
- Bollens S (1996) Diel vertical migration in zooplankton: trade-offs between predators and food. *Oceanus*, 39(1):19-20.
- Bollens SM, Frost BW (1991) Ovigerity, selective predation, and variable diel vertical migration in *Euchaeta elongata* (Copepoda: Calanoida). *Oecologia* 87:155–161. doi: [10.1007/BF00325252](https://doi.org/10.1007/BF00325252)
- Boyd CM, Smith SL, Cowles TJ (1980) Grazing patterns of copepods in the upwelling system off Peru. *Limnol Oceanogr* 25:583–596. doi: [10.4319/lo.1980.25.4.0583](https://doi.org/10.4319/lo.1980.25.4.0583)
- Broglia E, Johansson M, Jonsson P (2001) Trophic interaction between copepods and ciliates: effects of prey swimming behavior on predation risk. *Mar Ecol Prog Ser* 220:179–186. doi: [10.3354/meps220179](https://doi.org/10.3354/meps220179)
- Bruce VG (1970) The Biological Clock in *Chlamydomonas reinhardtii*. *J Protozool* 17:328–334. doi: [10.1111/j.1550-7408.1970.tb02380.x](https://doi.org/10.1111/j.1550-7408.1970.tb02380.x)
- Calbet A, Saiz E, Irigoien X, Alcaraz M, Trepas I (1999) Short communication. Food availability and diel feeding rhythms in the marine copepods *Acartia grani* and *Centropages typicus*. *J Plankton Res* 21(5)
- Calbet A, Landry M (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol Oceanogr* 49(1):51–57
- Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. *Aquat Microb Ecol* 38(2):157-67.
- Calbet A, Isari S, Martínez RA, Saiz E, Garrido S, Peters J, Borrat RM, Alcaraz M (2013) Adaptations to feast and famine in different strains of the marine heterotrophic dinoflagellates *Gyrodinium dominans* and *Oxyrrhis marina*. *Mar Ecol Prog Ser* 483:67-84
- Chisholm SW, Brand LE (1981) Persistence of cell division phasing in marine phytoplankton in continuous light after entrainment to light: Dark cycles. *J Exp Mar Bio Ecol* 51:107–118. doi: [10.1016/0022-0981\(81\)90123-4](https://doi.org/10.1016/0022-0981(81)90123-4)
- Christoffersen K (1994) Variations of feeding activities of heterotrophic nanoflagellates on picoplankton. *Mar Microb Food Web* 8:11-123
- Clark DR, Flynn KJ, Owens NJP (2002) The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. *New Phytol* 155:101–108. doi: [10.1046/j.1469-8137.2002.00435.x](https://doi.org/10.1046/j.1469-8137.2002.00435.x)

455 Conover RJ (1968) Zooplankton-life in a nutritionally dilute environment. Am Zool
456 8(1):107-118

457 Dolan JR, Šimek K (1999) Diel periodicity in *Synechococcus* populations and grazing
458 by heterotrophic nanoflagellates: Analysis of food vacuole contents. Limnol
459 Oceanogr 44:1565–1570. doi: [10.4319/lo.1999.44.6.1565](https://doi.org/10.4319/lo.1999.44.6.1565)

460 Duval WS, Geen GH (1976) Diel feeding and respiration rhythms in zooplankton.
461 Limnol. Oceanogr 21(6):823-829.

462 Edmunds LN (1965) Studies on synchronously dividing cultures of *Euglena gracilis*
463 Klebs (strain Z). I. Attainment and characterization of rhythmic cell division. J Cell
464 Comp Physiol 66:147–158. doi: [10.1002/jcp.1030660204](https://doi.org/10.1002/jcp.1030660204)

465 Edmunds LN (1988) Cellular and molecular bases of biological clocks: models and
466 mechanisms for circadian timekeeping. Springer-Verlag p 497

467 Eppley RW, Coatsworth JL (1966) Culture of the marine phytoplankter, *Dunaliella*
468 *tertiolecta*, with light-dark cycles. Arch for Mikrobiol 55:66–80. doi:
469 [10.1007/BF00409157](https://doi.org/10.1007/BF00409157)

470 Fenchel T (1990) Adaptive significance of polymorphic life cycle in protozoa:
471 responses to starvation and refeeding in two species of marine ciliates. J Exp Mar
472 Biol Ecol 136: 159-177

473 Fenchel T, Finlay BJ (1983) Respiration rates in heterotrophic, free-living protozoa.
474 Microb Ecol 9:99–122. doi: [10.1007/BF02015125](https://doi.org/10.1007/BF02015125)

475 Frost BW (1977) Feeding behavior of *Calanus pacificus* in mixtures of food particles.
476 Limnol Oceanogr 22:472-49

477 Gauld DT (1938) Diurnal Variation in the Feeding and Breeding of Zooplankton related
478 to the Numerical Balance of the Zoo-Phytoplankton Community. ICES J Mar Sci
479 13:323–337. doi: [10.1093/icesjms/13.3.323](https://doi.org/10.1093/icesjms/13.3.323)

480 Goodman DK (1987) Dinoflagellate cysts in ancient marine and modern marine
481 sediments. In: Taylor FJR (ed) The biology of dinoflagellates. Blackwell, Oxford,
482 pp 649-722

483 Guillard RRL (1975) Culture of Phytoplankton for Feeding Marine Invertebrates. In:
484 Culture of Marine Invertebrate Animals. Springer US, Boston, MA, pp 29–60

485 Heuschele J, Ceballos S, Borg CM, Bjærke O, Isari S, Lasley-Rasher R, Lindehoff E,
486 Souissi A, Souissi S, Titelman J (2014) Non-consumptive effects of predator
487 presence on copepod reproduction: insights from a mesocosm experiment. Mar
488 Biol 161(7):1653-66. doi: [10.1007/s00227-014-2449-z](https://doi.org/10.1007/s00227-014-2449-z)

489 Huntley M, Brooks ER (1982) Effects of age and food availability on diel vertical
490 migration of *Calanus pacificus*. Mar Biol 71:23–31. doi: [10.1007/BF00396989](https://doi.org/10.1007/BF00396989)

491 Jakobsen HH, Strom SL (2004) Circadian cycles in growth and feeding rates of
492 heterotrophic protist plankton. Limnol Oceanogr 49(6):1915-22

493 Jauzein C, Collos Y, Laabir M, Vaquer A (2011) Dark metabolism and carbon–nitrogen
 494 uncoupling in the toxic dinoflagellate *Alexandrium catenella* (Dinophyceae).
 495 Harmful Algae 11:73–80. doi: [10.1016/j.hal.2011.08.002](https://doi.org/10.1016/j.hal.2011.08.002)

496 Landry MR, Hassett R (1982) Estimating the grazing impact of marine
 497 microzooplankton. Mar Biol 67(3):283–8.

498 Liu H, Nolla H, Campbell L (1997) Prochlorococcus growth rate and contribution to
 499 primary production in the equatorial and subtropical North Pacific Ocean. Aquat
 500 Microb Ecol 12:39–47. doi: [10.3354/ame012039](https://doi.org/10.3354/ame012039)

501 Lowe CD, Keeling PJ, Martin LE, Slamovits C, Watts PC, Montagnes DJ (2010) Who
 502 is Oxyrrhis marina? Morphological and phylogenetic studies on an unusual
 503 dinoflagellate. J Plankton Res 33 (4):555–567. doi: [10.1093/plankt/fbq110](https://doi.org/10.1093/plankt/fbq110)

504 Mackas D, Bohrer R (1976) Fluorescence analysis of zooplankton gut contents and an
 505 investigation of diel feeding patterns. J Exp Mar Biol Ecol 25:77–85

506 Margalef R (1978) Life-forms of phytoplankton as survival alternatives in an unstable
 507 environment. Oceanologica acta 1(4):493–509.

508 Neveux J (2003) Diel dynamics of chlorophylls in high-nutrient, low-chlorophyll waters
 509 of the equatorial Pacific (180°): Interactions of growth, grazing, physiological
 510 responses, and mixing. J Geophys Res 108 (C12): 8140. doi:
 511 [10.1029/2000JC000747](https://doi.org/10.1029/2000JC000747)

512 Ng WHA, Liu H (2015) Diel variation of the cellular carbon to nitrogen ratio of
 513 *Chlorella autotrophica* (Chlorophyta) growing in phosphorus- and nitrogen-
 514 limited continuous cultures. J Phycol 51:82–92. doi: [10.1111/jpy.12254](https://doi.org/10.1111/jpy.12254)

515 Paasche E (1968) Marine Plankton Algae Grown with Light-Dark Cycles. II. Ditylum
 516 brightwellii and Nitzschia turgidula. Physiol Plant 21:66–77. doi: [10.1111/j.1399-3054.1968.tb07231.x](https://doi.org/10.1111/j.1399-3054.1968.tb07231.x)

518 Peruyeva YG (1977) Some experimental data on the fourth copepo- did stage of
 519 Calanus glacialis Jaschnov. Quantitative composition of the food. Oceanology
 520 17:587–590.

521 Prézelin BB (1992) Diel periodicity in phytoplankton productivity. In: Berman, T, Gons
 522 HJ, Mur LR (eds) The Daily Growth Cycle of Phytoplankton. Springer
 523 Netherlands, Dordrecht, pp 1–35

524 Putzeys S, Hernández-León S (2005) A model of zooplankton diel vertical migration off
 525 the Canary Islands: implication for active carbon flux. J Sea Res 53:213–222. doi:
 526 [10.1016/j.seares.2004.12.001](https://doi.org/10.1016/j.seares.2004.12.001)

527 Saiz E, Alcaraz M (1990) Pigment gut contents of copepods and deep phytoplankton
 528 maximum in the Western Mediterranean. J Plankton Res 12:665–672.

529 Saiz E, Rodriguez V, Alcaraz M (1992) Spatial distribution and feeding rates of
 530 *Centropages typicus* in relation to frontal hydrographic structures in the Catalan
 531 Sea (Western Mediterranean). Mar Biol 112:49–56. doi: [10.1007/BF00349727](https://doi.org/10.1007/BF00349727)

532 Saiz E, Tiselius P, Jonsson PR, Verity P, Paffenhöfer G (1993) Experimental records of
533 the effects of food patchiness and predation on egg production of *Acartia tonsa*.
534 Limnol Ocean 38(2):280-9

535 Saiz E, Calbet A (2011) Copepod feeding in the ocean: scaling patterns, composition of
536 their diet and the bias of estimates due to microzooplankton grazing during
537 incubations. Hydrobiologia 666:181–196. doi: [10.1007/s10750-010-0421-6](https://doi.org/10.1007/s10750-010-0421-6)

538 Saiz E, Sabatés A, Gili JM (2014) The zooplankton. In: Goffredo S, Dubinsky Z (eds)
539 The Mediterranean Sea. Springer Netherlands, pp. 183-211

540

541 Schmoker C, Hernandez-Leon S, Calbet A (2013) Microzooplankton grazing in the
542 oceans: impacts, data variability, knowledge gaps and future directions. J Plankton
543 Res 35:691–706. doi: [10.1093/plankt/fbt023](https://doi.org/10.1093/plankt/fbt023)

544 Stearns D (1983) Control of nocturnal vertical migration in the calanoid copepod
545 *Acartia tonsa* Dana in the Newport River Estuary, North Carolina. Ph.D. thesis,
546 Dept. of Zool. Duke Univ., pp 360

547 Stramski D, Reynolds RA (1993) Diel variations in the optical properties of a marine
548 diatom. Limnol Ocean 38:1347–1364.

549 Strom S (2001) Light-aided digestion, grazing and growth in herbivorous protists.
550 Aquat Microb Ecol 23:253–261. doi: [10.3354/ame023253](https://doi.org/10.3354/ame023253)

551 Sverdrup HU (1953) On Conditions for the Vernal Blooming of Phytoplankton. ICES J
552 Mar Sci 18:287–295. doi: [10.1093/icesjms/18.3.287](https://doi.org/10.1093/icesjms/18.3.287)

553 Sweeney BM, Hastings J (1958) Rhythmic cell division in populations of *Gonyaulax*
554 *polyedra*. J Protozool (3):217-24. doi: [10.1111/j.1550-7408.1958.tb02555.x](https://doi.org/10.1111/j.1550-7408.1958.tb02555.x)

555 Tarangkoon W, Hansen P (2011) Prey selection, ingestion and growth responses of the
556 common marine ciliate *Mesodinium pulex* in the light and in the dark. Aquat
557 Microb Ecol 62:25–38. doi: [10.3354/ame01455](https://doi.org/10.3354/ame01455)

558 Tiselius P, Hansen B, Jonsson P, Kiørboe T, Nielsen TG, Piontkovski S, Saiz E (1995)
559 Can we use laboratory-reared copepods for experiments? A comparison of feeding
560 behaviour and reproduction between a field and a laboratory population of *Acartia*
561 *tonsa*. ICES J Mar Sci Journal du Conseil 52(3-4):369-76.

562 Vault D, Marie D, Olson RJ, Chisholm SW (1995) Growth of *Prochlorococcus*, a
563 photosynthetic prokaryote, in the equatorial Pacific Ocean. Science
564 268(5216):1480.

565 Vault D, Marie D. (1999) Diel variability of photosynthetic picoplankton in the
566 equatorial Pacific. Journal of Geophysical Research 104:3297-310

567 Watts PC, Martin LE, Kimmance SA, Montagnes DJ, Lowe CD (2011) The distribution
568 of *Oxyrrhis marina*: a global disperser or poorly characterized endemic?. J
569 Plankton Res 33 (4): 579-589. doi: [10.1093/plankt/fbq148](https://doi.org/10.1093/plankt/fbq148)

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Figure captions

Graphics program used: KaleidaGraph

Fig. 1 a Time course of *Rhodomonas salina* concentration (cell $\cdot \text{ml}^{-1}$) and **b** cellular volume (μm^3) after inoculum in batch culture. In both graphics, the days sampled as representative of exponential and stationary growth phases are highlighted. White and black squares indicate light and night hours respectively. Error bars represent standard error.

Fig. 2 Day and night ingestion rates of *Gyrodinium dominans* (a, c) and *Oxyrrhis marina* (b, d), expressed either in terms of prey number and of prey volume, for each experimental condition (exponential and stationary growth). Error bars indicate standard error and asterisks represent significant differences (p<0.01).

Fig. 3 Ingestion rates (in cells and volume) of *Gyrodinium dominans* (a, c) and *Oxyrrhis marina* (b, d) as a function of previous feeding ($p < 0.01$) (either fed or starved), expressed either in terms of prey number and of prey volume. Error bars indicate standard error and asterisks represent significant differences ($p < 0.01$).

Fig. 4 Feeding rhythm index (quotient between day and night ingestion rates) of *Oxyrrhis marina* (BCN and BCN-2016 strains) and *Gyrodinium dominans* as a function of prey availability. a) Values in cells ml^{-1} . b) Values in volume ($\mu\text{m}^3 \text{ml}^{-1}$). Error bars show standard error.

Table 1 Prey and grazer concentrations (cell ml⁻¹) used in the experiments on the effect of food concentration on diel rhythms. Cell concentrations were selected according to Calbet et al. (2013) to include limiting, intermediate and saturating food conditions.

Grazer	Treatment	Prey concentration (cell ml ⁻¹)	Grazer concentration (cell ml ⁻¹)
GYRO-BCN	Limiting	4000 – 10000	600 – 850
	Intermediate	4x10 ⁴ – 6x10 ⁴	1500 – 2800
	Saturated	10x10 ⁴ – 11x10 ⁴	3000 - 3500
OXY-BCN	Limiting	8000 – 12000	192 – 240
	Intermediate	15000 – 25000	257 – 330
	Saturated	15x10 ⁴ – 20x10 ⁴	1607 - 2100
OXY-BCN-2016	Limiting	8000	192
	Medium	15000	257
	Saturated	15x10 ⁴	1607

Table 2 Day and night C:N, C:P and N:P molar ratios (average $\pm$ SE) of *Rhodomonas salina* under exponential phase (a) and stationary phase (b). Significance levels (p-value) of two-tailed t-test comparing day and night averages are also shown.

a)

EXPONENTIAL	C:N	C:P	N:P
Day	7.7 ± 0.14	104.4 ± 20.24	13.6 ± 2.86
Night	6.4 ± 0.29	63.6 ± 8.26	9.56 ± 1.05
p-value	<0.001	0.003	0.01

b)

STATIONARY	C:N	C:P	N:P
Day	16.2 ± 0.88	245.7 ± 11.30	15.2 ± 0.97
Night	15.7 ± 1.04	229.8 ± 14.49	14.7 ± 0.35
p-value	0.56	0.18	0.37

Table 3 Day and night C:N, C:P and N:P molar ratios (averages $\pm$ SE) of two-day starved (a) *Gyrodinium dominans* (Gyr-BCN) and (b) *Oxyrrhis marina* (OXY-BCN). Significance levels (p-value) of two-tailed t-test comparing day and night averages are also shown.

<i>Gyrodinium dominans</i>	C:N	C:P	N:P
Day	5.8 $\pm$ 0.07	46.0 $\pm$ 13.4	8.0 $\pm$ 2.4
Night	5.6 $\pm$ 0.06	39.4 $\pm$ 12.1	7.0 $\pm$ 2.2
p-value	0.01	0.55	0.64
b)			
<i>Oxyrrhis marina</i>	C:N	C:P	N:P
Day	5.8 $\pm$ 0.09	34.5 $\pm$ 8.2	6.0 $\pm$ 1.4
Night	5.7 $\pm$ 0.06	31.8 $\pm$ 8.0	5.6 $\pm$ 1.4
p-value	0.20	0.70	0.77