Predator chemical cue effects on the diel feeding behaviour of marine protists

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Abstract

We have assessed the effect of copepod chemical cues on the diel feeding rhythms of heterotrophic and mixotrophic marine protists. All phagotrophic protists studied exhibited relatively high diurnal feeding rates. The magnitude of the diel feeding rhythm, expressed as the quotient of day and night ingestion rates, was inversely related to the time that phagotrophic protists were maintained in the laboratory in an environment without predators. In the case of the recently isolated ciliate *Strombidium arenicola,* the rhythm was lost after a few months. When challenged with chemical alarm signals (copepodamides) from the copepod *Calanus finmarchicus* at realistic concentrations (0.6-6 pM), *S. arenicola* partially re-established diurnal feeding. Conversely, the amplitude of the diel feeding rhythm for the ciliate *Mesodinium rubrum* was not affected by copepodamides, although the 24 h integrated food intake increased by approximately 23%. For the dinoflagellates *Gyrodinium dominans* and *Karlodinium armiger*, copepodamides significantly reduced the amplitude of their diel feeding rhythms; significant positive effects on total daily ingestion were only observed in *G. dominans*. Finally, the dinoflagellate *Oxyrrhis marina,* isolated >20 years ago, showed inconsistent responses to copepodamides, except for an average 6% increase in its total ingestion over 24 h. Our results demonstrate that the predation risk by copepods effects the diel feeding rhythm of marine protists and suggests a species-specific response to predation threats.

Keywords: copepodamide, feeding rhythms, grazing, copepods, microzooplankton, dinoflagellate, ciliate

1. Introduction

Microzooplankton are key components of marine planktonic food webs, representing a crucial trophic link between primary producers and mesozooplankton [1, 2]. Despite their relevance, some key aspects of microzooplankton trophic behaviour and their impacts on planktonic food webs are still unclear. This is the case, for instance, for diel feeding rhythms. While laboratory-based studies with different protist species have repeatedly reported higher ingestion rates during the day-time (hereafter referred to as diurnal feeding) than during the night-time (e.g., [3-7]), the reasons for the existence of these rhythms are not yet well understood. Arias et al. [8] proposed that the diurnal feeding rhythm of marine protists could have evolved as a strategy to minimize the risk of predation, given that their main predators, copepods, typically exhibit nocturnal feeding (Fig. 1). Feeding by free-living protists involves motility, therefore increasing conspicuousness and encounter rates with predators [9]. Thus, an optimal microzooplankton might have developed an inverted feeding rhythm to that of its predator as a compromise between gathering food and avoiding predation [10-12]. Indeed, a predation-avoidance strategy has already been proposed to drive diel rhythms in larger zooplankton like copepods [13, 14], but such behavioural responses to predation have not yet been demonstrated in microzooplankton.

Copepods release different types of chemical cues in the surrounding waters [15] that induce defensive traits in their prey (Fig. 1). The most well-known are copepodamides [16], which induce toxin production in the dinoflagellate *Alexandrium minutum* [16] and in the diatom *Pseudo-nitzschia seriata* [17], a reduction of the chain length in the diatom *Skeletonema marinoi* [17] and an increase in the bioluminescence capacity in

55 various dinoflagellate species [18, 19]. Nevertheless, the effect of copepodamides on
56 the feeding activity of marine phagotrophic protists remains unexplored.

57 Within this framework, we aimed to evaluate the effects of predation risk on the diel
58 feeding rhythms of marine protists. We first explored how the time kept in predator-
59 free cultures affects the presence of diel feeding rhythms in several species of
60 heterotrophic and mixotrophic protists. Then, we simulated the presence of copepod
61 predators by using copepodamides and experimentally investigated the effects of the
62 threat of predation (mediated by chemical cues) on the rhythmic feeding activity of
63 these grazer protists.

64

65 **2. Materials and methods**

66 *(a) Prey and grazer cultures*

67 We used the heterotrophic ciliate *Strombidium arenicola* (strain ICM-ZOO-SA1-
68 2017), the mixotrophic ciliate *Mesodinium rubrum* (strain DK-2009), the
69 heterotrophic dinoflagellates *Gyrodinium dominans* (strain ICM-ZOO-GD1-2011)
70 and *Oxyrrhis marina* (strain ICM-ZOO-OM1-1995), and the mixotrophic
71 dinoflagellate *Karlodinium armiger* (strain ICM-ZOO-KA1-2013) as grazers in our
72 experiments. All strains were isolated from the NW Mediterranean Sea between 1995
73 and 2017, except for *M. rubrum*, which was isolated from Danish waters in 2009 (Dr.
74 Per J. Hansen, University of Copenhagen). Stock cultures were kept in a cold room at
75 19 ± 1°C and grown on 38 PSU autoclaved filtered seawater enriched with metals (1
76 mL metal stock per litre; [20]), provided with irradiance of 90 μE m^{-2} s^{-1} (white
77 fluorescent) and a 10:14 h L:D cycle. Grazers were fed the cryptophyte *Rhodomonas*

78 *salina* (strain K-0294) daily, except for *M. rubrum*, which was fed the cryptophyte
79 *Teleaulax amphioxeia* (strain K-1837) every other day. *R. salina* was isolated from
80 danish waters and *T. amphioxeia* was isolated from the Elsinore Harbour. Batch
81 cultures of *R. salina*, provided with gentle air bubbling, were grown in f/2 medium
82 and diluted daily to maintain exponential growth. *T. amphioxeia* was grown under the
83 same conditions but without air supply.

84

85 *(b) Diel grazing rhythm experiments*

86 We first analysed the permanence of diel feeding rhythms in the target species. Two
87 replicate experiments were conducted per each species, except for the recently
88 isolated *S. arenicola*. For this species, four experiments were carried out,
89 corresponding to 6 (October 2017), 10 (February 2018), 19 (November 2018) and 20
90 (December 2018) months after the time when it was isolated (April 2017).

91 Grazing experiments were conducted under saturated prey conditions, specific to each
92 studied species (Table 1; functional response data from Arias, unpublished; Calbet et
93 al. [21]; Martínez, unpublished; Fig. S1). In the experiments, *R. salina* was used as
94 prey for all grazers and it was offered in stationary phase to avoid day/night size
95 differences (see Arias et al. [3]). Prior to the experiments, the grazers were starved for
96 48 h to ensure that previously ingested prey-cells were completely processed prior to
97 the experiment [3, 22-24]. In the experiment setup, two suspensions were prepared:
98 one only with the prey to serve as a control for prey growth and another with the same
99 concentration of prey and the desired number of grazers. The grazing incubations
100 were conducted in 72 ml polyethylene culture flasks (three replicated experimental

and control flasks on each), which were incubated on a plankton wheel (0.2 r.p.m) from the beginning (9:00 a.m.) until the end of the day (7 p.m.), at 19 ± 1°C, and 90 μE m^{-2} s^{-1} irradiation; the experiment was then repeated for the night-time incubation under complete darkness (from 7 p.m. to 9 a.m.). Concentrations of prey and grazers were determined with a Beckman Coulter Multisizer III (100-μm aperture tube) at the beginning and the end of each incubation period.

(c) Effects of copepodamides on protist feeding behaviour

Copepodamides are surface-active and degrade over time in seawater, leading to an actual concentration (i.e., effective concentration) lower than the initially added concentration (i.e., nominal concentration) [25, 26]. For this reason, before undergoing grazing experiments we carried out a preliminary test to assess the effective concentrations of copepodamides at the starting point and during the experiments, and also to determine the most appropriate concentrations to be used (Fig. S2; see Supplementary Materials for the determination of effective concentrations methodology).

To test the effect of predation risk on the rhythmic feeding behaviour and the total daily ingestion (i.e., day and night sum) of the target grazers, we carried out diel feeding experiments using two copepodamide treatments, 1.4 and 18 pM initial concentrations (average effective concentrations during incubations of 0.6 and 6 pM, respectively; Table 2). Copepodamides were extracted from freeze-dried *Calanus finmarchicus* through a series of chemical separation steps (see Selander *et al*. [16] for further details). The lowest concentration used in our study was within the natural range of copepodamide concentrations (0.4-2 pM; [17, 26]). As concentrations may vary widely depending on the density of copepods or the proximity to the source, we

125 also included a higher concentration (average effective concentration of 6 pM) to
126 cover this range.

127 The experimental methodology used for the feeding incubations was the same as the
128 one described in the previous section (*Diel grazing rhythm experiments*), but in this
129 case, the control treatments had methanol added at the highest concentration used as
130 diluent for the copepodamide solution. Fresh copepodamide doses were prepared each
131 day and night. We conducted the experiments twice to ensure data robustness. The
132 experiments with *S. arenicola* were conducted after 19 and 20 months of laboratory
133 cultivation, when no diel feeding rhythm was apparent.

134 *(d) Statistical analysis*

135 To explore the existence of significant differences of grazers ingestion rates between
136 day-time and night-time, we applied t-tests comparing the results obtained from the
137 triplicate bottles of each treatment on the day-time and the night-time. We also
138 applied t-tests to determine the effect of copepodamides on the diel feeding rhythm of
139 the grazers in relation to the rhythm exhibited when no chemical cues were added.

140

141 **3. Results**

142 *(a) Laboratory time-dependent diel feeding rhythm*

143 For the whole group of protists studied, there was a negative relationship between the
144 time from isolation and the amplitude of the diel feeding rhythm, defined as the
145 quotient day/night ingestion rates (Fig. 2). In general, the magnitude of the rhythm
146 ranged from 1.5 (*O. marina*) to 3 times (*S. arenicola* and *G. dominans*) higher

147 ingestion rates during the day than during the night (Fig. 2). All species showed
148 significant differences between day and night ingestions ($p<0.01$ in all cases). The
149 rhythm was still detectable after 22 years of laboratory cultivation in *O. marina*.

150 Conversely, the diel feeding rhythm of the recently isolated ciliate *S. arenicola*
151 decreased more rapidly over time in a predator-free laboratory environment (Fig. 3a);
152 ingestion rates during day-time were 3 times significantly higher than during night-time
153 when first measured (t-test, $p<0.001$; October 2017), but these diel differences
154 completely disappeared after 19 months of maintenance in the laboratory (November
155 2018; t-test, $p>0.05$; Fig. 3a).

156 *(b) Effect of predation risk on the diel feeding rhythm of laboratory-cultured protists*

157 When exposed to grazer cues, under both concentrations of copepodamides, the diel
158 feeding rhythm of *S. arenicola* was partially reinstated (27-45% recovery relative to
159 the treatment without copepodamides; t-test, $p<0.05$ in all treatments; Fig. 3b; see
160 Table S1 for actual day and night ingestion rates). This enhancement of the diel
161 feeding rhythm did not consistently affect total daily ingestion (Table 3). The day and
162 night ingestion rate quotient of the other ciliate species, *M. rubrum*, also showed a
163 positive response to copepodamides (Fig. 4a), but it was weak (3-10% increase) and
164 not significant (t-test, $p>0.05$ in all cases). However, in this case, the total daily
165 ingestion increased by 23%, on average (Table 3).

166 Dinoflagellates were less consistent and showed variable responses to
167 copepodamides. The amplitude of the diel feeding rhythm of *G. dominans* decreased
168 by approximately 13% and 8% in copepodamide exposures of 0.6 and 6 pM,
169 respectively (t-test, $p<0.05$ in all cases; Fig. 4b; Table S1). Total ingestion over 24 h,

170 on the other hand, increased by 10%, on average (Table 3). *K. armiger* also
171 significantly reduced the feeding rhythm in a dose-dependent manner, 22% in 0.6 pM
172 and 46% in 6 pM copepodamide exposure (t-test, $p<0.05$ in all treatments; Fig. 4c;
173 Table S1). The total daily ingestion of this species was only significantly different
174 from the control in the higher (6 pM) copepodamide exposure in one of the two
175 replicated experiments (Table 3). Finally, the *O. marina* response to copepodamides
176 was inconsistent (Fig. 4d; Table S1); in the first experiment, the amplitude of the
177 feeding rhythm decreased 2-23% when exposed to copepodamides (t-test, $p<0.05$ for
178 the lowest copepodamide concentration), but in the second experiment, it increased
179 significantly by 8%-12% (t-test, $p<0.05$ in all treatments). The effects of
180 copepodamides on total ingestion (over 24 h) on this species ranged from non-
181 significant to a 11% reduction (Table 3).

182

183

184 **4. Discussion**

185 In this study, we provided the first evidence of a modulation in the diel feeding
186 behaviour of marine protist grazers in response to predator chemical cues. Moreover,
187 we also showed that copepodamides have the potential to reinstate the diel feeding
188 rhythm in a ciliate, whose inherent rhythmic behaviour was lost when reared under
189 predator-free laboratory conditions.

190

(a) Loss and recovery of the feeding rhythms in the laboratory: the particular case of ciliates

The absence of predators under laboratory rearing conditions appeared to be a probable factor inducing the loss of feeding rhythm in our strain of the ciliate *S. arenicola*. Similarly, the other protists studied also seemed to show a time-dependent weakening of their diel feeding rhythm, although at a much longer scale (years). Similar results were observed by Arias et al. [3] when comparing the feeding rhythm amplitude of two strains of the dinoflagellate *O. marina* isolated in different years (1995 and 2016), with the newest isolated strain showing the highest amplitude feeding rhythm, although we cannot exclude inter-strain variability.

The fading of a diel feeding rhythm in the absence of predators in the laboratory has already been documented for marine copepods [27], and the presence of fish has also been reported to sharply enhance their diel feeding cycle [28], although the role of chemical cues alone might not be so clear [29, 30]. However, the physical presence of fish can induce changes in some behavioural and morphological traits of copepods. For example, fish presence has been reported to induce diapause in copepods from freshwater ecosystems [31], as well as mating behaviour alterations [32], changes in body and clutch sizes [33], and variations in the pigmentation level used as photoprotection [34]. Other groups, such as freshwater rotifers and cladoceran, however, are more prone to respond to predator chemical cues. For instance, freshwater water fleas develop behavioural (e.g., [35]), morphological (e.g., [36]) and life-history trait (e.g., [37]) responses as anti-predator defences to predator exudates or physical presence. Additionally, rotifers display morphological responses, involving the development and elongation of spines and appendages with the

consequent increment in body size, to kairomones produced by copepods [43]. Similar responses have been described in dinoflagellate defensive mechanisms as a response to copepod chemical alarm signals. Lindström et al. [18] reported an increase in the total bioluminescence capacity of the long-term laboratory-cultivated (9-14 years) dinoflagellates *Lingulodinium polyedra* and *Alexandrium tamarense* when exposed to copepodamide dose treatments. Similarly, the production of toxic secondary metabolites in dinoflagellates (described as another defence mechanism against predators) is also reduced when organisms are cultivated in the laboratory [18], but it is also restored under exposure to waterborne copepod cues [39] and copepodamides [16].

The recovery of the diel feeding rhythm in *S. arenicola* when exposed to copepodamides resulted in a significant decrease in ingestion rates during the night (see Table S1), supporting the hypothesis of a relationship between feeding rhythm and threat of predation. The effect of predation threat also translated into the decrease in the total ingestion rate observed in this species. In contrast, in *Mesodinium rubrum,* feeding rhythms were not significantly affected, and the total daily ingestion rate increased when exposed to copepodamides. Therefore, the two ciliates studied responded differently to predator chemical cues. The difference may have resulted from behavioural differences between species. It is known that predation risk to ciliates is determined by their escape ability [9, 40]. In our study, *S. arenicola*, such as other *Strombidium* species, was expected to have a relatively low escape ability [40]. Consequently, at night, when copepods ascent to surface layers and may overlap with ciliates, this species may benefit from reduced nocturnal feeding (which implies lower swimming activity) to reduce conspicuousness and hence safeguard its survival. Conversely, when predators are absent, continuous feeding seems to be more

advantageous. *M. rubrum*, on the other hand, exhibited a very different swimming behaviour based on a combination of long motionless periods interspersed with shorter periods of quick jumps. Previous studies have highlighted the effective escape response of *M. rubrum* when surrounded by copepods, which substantially reduces its vulnerability to predator mortality in comparison to that of other planktonic ciliates [41]. In fact, *M. rubrum* is characterized by an extremely high swimming speed for a protist (at over 5 mm s^{-1} and up to 8.5 mm s^{-1}, at least momentarily; [42]), approximately an order of magnitude faster than most other ciliates [43]. Therefore, the non-significant response of *M. rubrum* to copepodamides may be based on its high capability to escape from predators, which may make it less necessary for this species to largely modify its diel feeding behaviour.

(b) Contrasting responses of dinoflagellates to copepodamides

The general response of dinoflagellates to copepodamide exposure was a decrease in the amplitude of the diel feeding rhythm, except for *O. marina*, which did not present a clear response. Regarding the heterotrophs *G. dominans* and *O. marina*, the variation in the amplitude of the diel feeding rhythm was caused by an unequal increase in both diurnal and nocturnal feeding and a consequent significant increase in total daily ingestion rates (Table S1). In contrast to ciliates, dinoflagellates are not able to escape from copepods due to their limited swimming capacity [40]. Thus, we believe that when threatened by predation, heterotrophic dinoflagellates may increase total daily prey uptake, independent of a dictated diel feeding rhythm, to maximize their energy intake for reproduction and ensure the rapid growth of the population, guaranteeing their survival. In environments with high predation risks, faster growth

264 has been suggested as an adaptive response to outgrow the hunting impact of the
265 predator in the population [44]. An increase in the prey growth rate as a defence
266 response to predation risk has also been described in water fleas [45, 46].

267 In the particular case of *O. marina*, the ambiguous results of the effect of predation risk
268 on the diel feeding rhythm (increasing *versus* decreasing its amplitude) could also be
269 associated with the habitat of the species. This dinoflagellate typically thrives in
270 intertidal pools, and shallow waters [47-49], which might be environments where diel
271 migration is probably not as relevant than in open, deeper aquatic domains. Hence, this
272 dinoflagellate may not have experienced the necessity to evolve predator-defence
273 mechanisms associated to diel rhythms.

274 Both *K. armiger* and *G. dominans* showed a reduction in the magnitude of feeding
275 rhythms when exposed to copepodamides. However, in contrast to *G. dominans*, *K.*
276 *armiger* did not consistently increase its total ingestion rate. The pattern observed in *K.*
277 *armiger* with a decrease in diurnal feeding and an increase in nocturnal feeding might
278 be partially related to the grazer photosynthetic activity. This dinoflagellate presents
279 higher growth rates when feeding on microalgal prey (Li et al., 1999; Berge et al.,
280 2008a; 2012) and, under saturated prey conditions, phagotrophy represents the main
281 source of carbon (Berge, 2016); however, when threaten by predators, the strategy of
282 *K. armiger* might be based on boosting photosynthetic activity during the day and
283 devote night hours to feed when they have the possibility to catch higher size prey (no
284 upper prey size limit has been described for this dinoflagellate), by which they adquire
285 essential growth factors (Berge et al., 2008). Nevertheless, we consider that this
286 particular behaviour might be also related to the capability of *K. armiger* to produce
287 karmitoxin, a toxin that can cause the rapid (within minutes; [50]) immobilization and

mortality of copepods [51]. Toxin production in dinoflagellates has been reported to be induced by the presence of copepods and their chemical signals [52, 53] and, recently, by copepodamides [16]. Several dinoflagellates have efficient grazer deterrent traits that alone probably allow them to co-exist with copepods [19, 54].

In this study we have demonstrated that the risk of predation by copepods can strongly affect the diel feeding rhythms of micrograzers, hence becoming an important trigger of such rhythmicity. However, other triggers may exist and, moreover, other factors are already known to modify this rhythmic activity. For instance, the feeding behaviour of marine protists is widely modified by prey availability, with the major differences between day and night ingestion rates under saturated food conditions, and poorly marked or inexistent rhythms under food limitation [3]. Moreover, in natural communities, the feeding patterns of marine protists have been suggested to vary according to prey and grazers species composition [55].

Finally, diel feeding rhythms in marine protists become an important event to consider when addressing grazing approaches *in situ* [56]. Not talking into account this rhythmic activity may lead to considerable vias or incorrect interpretation of the results as it involves important differences depending on the phase of the diel cycle.

5. Final remarks

In this study, we have shown that predation threat can affect the feeding behaviour of several heterotrophic and mixotrophic protist species. The overall pattern of a gradual decrease in the diel feeding rhythm in long-term predator-free laboratory cultures may

311 indicate, the importance of predation risk in modulating feeding behaviour. However,
312 given we only have the whole time-course of one strain of one species and that other
313 evidences may be slightly marginal we cannot disregard other factors, such us strain
314 variability and loss of diversity, taking place. Moreover, the diversity of the
315 responses to copepodamides as a proxy for predation threat by copepods, their main
316 natural predator, suggests a species-specific response, depending on the physiological
317 (e.g., deterrent production), behavioural (e.g., hydrodynamic conspicuousness and
318 escape ability) and ecological (e.g., habitat) traits of the grazers. Nonetheless, we
319 should consider that the risk of predation might not be the only trigger of the diel
320 feeding rhythm in all marine protists.
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322
323 **Supplementary information.** Supplementary information is available online at
324 *Microbial Ecology* and includes (a) the functional response of the ciliate *Strombidium*
325 *arenicola*, (b) a detailed explanation of the methodological process conducted to
326 determine copepodamide effective concentrations and the resultant plot of
327 copepodamide effective concentration throughout the incubation, and (3) a table
328 containing day-time and night-time ingestion rates per grazer species studied in each
329 experiment.
330
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Graphics program. All Figures have been elaborated using KaleidaGraph, except for Figure S2 which was elaborated with MATLAB.

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Table 1. Prey (*Rhodomonas salina*) and grazer concentrations (cells mL^{-1}) used in the feeding experiments. The period of time the grazer cultures were maintained under laboratory conditions is also shown.

Table 2. Initial, final and average effective concentrations of copepodamides during the feeding incubations. The half-life (T1/2) of the copepodamides is also provided.

Table 3. Total daily ingestion rates (day+night sum; prey μm^3 $grazer^{-1}$ day^{-1}) of the studied grazers under the different copepodamide concentrations. The percentage of variation with respect to the control treatments is also provided. Data from Experiment 1 and Experiment 2 are presented separately. ANOVA Dunnett test p-values are shown. *n.s.* indicates no significant differences.

Fig. 1 Illustration of microzooplankton feeding during the day and during the night, when they are exposed to increased threat of predation by copepods.

Fig. 2 Diel feeding rhythms, as the quotient between day and night ingestion rates, of *S. arenicola*, *M. rubrum*, *G. dominans*, *K. armiger* and *O. marina* as a function of the time in culture since isolation. All day ingestion rates were significantly higher than the night ingestion rates (t-test, $p<0.01$). Dashed lines indicate the value of equal day and night ingestion rates (i.e., non-existence of diel feeding rhythm), and error bars show the standard deviation.

Fig. 3 (a) Temporal evolution in the diel feeding rhythm of the ciliate *S. arenicola*, expressed as the quotient between day and night ingestion rates, from isolation (October 2017) until December 2018. Asterisks indicate significant differences between day and night ingestion rates (t-test, $p<0.001$) (b) Recovery of the diel feeding rhythm in *S. arenicola*, after 19 and 20 months from isolation, as a function of copepodamide effective concentrations. Yellow and green symbols denote two independent experiments. Asterisks indicate significant differences between copepodamide treatments relative to the control (t-test, $p<0.05$). Dashed lines indicate the values of equal day and night ingestion rates (i.e., non-existence of diel feeding rhythm). Error bars show the standard errors.

Fig. 4 Diel feeding rhythms, as the quotient between day and night ingestion rates, of (a) *M. rubrum*, (b) *G. dominans*, (c) *K. armiger*, and (d) *O. marina* as a function of copepodamide effective concentrations. Yellow and green denote two independent experiments. Asterisks indicate significant differences between copepodamide treatments relative to the control (t-test, * $p<0.05$). Dashed lines indicate the values of equal day and night ingestion rates (i.e., non-existence of diel feeding rhythm). Error bars show the standard errors.

Fig. S1 Ingestion rate of the ciliate *Strombidium arenicola* (μm^3 $grazer^{-1}$ h^{-1}) as a function of prey concentration (μm^3 mL^{-1}). Error bars show standard error.

Fig. S2 Effective concentration (nM) of copepodamides during 10h incubation. Closed circles represent the average data from the sampling time points and shaded area is the error interval (standard deviation).

Table S1 Day and night ingestion rates (in terms of prey volume ingested, μm^3 $grazer^{-1}$ $hour^{-1}$) for each of the studied grazer species as a function of copepodamides treatments (Control, 0.6 pM and 6 pM). Rates are differentiated between Experiment 1 and Experiment 2. Average ± standard error are shown. *p*-values from one-way ANOVA followed by a Dunnet's test are presented to show the significance level of each copepodamides treatment with respect to the correspondent control in each phase (day and night).

Table 1

Grazer	Time since isolation	Prey concentration (cell mL^{-1})	Grazer concentration (cell mL^{-1})
Strombidium arenicola	6 months	46079 – 48952	175 – 343
	10 months	75091 – 77118	259 – 387
	19 months	78094 – 80915	206 – 388
	20 months	81544 - 84929	272 - 462
Mesodinium rubrum	8 years	10570-12860	1510-2988
Gyrodinium dominans	6 years	100700 - 110500	3000 - 3580
Karlodinium armiger	4 years	100000-111800	6130-7500
Oxyrrhis marina	22 years	140010-160500	1705-2360

Table 2

Initial concentration (pM)	Final concentration (pM)	Average effective concentration (pM)	$T_{1/2}$ (h)
1.4	0.2	0.6	6.2
18	2	6	3.2

Table 3

		EXPERIMENT 1			EXPERIMENT 2		
Species	**Treatment**	**Ingestion (avg ± SE)**	**% variation**	***p***	**Ingestion (avg ± SE)**	**% variation**	***p***
	Control	51229 ± 1782	0	-	26285 ± 463	0	-
S. arenicola	1.4	46374 ± 2312	-9.5	*n.s.*	25422 ± 751	-3.3	*n.s.*
	18	44325 ± 443	-13.5	<0.05	24168 ± 392	-8.1	*n.s.*
	Control	228 ± 0.7	0	-	732 ± 14	0	-
M. rubrum	1.4	315 ± 9.1	38.1	<0.001	817 ± 16	11.6	<0.05
	18	275 ± 7.8	20.6	<0.01	892 ± 20	21.8	<0.001
	Control	6078 ± 68	0	-	7307 ± 91	0	-
G. dominans	1.4	6718 ± 53	10.5	<0.001	8061 ± 35	10.3	<0.001
	18	6997 ± 43	15.1	<0.001	7704 ± 17	5.4	<0.01
	Control	2448 ± 46	0	-	1807 ± 14	0	-
K. armiger	1.4	2305 ± 58	-5.8	*n.s.*	1753 ± 12	-3.0	*n.s.*
	18	2286 ± 23	-6.6	*n.s.*	2118 ± 23	17.2	<0.001
	Control	13305 ± 116	0	-	17035 ± 77	0	-
O. marina	1.4	14442 ± 253	8.5	<0.01	17749 ± 81	4.2	<0.05
	18	13509 ± 54	1.5	*n.s.*	18849 ± 192	11	<0.001

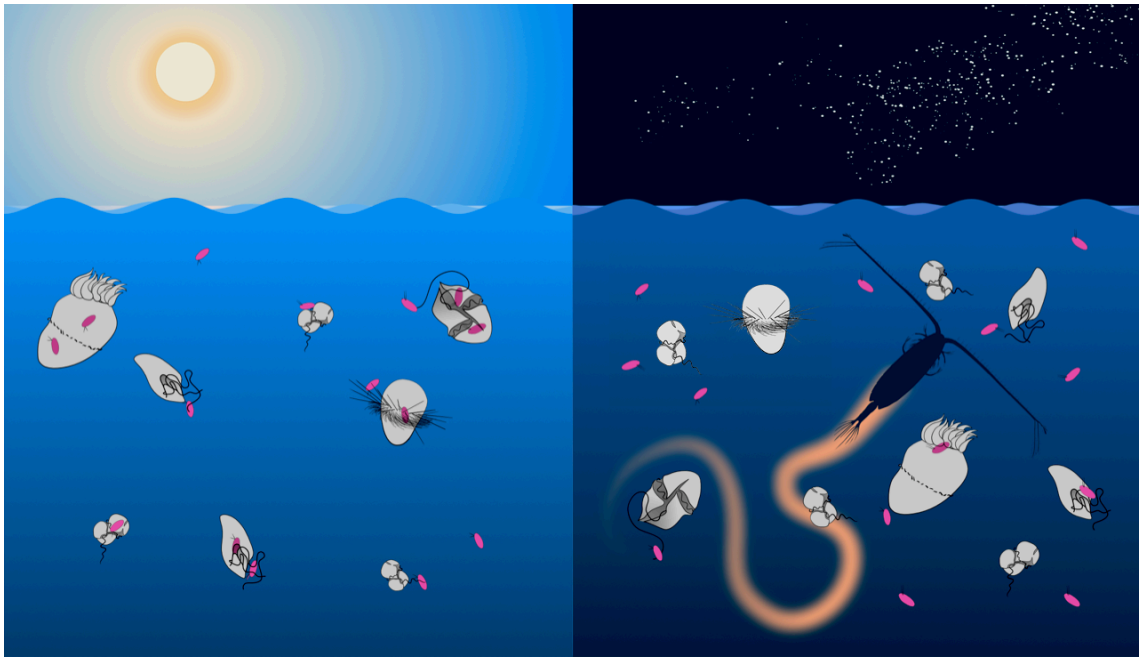

Figure 1

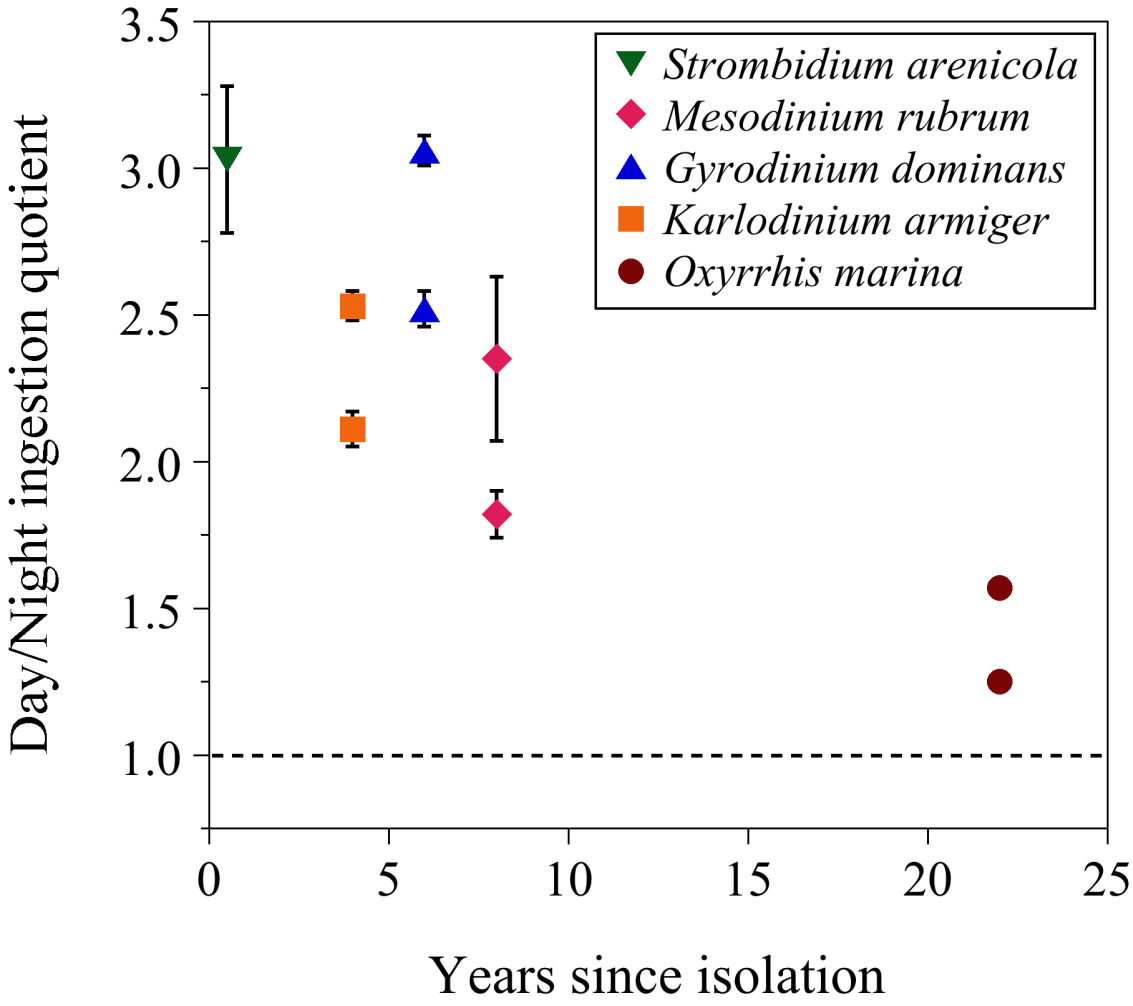
Day/Night ingestion quotient
3.5
3.0
2.5
2.0
1.5
1.0
0
5
10
15
20
25
Years since isolation
Strombidium arenicola
Mesodinium rubrum
Gyrodinium dominans
Karlodinium armiger
Oxyrrhis marina

Figure 2

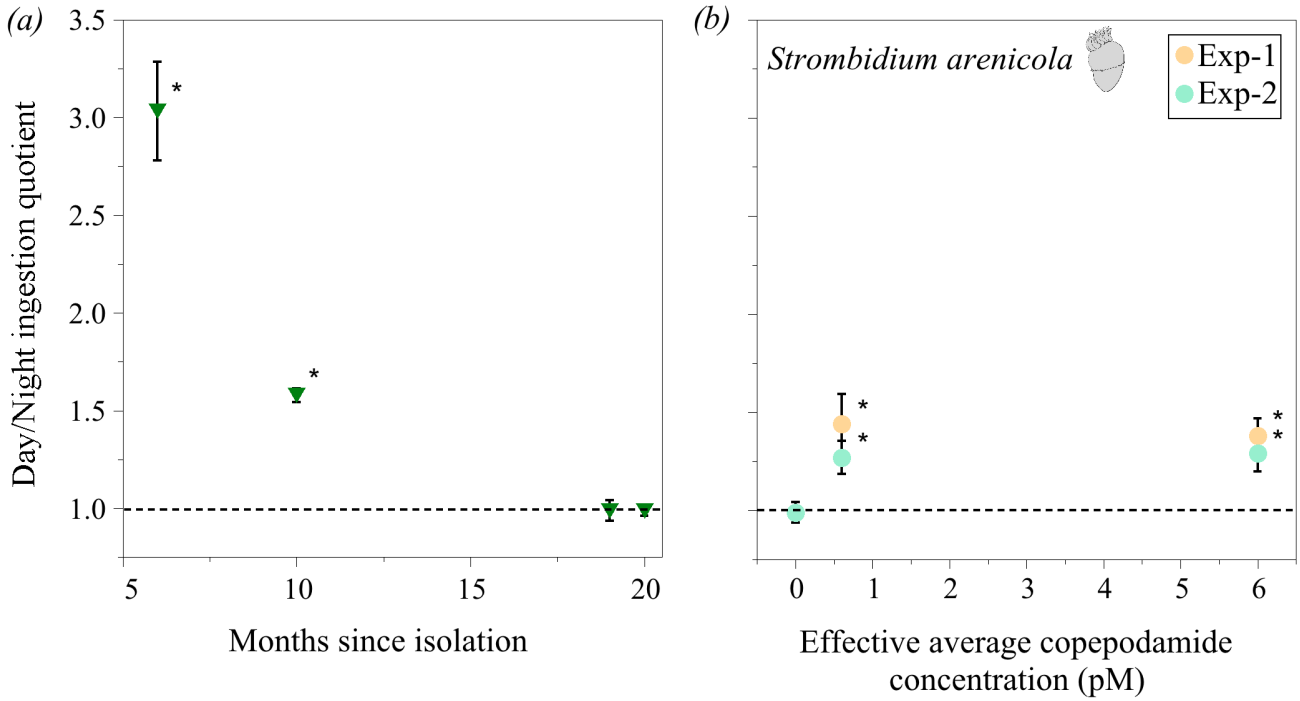
(a)
3.5
3.0
2.5
2.0
1.5
1.0
Day/Night ingestion quotient
*
*
5
10
15
20
Months since isolation
(b)
Strombidium arenicola
Exp-1
Exp-2
*
*
*
*
0
1
2
3
4
5
6
Effective average copepodamide concentration (pM)

Figure 3

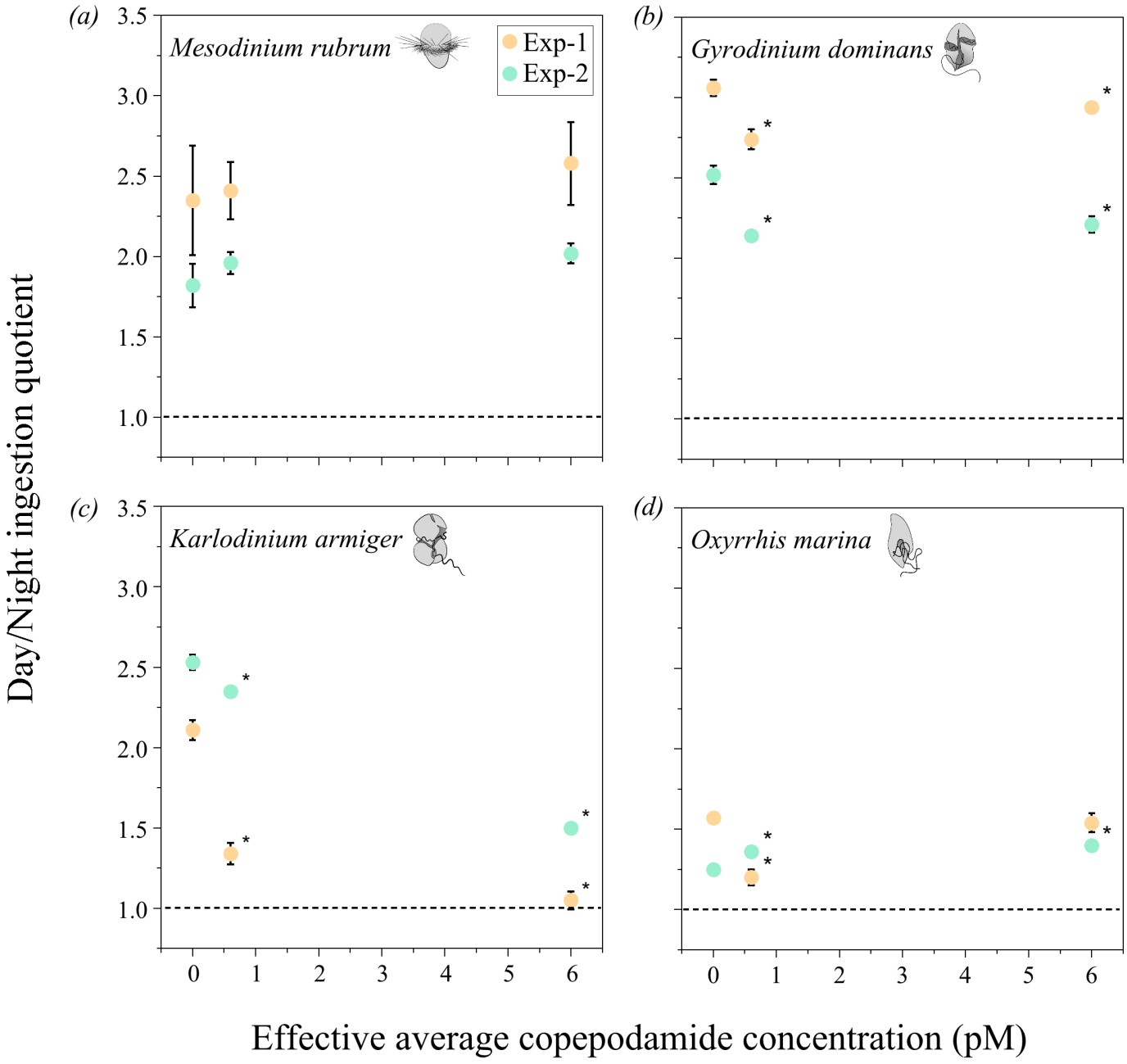
(a)
Mesodinium rubrum
Exp-1
Exp-2
(b)
Gyrodinium dominans
(c)
Karlodinium armiger
(d)
Oxyrrhis marina
Day/Night ingestion quotient
Effective average copepodamide concentration (pM)
3.5
3.0
2.5
2.0
1.5
1.0
0
1
2
3
4
5
6
*

Figure 4

Microbial Ecology

Predator chemical cue effects on the diel feeding behaviour of marine protists

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a) Functional response of the recent isolated ciliate Strombidium arenicola

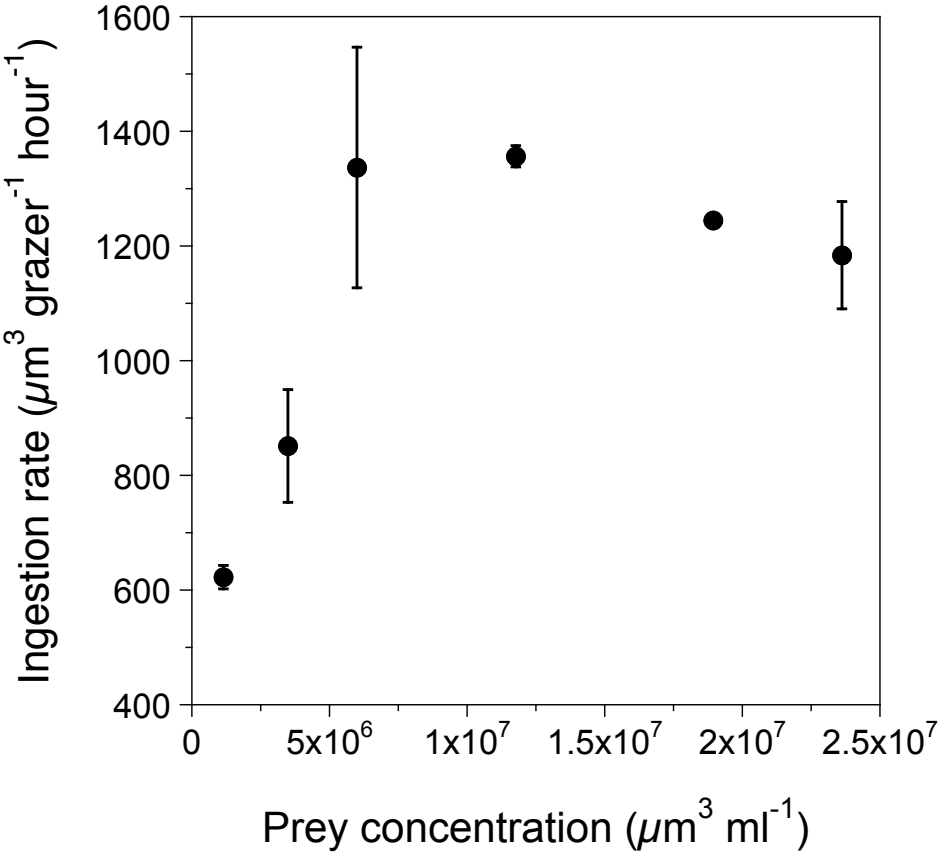

Figure S1

b) Determination of copepodamides concentrations

As copepodamides are surface-active and degrade over time, a preliminar test was carried out to measure the effective concentrations in the experiments over time. Copepodamides were extracted from freeze-dried *Calanus finmarchicus*, both male and female, through a series of chemical separation steps (see Selander et al., 2015 for details). The experimental procedures to assess the losses of copepodamide were performed in identical conditions to that of the feeding experiments (see below section).

Four sets of suspensions in FSW medium were prepared with mixtures of the desired prey and grazer concentrations, with copepodamides added at the following nominal concentrations: 0 (only adding methanol, the diluent), 0.01, 0.1 and 1 nM. Each suspension was split into twelve 72 ml polyethylene culture flasks, to get three replicates per each copepodamide concentration at every sampling time: t=0 (initial samples), 2, 5, and 10 (final samples) hours. Flasks were all incubated on a plankton wheel (0.2 r.p.m) at 19 ± 1ºC, and an irradiation of 90 μE m^{-2} s^{-1}. The triplicate samples from each concentration removed at every sampling time were loaded onto solid-phase extraction (SPE) columns (Evolute Express ABN, 100 mg, 3ml, Biotage). The columns were de-salted with 1 column volume MilliQ water and the compounds eluted into 3 ml methanol. The methanol evaporated and the copepodamides were then resolved in a small (80 μl) volume before analysis on an Agilent 1260 Infinity HPLC system connected to an Agilent 6410 Triple Quad LC/MS (see Selander et al., 2015 for further details).

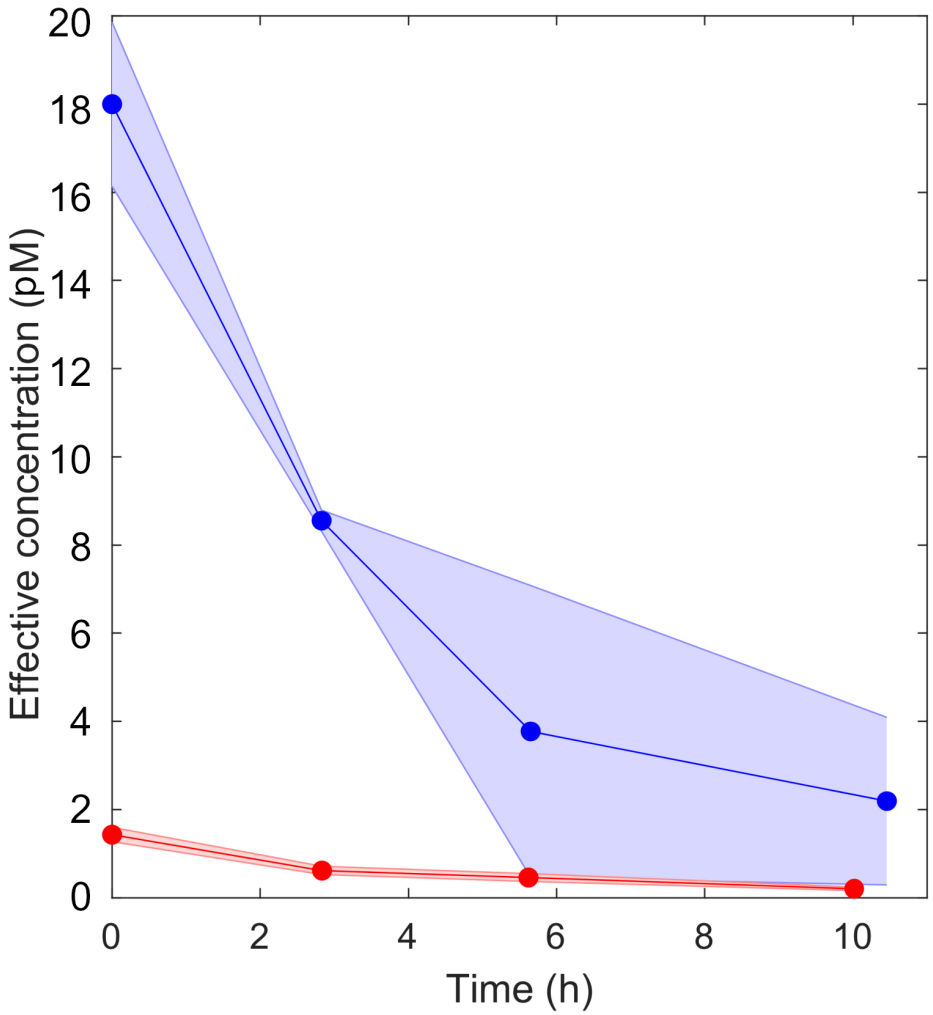

Figure S2

Table S1

c) Total daily ingestions of the target grazers

	EXPERIMENT 1										EXPERIMENT 2									
	Control (Methanol)		0.6 pM				6 pM				Control (Methanol)		0.6 pM				6 pM			
Species	**Day ±SE**	**Night ±SE**	**Day ±SE**	**p-value**	**Night ±SE**	**p-value**	**Day ±SE**	**p-value**	**Night ±SE**	**p-value**	**Day ±SE**	**Night ±SE**	**Day ±SE**	**p-value**	**Night ±SE**	**p-value**	**Day ±SE**	**p-value**	**Night ±SE**	**p-value**
S. arenícola (Oct 17)	810.9 ±27.7	267.4 ±20.4																		
S. arenícola (Feb 18)	2590.5 ±55.4	1640.2 ±12.7																		
S. arenícola (Nov-Dec18)	2120.6 ±22.2	2144.5 ±111.6	2347.7 ±97.4	n.s.	1635.5 ±161.9	<0.05	2199.1 ±83.8	n.s.	1595.3 ±83.4	<0.05	1087.9 ±22.0	1100.4 ±17.8	1206.0 ±52.7	n.s.	953.7 ±48.3	n.s.	1160.7 ±40.0	n.s.	897.2 ±55.2	<0.05
M. rubrum	14.3 ±0.9	6.1 ±0.6	19.9 ±0.3	<0.001	8.3 ±0.5	<0.05	17.8 ±0.2	<0.01	6.9 ±0.5	n.s.	41.4 ±0.4	22.7 ±1.0	47.7 ±1.4	<0.01	24.3 ±0.4	n.s.	52.7 ±0.9	<0.001	26.0 ±0.8	<0.05
G. dominans	417.1 ±6.9	136.2 ±0.3	444.7 ±3.7	<0.05	162.2 ±3.4	<0.001	474.0 ±5.2	<0.001	161.2 ±0.7	<0.001	470.0 ±3.5	186.2 ±4.0	487.4 ±3.2	<0.05	227.6 ±3.1	<0.001	472.0 ±5.4	n.s.	213.2 ±4.3	<0.01
O. marina	702.4 ±10.0	448.7 ±1.3	666.8 ±24.3	n.s.	555.3 ±9.2	<0.001	706.8 ±20.7	n.s.	460.1 ±11.0	n.s.	803.5 ±9.2	642.9 ±1.2	873.0 ±10.2	<0.01	644.2 ±11.5	n.s.	941.4 ±6.2	<0.0001	673.9 ±10.8	n.s.
K. armiger	147.2 ±4.1	69.7 ±0.7	112.9 ±5.4	<0.01	84.0 ±1.2	<0.0001	97.8 ±4.8	<0.001	93.5 ±2.0	<0.0001	116.4 ±1.9	45.9 ±0.5	109.9 ±0.8	<0.05	46.7 ±0.3	n.s.	109.7 ±1.2	<0.05	73.0 ±0.8	<0.0001