



# Protistal Grazers Increase Grazing on Unicellular Cyanobacteria Diazotroph at Night

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In this study, we have for the first time analyzed diel microzooplankton grazing selectivity on unicellular cyanobacterial diazotroph (i.e., *Crocospaera watsonii* WH8501) and non-diazotrophic unicellular microalga (i.e., *Chlorella autotrophica*). A mixed diet consisting of these two phytoplankton was supplied to four species of protistal grazers during daytime and nighttime, respectively. *C. watsonii* fixes nitrogen during nighttime and showed a stronger diel pattern of cellular C:N ratio than *C. autotrophica*. All four grazers ingested more nighttime *C. watsonii* than daytime *C. watsonii*, suggesting the diazotroph became more nutritious (inferred by C:N ratio) and thus a preferred prey for grazers when it fixes nitrogen. In particular, *Oxyrrhis marina* changed from preferring *C. autotrophica* during daytime to preferring *C. watsonii* during nighttime. The rest grazers showed species-specific grazing preferences, which could be explained by extracellular polysaccharide production of *C. watsonii*, feeding mode, cingulum size and cell size of grazers.

**Keywords:** C:N ratio, *Crocospaera*, *Chlorella*, protist, grazing selectivity

## INTRODUCTION

Nitrogen (N) is essential to the growth and metabolism of life, and it often limits primary productivity in the ocean (Moore et al., 2013). N<sub>2</sub> fixation by diazotroph is an important source for biologically available N in the euphotic zone, in which its significance equals or exceeds the flux of dissolved inorganic N from deep waters (Capone et al., 2005). In oligotrophic waters, the fixed N is especially a significant N source, contributing to approximately half of primary production (Karl et al., 1997). The fixed N can be released to the marine environment or transferred to other organisms through endogenous and exogenous processes. For example, the most studied diazotroph *Trichodesmium* could release its fixed N directly (Ohlendieck et al., 2000), through extracellular release (Capone et al., 1994), viral cell lysis (Ian et al., 2004), programmed cell death (Berman-Frank et al., 2004) and grazing (O'Neil et al., 1996). Unlike *Trichodesmium*, how the unicellular cyanobacterial diazotrophs (UCDs) transfer their fixed N to the environment and other organisms is unclear. In recent years, there was a growing concern about trophic interaction between UCDs and higher trophic levels (Wilson et al., 2017; Horii et al., 2018), with the majority of

related studies focusing on mesozooplankton (0.2–20 mm) grazing on UCDs (Scavotto et al., 2015; Conroy et al., 2017).

However, it should be noted that microzooplankton (<200  $\mu\text{m}$ , mainly protists) are the dominant grazers of phytoplankton in the marine environments, especially in oligotrophic waters (Calbet and Landry, 2004). Microzooplankton consume 60–75% of primary production (i.e., daily phytoplankton growth) from estuarine and coastal waters to oligotrophic open ocean (Calbet and Landry, 2004), greatly exceeding the consumption by mesozooplankton such as copepods (Gifford et al., 1995). Therefore, microzooplankton grazing plays an essential role in transferring energy and nutrients from phytoplankton to the consumers of higher trophic levels (John and Davidson, 2001; Strom et al., 2001). Hence, microzooplankton should also be major grazers of UCDs in oligotrophic waters and they might play an important role in transferring fixed N from UCDs to higher trophic levels and to other phytoplankton by releasing N to the surrounding water during grazing. However, the rate and selectivity of microzooplankton grazing on UCDs are unclear.

The grazing rate and selectivity of microzooplankton were proposed to be affected by various cellular properties of the prey (Gonzalez et al., 1990, 1993; González et al., 1990; Monger and Brown, 1999; Breckels et al., 2011). However, the nutritional quality of prey has been increasingly regarded a major factor in prey selection of protists (Verity, 1991; John and Davidson, 2001; Shannon et al., 2007; Siuda and Dam, 2010; Ng et al., 2017). Generally, herbivores have a lower C:N ratio (with a C:N ratio ranging 4–6.3; Koski, 1999; Pertola et al., 2002; Ng et al., 2017) compared to phytoplankton (with a C:N ratio ranging 6–10; Quigg et al., 2003). According to Liebig's law of the minimum, phytoplankton N contents may determine the growth efficiency of the herbivores in terms of C (i.e., the efficiency of herbivores consuming carbon and converting it into biomass; Hessen et al., 2003). Thus, the C:N ratio of phytoplankton is considered to represent the nutritional value for grazers, with a lower C:N ratio considered as a hint of higher nutritional value (John and Davidson, 2001). Additionally, a number of studies have reported diel variation of grazing behavior of protists on marine phytoplankton (Dolan and Simek, 1999; Suzanne, 2001; Jakobsen and Strom, 2004), which could be partially explained by the diel variation in the nutritional quality of prey (Ng et al., 2017).

So far, studies on the effect of prey nutritional quality on protistan grazing have yielded inconsistent results. When protists were fed with mixtures of prey, both ciliates (Verity, 1991) and microflagellates (John and Davidson, 2001) showed higher ingestion rates on prey with low C:N ratio (i.e., high nutritional quality). Opposing results were found when protists were fed with single prey (Ng et al., 2017). These inconsistent results were obviously related to different experimental designs (i.e., single prey vs. mixed prey grazing experiments). When there is no choice of food, i.e., in single prey experiment, higher ingestion rates on low nutritional quality prey could be a result of compensatory feeding, where grazer compensates for poor nutritional quality (e.g., high C:N) by increasing food consumption to acquire sufficient nutrient (e.g., N)

(Siuda and Dam, 2010). Therefore, it is inadequate to use single prey experiments for studying zooplankton feeding selectivity.

There are three distinct groups of UCDs in the ocean (UCYN-A, UCYN-B and UCYN-C). UCYN-A and UCYN-B are widespread in tropical and subtropical oceans, but UCYN-A can extend their distribution to higher latitudes (Zehr et al., 2001; Martínez-Pérez et al., 2016). Based on a recent study of the distribution of diazotrophs in North Pacific Ocean, the abundance of UCYN-B ( $7.0\text{--}8.4 \times 10^9$  copies  $\text{m}^{-2}$ ) was comparable to, or higher than that of UCYN-A ( $4.7\text{--}7.4 \times 10^9$  copies  $\text{m}^{-2}$ ) in lower latitudes (Shiozaki et al., 2017). UCYN-C is the least studied and descriptions about UCYN-C are rare. These three groups of UCDs may form blooms or dominate diazotrophic community in oligotrophic waters (Robidart et al., 2014; Bonnet et al., 2016; Wilson et al., 2017). Among the groups of the UCDs, only the UCYN-B (i.e., *Crocospaera watsonii*) is cultivated. *Cyanothece* sp. TW3 was proposed as the only representative culture of UCYN-C (Taniuchi et al., 2012). However, the DNA similarity between *nifH* gene sequence of *Cyanothece* sp. TW3 and environmental clones of UCYN-C was only 92% (Foster et al., 2007; Cheung et al., 2017). *C. watsonii* exhibits a diel pattern of C:N ratio, with the cellular C:N ratio increasing during the light period and decreasing during the dark period (Dron et al., 2012). This is because *C. watsonii* conducts photosynthesis during daytime and  $\text{N}_2$  fixation during nighttime. Considering that prey with low C:N ratio is more nutritious and it could influence the grazing behavior of grazers (John and Davidson, 2001; Shannon et al., 2007; Ng et al., 2017), we hypothesize that *C. watsonii* is selectively grazed by microzooplankton when it is fixing nitrogen during nighttime. To test this hypothesis, a mixed diet consisting of *C. watsonii* and *Chlorella autotrophica* (non-diazotrophic unicellular microalgae with a similar size to *C. watsonii*) was used to feed four species of marine protists (*Oxyrrhis marina*, *Euplotes* sp., *Scuticociliate* sp. and *Lepidodinium* sp.) during daytime and nighttime. With this study, we aim to provide first insight to the diel pattern of microzooplankton grazing on UCD and their selectivity between diazotrophic and non-diazotrophic unicellular microalgae.

## MATERIALS AND METHODS

### Culture Conditions of Algae and Protists

All cultures used in this study were maintained at 23.5°C with a 12:12 h light:dark cycle at the light intensities of 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for at least 4 weeks before the grazing experiments. The cell sizes of prey and grazers were listed in **Table 1**. Two batches of cultures were kept under identical culture conditions but in a reversed 12:12 h light:dark cycle. This means when one batch of cultures was at the beginning of the light period, another batch of cultures was at the beginning of the dark period.

*C. watsonii* WH8501 was maintained in Scripps Oceanographic medium (Tuit et al., 2004) and *C. autotrophica* was maintained in *f/2* medium (Guillard and Ryther, 1962). Sterile dilution techniques were applied periodically to keep the prey in exponential growth phase. Starting from 2 weeks before the grazing experiments, semi-continuous cultures were

**TABLE 1** | Grazers and prey used in this study.

Grazer	ESD(±SD) (μm)	Prey	ESD(±SD) (μm)
<i>O. marina</i>	22.6 (±4.4)	<i>C. watsonii</i> WH8501	3.9 (±1.1)
<i>Euplotes</i> sp.	45.6 (±14.0)	<i>C. autotrophica</i>	3.5 (±1.1)
<i>Scuticociliate</i> sp.	19.1 (±4.2)		
<i>Lepidodinium</i> sp.	17.3 (±2.2)		

The cell size ESD, equivalent spherical diameter was measured using FlowCam.

diluted every 3 days. All four grazers, heterotrophic dinoflagellate *O. marina*, ciliates *Euplotes* sp. and *Scuticociliate* sp., and the mixotrophic dinoflagellate *Lepidodinium* sp. were maintained in autoclaved, filtered (0.2 μm) seawater. *Lepidodinium* sp. and *O. marina* were fed with *Rhodomonas* sp. daily. *Scuticociliate* sp. and *Euplotes* sp. were supplemented with rice grains to enrich natural bacteria as food. Grazers were starved for 1 day before the grazing experiments.

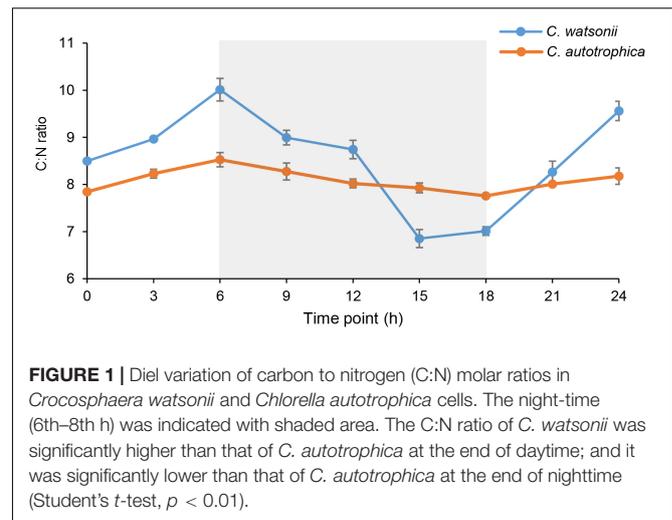
## Measurement of Cellular Content of Carbon and Nitrogen of Prey

Cellular C and N content of *C. watsonii* and *C. autotrophica* were determined every 3 h over a 24 h period to investigate the diel variation of cellular C:N ratio.

Ten ml of *C. watsonii* and *C. autotrophica* cultures in exponential growth phase were filtered onto pre-combusted (550°C, 5 h) glass-fiber filters (Whatman GF/A) and stored at -80°C before analysis using a CHNS elemental analyzer (Thermo Fisher Scientific Inc., Waltham, MA, United States). Three replicates of culture samples were collected and analyzed at each time point.

## Diel Grazing Experiment on Mixed Prey

The abundance of grazers and prey was quantified with a FlowCam (FlowCam 8000, Fluid Imaging Technologies, Yarmouth, ME, United States) right before the grazing experiments. Diel grazing experiments were carried out with ~40 ml final volume in 50 ml flasks. *C. watsonii* and *C. autotrophica* were mixed in a 1:1 ratio and the ratio of grazers to each prey was around 1:250~1:1000. For grazing experiments with *O. marina*, *Scuticociliate* sp. or *Lepidodinium* sp., each grazer and the mixture of prey were added to the flasks, followed by adding Scripps Oceanographic medium to make the concentration of grazers and each prey to around 50~170 cells ml<sup>-1</sup> and 50000~150000 cells ml<sup>-1</sup>, respectively. For grazing experiments with *Euplotes* sp. as grazer, the concentration of grazer and each prey was approximately 13 and 13000 cells ml<sup>-1</sup>, respectively. Each grazer was fed with a mixed prey during daytime (4 h before the end of light period) and nighttime (4 h before the end of dark period), respectively, representing the highest and lowest prey C:N ratio at the end of the light and dark period (Figure 1). In parallel, treatments without grazers were carried out as control. The grazing experiments lasted 4 h in darkness (Fulton, 1988; John and Davidson, 2001; Chen et al., 2010; Chrzanowski and Foster, 2014) and all the treatments were carried out in triplicates. To determine the abundance of prey, 1.8 ml of samples were collected from each



**FIGURE 1** | Diel variation of carbon to nitrogen (C:N) molar ratios in *Crocospaera watsonii* and *Chlorella autotrophica* cells. The night-time (6th–8th h) was indicated with shaded area. The C:N ratio of *C. watsonii* was significantly higher than that of *C. autotrophica* at the end of daytime; and it was significantly lower than that of *C. autotrophica* at the end of nighttime (Student's *t*-test,  $p < 0.01$ ).

treatment at the beginning and end of the grazing experiments. These samples were fixed with 50 μl 20% paraformaldehyde solution (0.5% final concentration, Guo et al., 2014) and stored at -80°C until analysis using a Becton-Dickinson FACSCalibur flow cytometer. Samples for determining the concentration of grazers were fixed with formaldehyde solution (4% final concentration, Herfort et al., 2011) at the end of the experiments and analyzed with a FlowCam.

## Data Analysis

Growth rate ( $\mu$ , h<sup>-1</sup>) of the prey was assumed to be exponential during the grazing experiment and was calculated as

$$\mu = \frac{\ln C_t - \ln C_0}{t}$$

where  $C_0$  and  $C_t$  were the concentration of prey (cell ml<sup>-1</sup>) at the beginning and end of each incubation experiment, and  $t$  was the incubation time (h).

Ingestion rate (IR, cell predator<sup>-1</sup> h<sup>-1</sup>) was calculated according to Harris, 2000,

$$IR = \frac{\mu_c - \mu_g}{C_g} * C_p$$

where  $C_p$  and  $C_g$  were the concentration of prey (cell ml<sup>-1</sup>) and grazer (predator ml<sup>-1</sup>) in each incubation experiment, and  $\mu_c$  (h<sup>-1</sup>) and  $\mu_g$  (h<sup>-1</sup>) were the growth rate of prey in the prey-only control and grazing treatments.  $C_g$  was the concentration of grazer at the end of each grazing experiment. The significance of the difference between the ingestion rate on two prey was verified by the Student's *t*-test.

The average concentration of prey ( $C_p$ ) was calculated as

$$C_p = \frac{C_0 * (1 - e^{\mu_g * t})}{(-\mu_g) * t}$$

Prey selectivity of protists on each prey was quantified using the selectivity index  $\alpha$  (Chesson, 1983):

$$\alpha_i = \frac{r_i/n_i}{\sum_{i=1}^m r_i/n_i}$$

where  $r_i$  was the proportion of prey  $i$  in the diet,  $n_i$  was the proportion of prey  $i$  in the environment.

## RESULTS

### Prey Diel C:N Stoichiometry

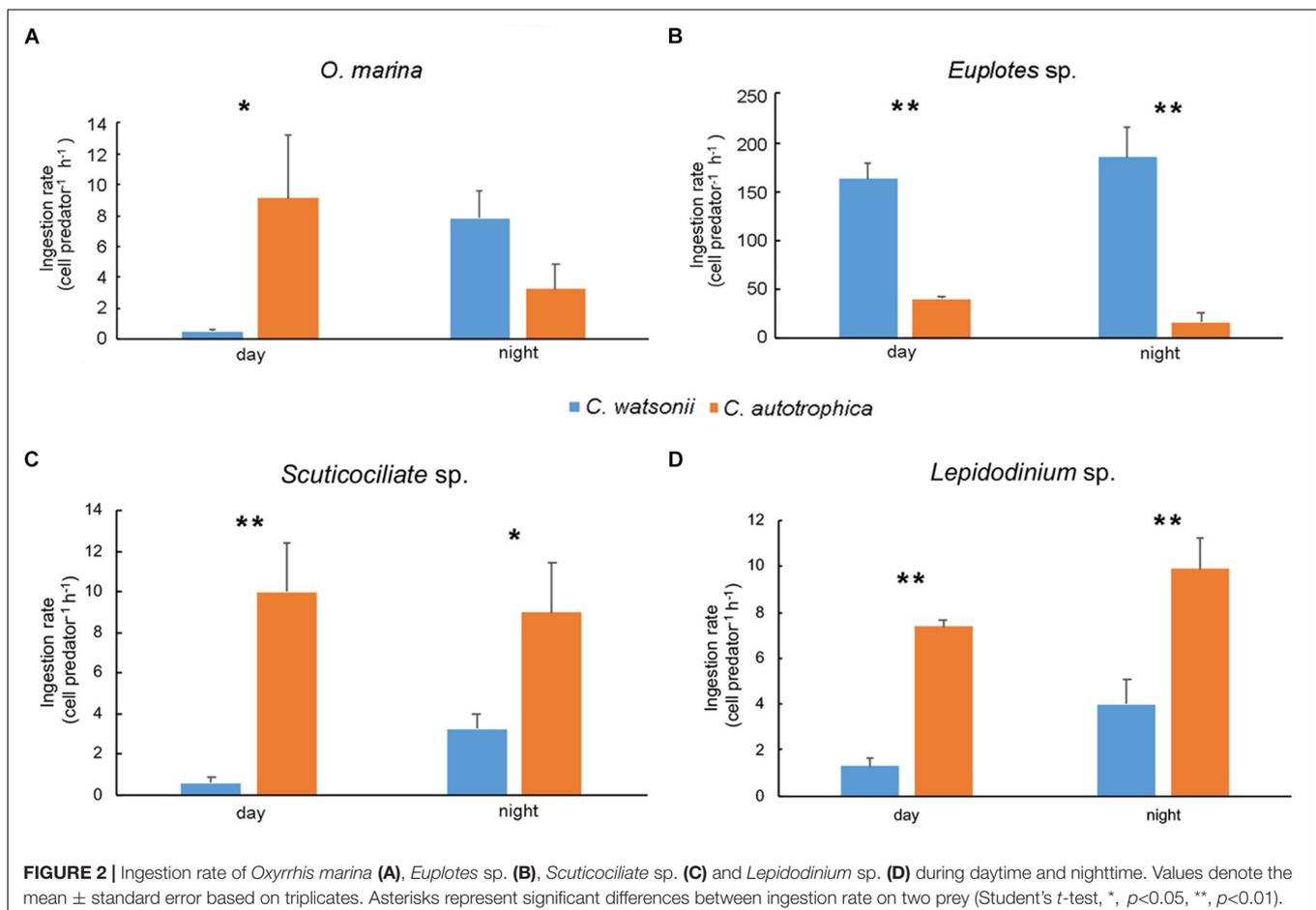
Both *C. watsonii* and *C. autotrophica* showed a diel pattern of cellular C:N molar ratio that increased during daytime and decreased during nighttime (Figure 1). The range of the diel variation of C:N ratio was larger in *C. watsonii* than in *C. autotrophica*. The C:N ratio of *C. watsonii* varied from a maximum of 10.0 at the end of daytime to 6.9 during nighttime while that of *C. autotrophica* ranged from 8.5 to 7.8.

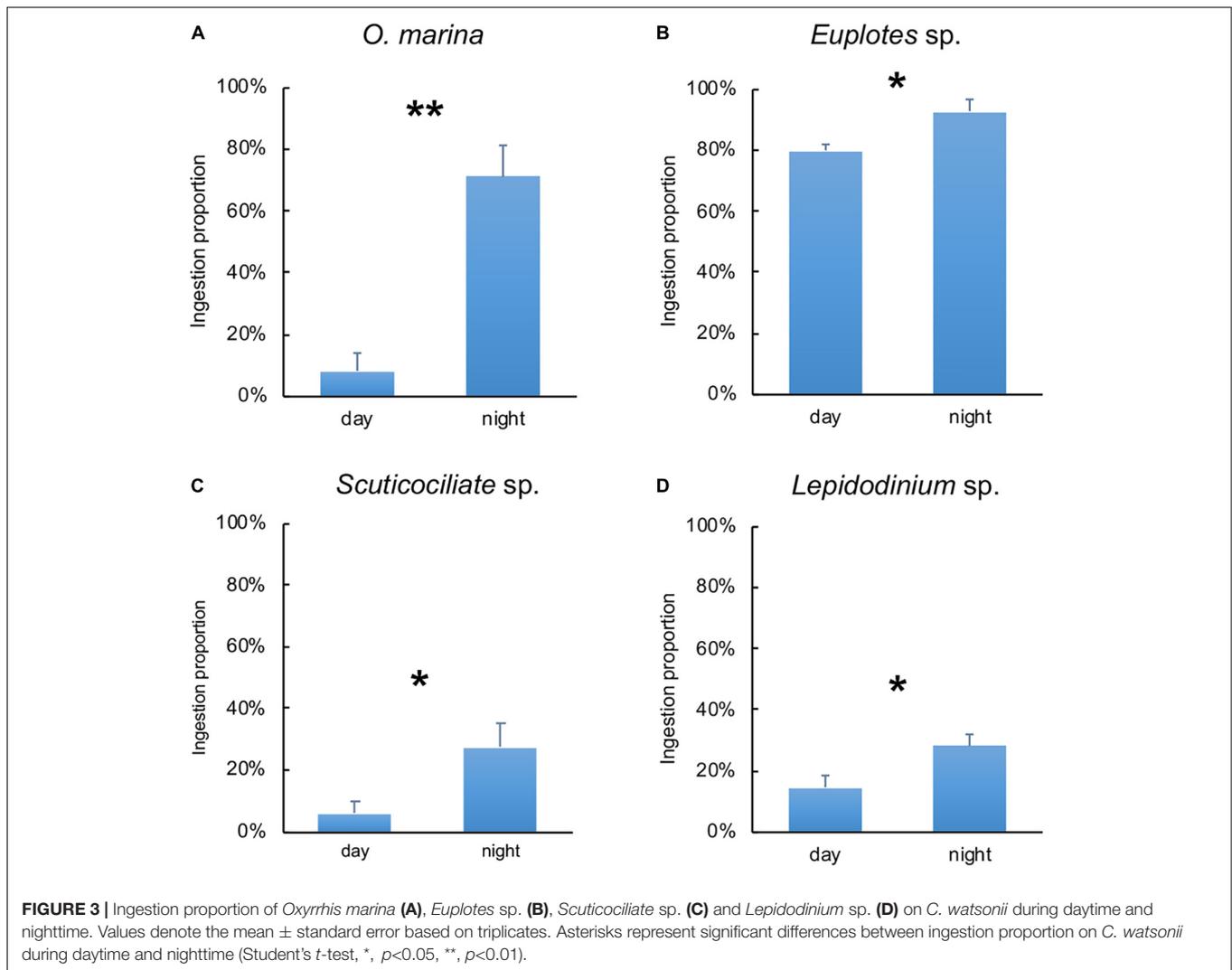
### Diel Mixed Prey Grazing Experiment

In general, all the grazers ingested more *C. watsonii* during nighttime than during daytime (Figure 2). In particular, *O. marina* ingested more *C. watsonii* (7.82 cell predator<sup>-1</sup> h<sup>-1</sup>)

than *C. autotrophica* (3.24 cell predator<sup>-1</sup> h<sup>-1</sup>) during nighttime, while it preferred *C. autotrophica* (9.11 cell predator<sup>-1</sup> h<sup>-1</sup>) over *C. watsonii* (0.54 cell predator<sup>-1</sup> h<sup>-1</sup>) during daytime. The other grazers showed species-specific grazing preferences. *Euplotes* sp. ingested *C. watsonii* (163.17 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and 184.75 cell predator<sup>-1</sup> h<sup>-1</sup> during nighttime) at a significantly higher rate than *C. autotrophica* (41.03 cell predator<sup>-1</sup> h<sup>-1</sup> and 16.68 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and night-time, respectively). In contrast, *Scuticociliate* sp. ingested much less *C. watsonii* (0.57 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and 3.25 cell predator<sup>-1</sup> h<sup>-1</sup> during nighttime) than *C. autotrophica* (9.97 cell predator<sup>-1</sup> h<sup>-1</sup> and 8.98 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and nighttime, respectively). Similarly, *Lepidodinium* sp. grazed on *C. autotrophica* at a rate of 7.38 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and 9.89 cell predator<sup>-1</sup> h<sup>-1</sup> during nighttime, which was significantly higher than the ingestion of *C. watsonii* (1.28 cell predator<sup>-1</sup> h<sup>-1</sup> and 3.99 cell predator<sup>-1</sup> h<sup>-1</sup> during daytime and night-time, respectively). Mean prey growth rates in the control experiments, and grazing mortality rates and net growth rates in all grazing experiments was shown in Supplementary Table S1.

The diel pattern of ingestion proportion of *O. marina*, *Euplotes* sp., *Scuticociliate* sp. and *Lepidodinium* sp. on *C. watsonii* revealed that the ingestion proportion increased





during nighttime (Figure 3). During nighttime, the ingestion proportion of *O. marina* on *C. watsonii* was around 71%, nearly nine times more than during daytime (8%). The ingestion proportion of *Euplotes* sp. on *C. watsonii* was 92% in the dark, higher than that in the light (80%). The ingestion proportion of *Scuticociliate* sp. on *C. watsonii* increased by more than four times from 6% to 27%. There was also a twofold increase in the ingestion proportion of *Lepidodinium* sp. on *C. watsonii* during nighttime. The selectivity index also indicated that all the grazers appeared to be more selective toward *C. watsonii* during nighttime (Table 2), when *C. watsonii* was richer in nitrogen (lower C:N ratio) than that of *C. autotrophica*.

## DISCUSSION

### Comparison of Diel C:N Ratio Patterns of *C. watsonii* and *C. autotrophica*

The diel C:N ratio pattern of *C. watsonii* has never been compared with that of other non-diazotrophic phytoplankton in the same

study. In our result, *C. watsonii* and *C. autotrophica* exhibited a diel C:N ratio variation with similar pattern (higher C:N ratio during daytime than during night-time), while the magnitude of the diel variation was much greater in the case of *C. watsonii* (Figure 1). During nighttime, the C:N ratio of *C. watsonii* decreased and the decreasing trend suddenly accelerated after 6 h in darkness (Figure 1), which agreed with the previous findings about C and N metabolism of *C. watsonii* during nighttime. *C. watsonii* was found to enhance respiration during the late light-early dark period for creating an oxygen depleted condition (Shi et al., 2010; Dron et al., 2012), and to promote the onset of the oxygen-sensitive nitrogenase at 3–6 h after entering dark phase (Dron et al., 2012). In contrast, the non-diazotrophic *C. autotrophica* showed a higher C:N ratio than *C. watsonii* at the end of nighttime. During daytime, it was interesting that the C:N ratio of *C. watsonii* was higher than that of *C. autotrophica*, which indicated that the C fixation through photosynthesis may exceed the requirements for N assimilation. Considering  $N_2$  fixation is an energy expensive process and requires respiration of carbohydrate as energy source (Mullineaux et al., 1980; Mitsui

**TABLE 2** | Manly–Chesson selectivity index  $\alpha$  of grazers feeding on mixed prey *C. watsonii* and *C. autotrophica* during daytime and night-time.

Grazer	Phase	Selectivity index $\alpha$	
		<i>C. watsonii</i>	<i>C. autotrophica</i>
<i>O. marina</i>	Day	0.04	0.96
	Night	0.71	0.29
<i>Euplotes</i> sp.	Day	0.80	0.20
	Night	0.91	0.09
<i>Scuticociliate</i> sp.	Day	0.06	0.94
	Night	0.28	0.72
<i>Lepidodinium</i> sp.	Day	0.17	0.83
	Night	0.20	0.80

The selectivity index  $\alpha$  varies between 0 (complete discrimination) and 1 (complete preference).  $\alpha > 0.5$  indicates a significant preference for the particular prey,  $\alpha = 0.5$  indicates non-selective grazing and  $\alpha < 0.5$  indicates discrimination against the particular prey (Chesson, 1983).

et al., 1986), *C. watsonii* may enhance photosynthesis compared to non-diazotrophic phytoplankton, resulting in a higher C:N ratio during daytime.

### Increased Grazing on UCDs by Microzooplankton During Night Time

Despite the fact that different grazers showed specific preferences for different prey, all showed higher selectivity for *C. watsonii* during nighttime (with the lowest C:N ratio) than during daytime (Figure 3). In particular, *O. marina*, a widely distributed free-living protist in marine ecosystems (Watts et al., 2011), closely followed the changes in C:N ratio over time with its grazing behavior. *O. marina* preferentially grazed on diazotrophs when they had a lower C:N ratio during nighttime and switched to prefer *C. autotrophica* when they had a lower C:N ratio during daytime. Therefore, our results are partially in line with the previous finding that prey C:N ratio determines the protistan grazing selectivity (Verity, 1991; John and Davidson, 2001), which suggested that the C:N ratio of prey is at least acting as a secondary controlling factor on protistan grazing selectivity. Additionally, given that mixed-prey experiments (Verity, 1991; John and Davidson, 2001) were much rarer than single-prey experiments (Shannon et al., 2007; Siuda and Dam, 2010; Ng et al., 2017), our results provide a more realistic assessment of microzooplankton grazing selectivity.

Moreover, our results suggest that microzooplankton tends to ingest more *C. watsonii* when the latter is fixing nitrogen during nighttime, independent of the grazer species. Although different microzooplankton show their preference on different kinds of phytoplankton prey, they seem to ingest more nutritious actively nitrogen fixing UCDs during nighttime as a supplement of bioavailable nitrogen. This could be an indication of the importance of diazotrophs as a nitrogen source to the microzooplankton in nitrogen limited oceanic waters. Furthermore, it has been reported that phytoplankton nutritional quality (C:N ratio) is important in regulating microzooplankton  $\text{NH}_4^+$ -N excretion, in which the grazers excrete more  $\text{NH}_4^+$  when they were fed with more nutritious prey (Lehrter et al.,

1999; Davidson et al., 2005). The excretion of dissolved inorganic N from zooplankton could stimulate bacterial/phytoplankton activity in nitrogen limited ocean (Ikeda and Motoda, 1978; Carman, 1994; Legendre and Rassoulzadegan, 1995). Microzooplankton has been proposed to be the dominant grazers of phytoplankton in oligotrophic oceans where the microbial loop is the dominant component of the planktonic food web (Jackson, 1980; Calbet, 2008). In the light of our finding, the grazing pattern of protists on diazotrophs might play a role in mediating protistal nutrient excretion and hence diel variation in bacterial/phytoplankton activity in oligotrophic waters. It should be noted that another dominant UCD *Candidatus Atelocyanobacterium thalassa* (UCYN-A) fixes N during daytime and they were found to be able to grow in higher latitude regions than *C. watsonii* (Sohm et al., 2011b; Martínez-Pérez et al., 2016; Shiozaki et al., 2017; Tang et al., 2019a,b). UCYN-A is a symbiont and the sizes of the UCYN-A1 and UCYN-A2 cell consortia are 1–3 and 4–10  $\mu\text{m}$  in diameter, respectively (Farnelid et al., 2016). In general, protists graze on prey that are about 1/10 of their own size (Kirchman, 2012). Therefore, protistal microzooplankton (<200  $\mu\text{m}$ ) might be also the important grazers of UCYN-A. Diel protistal grazing activity on different UCDs and the effects on bacterial/phytoplankton growth and metabolism in different oligotrophic ecosystems worth to be further studied.

### Other Factors Possibly Influencing the Grazing Selectivity

The grazer specific preference for *C. watsonii* (*Euplotes* sp.) and *C. autotrophica* (*Scuticociliate* sp. and *Lepidodinium* sp.) indicated that prey selection of protists is complicated; it is not determined by a single factor of the nutritional quality of the prey, but a number of other cellular properties of the prey and the feeding mode of the grazers. Considering that the cell size of prey also plays a crucial role in determining the grazing selectivity of protists (Chrzanowski and Simek, 1990; Gonzalez et al., 1990), we have chosen *C. autotrophica*, which has a similar size to *C. watsonii* (Table 1). There was a slight diel variation of cell size, in which the cell size of both prey types slightly increased during daytime (Supplementary Figure S1). Therefore, we suggest that the diel variation in prey cell size did not contribute significantly to diel grazing selectivity on *C. watsonii*. Besides cell size, cell surface characteristics, like extracellular polysaccharides (EPS), have been reported to adhere to the cilia and clog the feeding apparatus of ciliates and hence inhibit their grazing (Liu and Buskey, 2000). Moreover, EPS could cause aggregation of cells through adsorption and stickiness (Lüring and Donk, 2000). It has been reported that *C. watsonii* could produce significant amounts of EPS (Sohm et al., 2011a). Regarding the grazers, both *O. marina* and *Lepidodinium* sp. are phagotrophic dinoflagellates, grazing on prey by raptorial feeding (Jeong et al., 2010). Although the cell sizes of the two protists are similar (Table 1), *O. marina* is heterotrophic with a large cingulum, whereas *Lepidodinium* sp. is mixotrophic with a smaller cingulum (Hansen et al., 2007; Jeong et al., 2010). Hence, *O. marina* may have an advantage in engulfing larger prey cells, which may facilitate its ingestion on *C. watsonii* bounded by the EPS and even the aggregation

of *C. watsonii*. In addition, although both *Euplotes* sp. and *Scuticociliate* sp. are filter-feeding ciliates, the former is two times larger in cell size than the latter (Table 1). It has been reported that *Euplotes* sp. prefers to feed on bacterial aggregates rather than on free-living bacteria (Albright et al., 1987), which may explain why the *Euplotes* sp. preferred *C. watsonii* to *C. autotrophica*.

## AUTHOR CONTRIBUTIONS

LD and SC conducted the experiment and wrote the manuscript. HL designed the experiment and wrote the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00135/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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