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Effect of light on the metabolism of the foraminifera *Cribroelphidium selseyense* lacking photosymbionts and kleptoplasts

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ABSTRACT

Foraminifera are essential contributors to the marine carbon and nitrogen cycle. A small group of foraminifera hosts symbiotic microalgae and kleptoplasts and irradiance is a key variable influencing their metabolism. However, the majority of foraminifera is fully heterotrophic, and whether irradiance influences food ingestion patterns has remained an open question. We studied the food uptake of fully heterotrophic *Cribroelphidium selseyense* specimens exposed to varying light-dark cycles. Specimens obtained from the Baltic Sea were fed with lyophilised, isotopically labelled diatoms from the species of *Phaeodactylum triconutum*, to estimate the rate of food ingestion. We exposed the specimens to different light-dark cycles (0:24, 8:16, 16:8, 24:0 = light: dark) and irradiance intensities (0, 50, 100 and 200 μmol photons m^{-2} s^{-1}) in this experiment. Differences in light-dark regime did not affect the food uptake rates of *C. selseyense*. Irradiance intensity, however, strongly affected food uptake, increasing with incubation time from day 1 to day 15. In parallel, the food uptake decreased with higher irradiance intensity. Therefore, we can conclude irradiance intensity and not the light-dark cycle affected food uptake of fully heterotrophic *C. selseyense*, leaving the mechanisms of how light intensity regulates food intake being unresolved yet.

1. Introduction

Foraminifera from the class Sarcodina are unicellular protists and are mainly found in marine environments; some taxa also inhabiting freshwater and terrestrial environments [1,2]. They are essential components of the global aquatic carbon and nitrogen cycles [3, 4]. Foraminifera occur in large numbers in both, shallow marine areas, with more than 80 individuals per 10 x cm^2 [5], and in the deep sea (up to 190 individuals per 10 cm^2, [6]), where they ingest and metabolize large amounts of non-living organic matter into their biomass [7,8]. Physical parameters such as water temperature, salinity, and pH are of particular importance for their fitness [9–11].

Foraminifera are encased by single or multi-chambered solid housings, called tests, their size ranging from a few μm up to several cm. The tests are mainly constructed of calcite, but also other materials are used such as protein, sediment particles, aragonite, and silica [12]. They can reproduce both sexually and asexually and feed on phytodetritus, which they ingest via a pseudopodial network [41]. Food uptake and feeding behaviour depend on several factors, such as the temperature and salinity of sea water [9,10], and the type and quality of the food source [13,14].

Basal foraminifera neither contain photosymbionts or kleptoplasts, but more recently evolved forms developed these additional sources of energy generation to optimize their metabolism. The ecological advantage of this different lifestyles is that foraminifer, which host symbionts, can have positive feedbacks from their symbionts in the form of food exchange (sugar and amino acid export from kleptoplasts to foraminiferal hosts) [15]. So far, only nine benthic (no planktonic) foraminifera genera out of 1400 [16] are known to host kleptoplasts [17–26].

In a pilot study dealing with food uptake at different salinities, [42] observed that food ingestion of *Cribroelphidium selseyense* (formerly *Elphidium excavatum*) lacking photosymbionts and kleptoplasts might be

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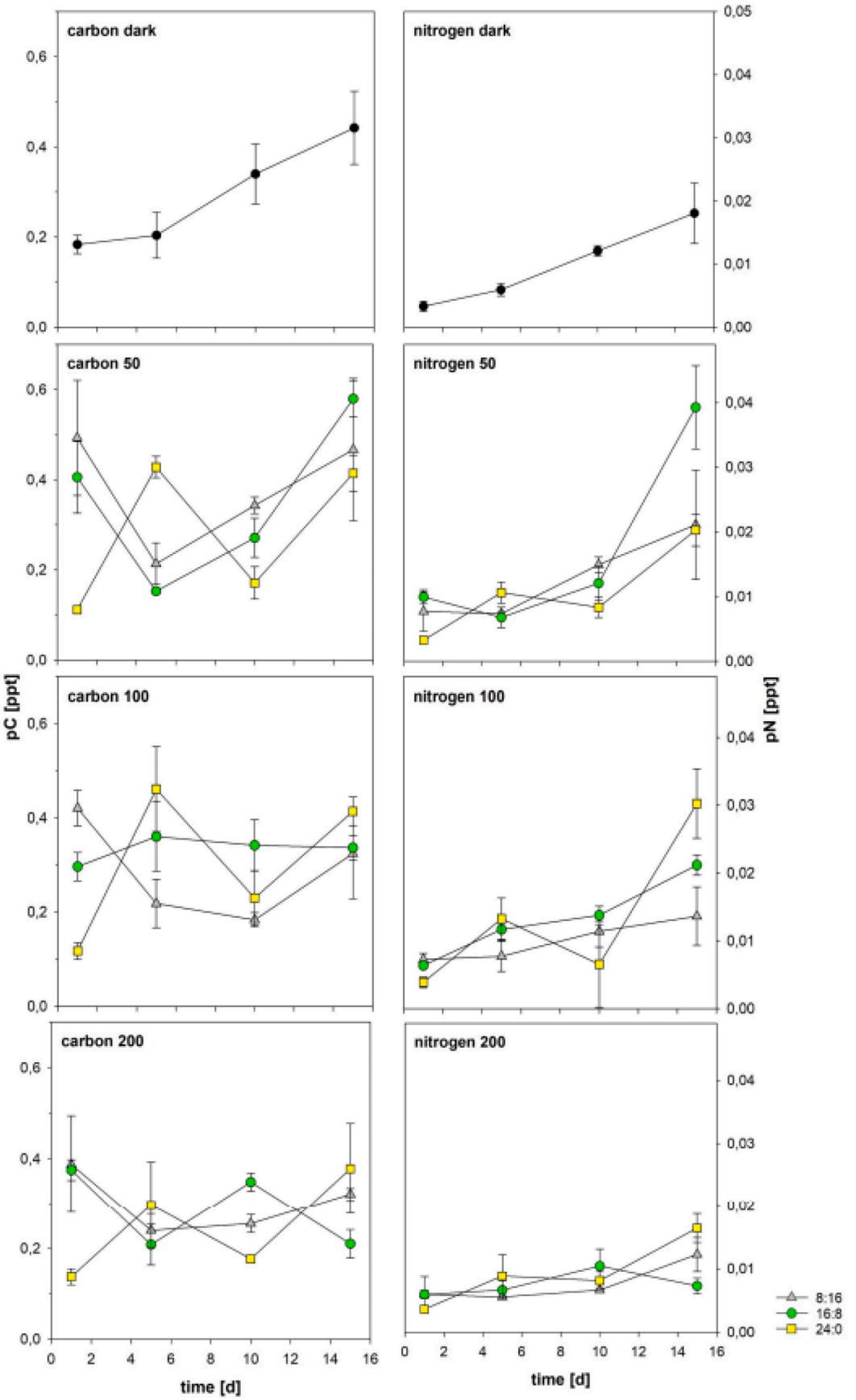

Fig. 1. Food C and N uptake by *C. selseyense* under different light-dark cycles and irradiances. The pC and pN values (50, 100, 200) correspond to the light intensity given in µmol photons m^{-2} s^{-1}. The black dots represent the mean values of 3 replicates, the error bars indicate the standard deviation. The light cycles are described in the legend and stand for a light-dark regime.

Table 1
Statistical evaluation of the effects of light: dark cycle, light intensity and the interaction of time and intensity on pC and pN across all times (day 1, 5, 10 and 15). Df = degree of freedom. Statistically significant values are depicted in bold.

light-dark cycle	parameter	pC/ pN	Df	Mean Square	F - ratio	p -value
0:24	time	pC	3	0.044323	12.71	**0.002**
	time	pN	3	0.000130	20.59	**<0.001**
8:16	time	pC	3	0.084880	14.2	**<0.001**
	time	pN	2	0.029561	4.95	**0.014**
	intensity	pC	3	0.000156	13.49	**<0.001**
	intensity	pN	2	0.000799	6.91	**0.003**
	interaction	pC	6	0.006468	1.11	0.388
	interaction	pN	6	0.000015	1.48	0.245
16:8	time	pC	3	0.032575	2.96	**0.047**
	time	pN	2	0.014067	1.28	0.293
	intensity	pC	3	0.000433	10.5	**<0.001**
	intensity	pN	2	0.000270	6.54	**0.004**
	interaction	pC	6	0.047034	23.46	**<0.001**
	interaction	pN	6	0.000183	31.23	**<0.001**
24:0	time	pC	3	0.182170	51.84	**<0.001**
	time	pN	2	0.010165	2.89	0.071
	intensity	pC	3	0.000582	39.53	**<0.001**
	intensity	pN	2	0.000056	3.78	**0.034**
	interaction	pC	6	0.005749	1.95	0.114
	interaction	pN	6	0.000038	4.31	**0.004**

influenced by light supply. They found that the food uptake of foraminifera was much higher if they were incubated at low light, in contrast to absolute darkness or high light. This interesting observation was hitherto unexplored, and a detailed investigation could significantly contribute to a better understanding of the metabolic cycle of fully heterotrophic foraminifera. One explanation for temporal light-dependant food ingestion patterns of heterotrophic foraminifera is the changes in the availability of their main food microalgae. Phytobenthos accumulates at the sediment surface during the day (e.g., [27]). On the contrary, benthic algae deplete low-molecular storage compounds due to catabolic activities at night, reducing their nutritional value. We therefore tested the food uptake rates of *C. selseyense* at different light cycle regimes and at different irradiance intensities. We hypothesize that food uptake of *C. selseyense* is lower under continuously dark conditions compared to daylight, peaks at low light intensities, and will decrease under highlight conditions.

2. Methods

We collected sediment during a sampling campaign with the research vessel F.S. Alkor in July 2020 in the Kiel Fjord (Baltic Sea, Germany), by means of a box-corer at a water depth of 14 m. The sampling area has sediments rich in organic compounds. It is partly dysoxic and the sea water is turbid [28]. Only 1% of the light at the sea surface reaches the seafloor at 10.7 m [29]. The light intensity at the sea water surface is about 280 μmol$*m^{-2}*s^{-1}$ between August and October [29]. According to Rohde et al. [29]; Fig. 1] mean light intensities of 200, 100 and 50 μmol photons m^{-2} s^{-1} as applied in this study correspond to a light supply at 1, 2.5 and 4 m water depth in the Kiel Fjord.

After wet-sieving the sediments through a 125 μm sieve with sea water, the fraction >125 μm was placed in a cool box and covered with sea water for transport. Back in Vienna, main cultures were maintained in an aquarium at 20 °C and a light dark cycle of 8:16 h at 50 μmol photons m^{-2} s^{-1}. In the last years we observed, that under these conditions, the culture remained intact for several months, as both algae and foraminifera reproduced, however it is not clear if the used 50 μmol photons m^{-2} s^{-1} are optimal for them. The foraminifera used for this experiments were removed from the main culture the very next day after arriving in Vienna, thus, the individuals had no time to acclimatize to a certain light intensity from the main culture.

For feeding experiments, we used the diatom species *Phaeodactylum tricornutum* cultivated in f/2 medium [30] at a light-dark cycle of 16:8 h at 20 °C. The culture medium (f/2) was enriched with ^{13}C (1.5 mmol L^{-1} $NaH^{13}CO_3$) and ^{15}N (0.44 mmol L^{-1} $Na^{15}NO_3$) to produce an isotopically labelled food source. For preparing the food source, the diatom cultures were centrifuged after the culture became an intense brownish colour, which correlates to a healthy and dense alga growth at 1200 rpm (rounds per minute) for 10 min. The algal pellet was rinsed three times with artificial sea water [31], then frozen in liquid nitrogen and lyophilized at −55 °C and 0.180 mbar for 3 days.

For the experiments, 60 foraminifera (>125 μm) for every treatment and every harvest were taken from the raw culture. The specimens were then split into three replicates (20 organisms per replicate). The foraminifera were carefully cleaned from adhering particles with a brush and rinsed repeatedly with sterile-filtered seawater to minimize growth of other potential food sources, and were then transferred into crystallization dishes filled with 250 ml sterile filtered (0.45 μm) sea water from the sampling site. We only took individuals with intact cytoplasm, which is an indication for healthy organisms in good condition. All individuals were first tested if they have any photosynthetic chloroplasts (or kleptoplasts) using PAM (photoacoustic microscopy) chlorophyll fluorescence imaging – Imaging PAM Microscopy Version – Walz GmbH). No individual showed a presence of active chloroplasts/kleptoplasts or photosynthetic symbionts. The incubation experiments took place in crystallization dishes at 18 °C, under different light-dark cycles, i.e., 8:16 h, 16:8 h and 0:24 h for 1, 5, 10 and 15 days. To avoid temperature variation during the experiments, they were carried out in a cold room with a constant temperature. Additionally the used light source was an LED-lamp which produces no heat during usage. All cycles were run at 200, 100, 50 μmol photons $m^{-2}s^{-1}$; additional experiments were conducted under dark conditions. As three replicates were run per treatment, the overall sample number was 120. We added 5 mg lyophilised algae powder as a food source to each replicate at the beginning of the experiments. As we still observed food particles after the experiments were finished, adequate food was available during the experiment. The dishes were sealed with parafilm to avoid evaporation during the experiment. After harvest, the foraminifera were cleaned from food particles, rinsed 3 times with distilled water and transferred into Sn capsules. Individuals were then dried at room temperature for 3 days; tests were dissolved with 10 μl HCl (4%) at 50 °C inside the Sn capsules and the remaining protoplast weighed.

Carbon and nitrogen contents and their isotopic ratios were measured at the Stable Isotope Laboratory for Environmental Research (SILVER) of the University of Vienna. To quantify the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, an isotope mass spectrometer was used (IRMS, Delta^{PLUS}, coupled via a ConFlo III interface to an elemental analyser EA 1110, Thermo Finnigan). The amount of incorporated C and N during the experiments were calculated as follows: X corresponds to the heavy isotopes of C an N. The atomic percent (at.%) were calculated using the international standards (for C = Vienna PeeDee Belemnite R_{VPDB} = 0.0112372: for atmospheric $N = R_{atmN} = 0.0036765$). The first step was to calculate the $\delta^{13}C$ and $\delta^{15}N$ of the samples as described in the following equation:

$$\delta X = (Rsample / Rstandard - 1) \times 1000,$$

where R describes the heavy isotopic ratio ^{13}C:^{12}C and ^{15}N:^{14}N, respectively. Afterwards the amount of at.% C and N in the samples was calculated:

$$at.\% = \frac{100 \times R_{standard} \times \left(\frac{\delta X_{sample}}{1000+1}\right)}{1 + R_{standard} \times \left(\frac{\delta X_{sample}}{1000+1}\right)}$$

The at.% X calculated represents the total at.% of heavy isotopes located in the cells. The natural number of heavy isotopes was corrected by subtraction of the amount of at.% X measured for untreated foraminifera (natural abundance). This so-called excess (E) of heavy isotopes

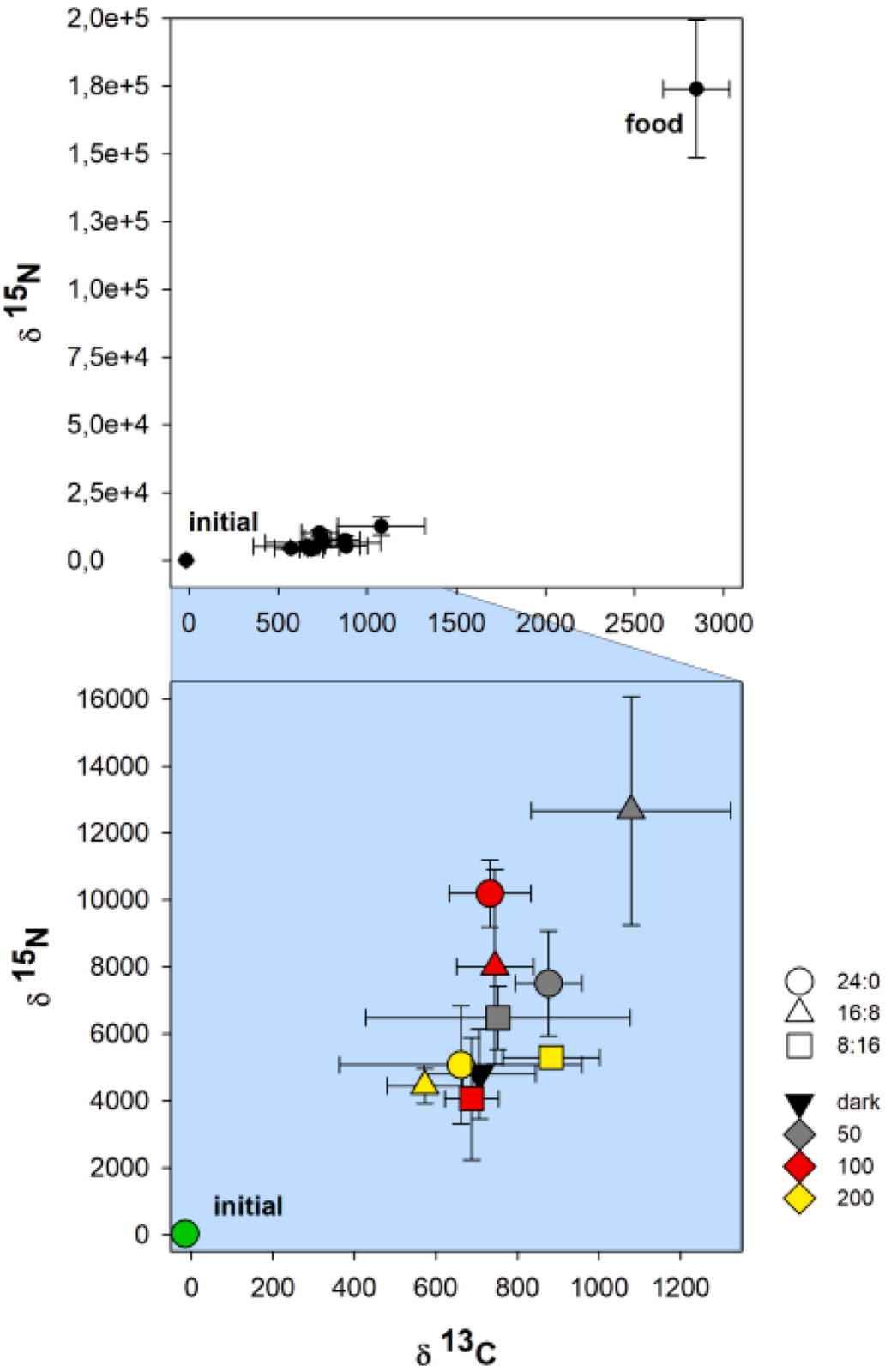

Fig. 2. Isotopic ratios of carbon and nitrogen of foraminifera cytoplasm at the beginning of the experiments ("initial") and after 15 days of incubation. Different symbols depict the light-dark cycles and the colour represents the light intensity in μmol photons m-2 s-1.

was calculated after [43]:

$$E = \frac{atomX_{sample} - atomX_{background}}{100}.$$

Afterwards, C and N incorporated during the experiment were calculated as follows:

$$I_{iso}\left[\mu g mg^{-1}\right] \text{ or } \left[\mu g ind^{-1}\right] = E \times C(N)\left[\mu g mg^{-1}\right] \text{ or } \left[\mu g ind^{-1}\right].$$

Food uptake, called phytodetrital C (pC) or N (pN) uptake was then

Table 2
Statistical evaluation (One-way ANOVA based on 3 replicates) of the δ15N and δ13C values between the start point (blank, time = 0) and the uptake after 15 days. Significant values are in bold.

cycle	element	Df	F-Ratio	p-value
0:24	C	1	83.51	<0.001
	N	1	37.89	0.004
8:16	C	1	45.71	<0.001
	N	1	35.84	<0.001
16:8	C	1	27.04	<0.001
	N	1	10.97	0.008
24:0	C	1	68.9	<0.001
	N	1	26.02	<0.001

Table 3
Statistical evaluation (One-way ANOVA based on 3 replicates) of the $\delta^{15}N$ and $\delta^{13}C$ values within the light cycles depending on different light intensities. Significant values are in bold.

cycle	element	Df	F-Ratio	p-value
8:16	C	2	0.72	0.524
	N	2	3.09	0.119
16:8	C	2	7.7	**0.022**
	N	2	7.49	**0.023**
24:0	C	2	1.77	0.248
	N	2	11.69	**0.009**

calculated as described below:

$$pX = \frac{I_{iso}}{\frac{at.\%Xphyto}{100}},$$

where at.% X_{phyto} represents the isotopic values of the labelled food source.

Shapiro-Wilk-tests were run to test for the normality of the results. Two-way ANOVA was applied to test effects of light intensity and time, as well as light-dark cycle and time on pC and pN (significant cut of: $\alpha = 0.05$). One-way ANOVA was applied to test for differences in food uptake between day 0 and day 15 of each treatment (light cycle and intensity). All statistical tests were performed using StatGraphics Centurion 18.

3. Results

3.1. Food uptake under different light-dark regimes

The food uptake of *C. selseyense* at different light-dark cycles is shown in Fig. 1. Data used for calculation and for the graphs (pC/pN values, additionally statistical data) can be found in the supplement.

Both pC ($p = 0.008$) and pN ($p < 0.001$) were normally distributed: therefore, two-way ANOVA were applied to test for significant effects of time, light intensity and light-dark cycle across the whole experimental time (day 1, 5, 10 and 15) (Table 1).

- 0:24 (light: dark): pC ($p < 0.001$) and pN ($p < 0.001$) increased significantly with time, but was not affected by light intensity.
- 8:16 (light: dark): Time ($p < 0.001$) and light intensity ($p = 0.014$) significantly decreases pC and pN (both: $p < 0.001$).
- 16:8 (light: dark): pC was significantly affected by time ($p = 0.048$), but not by light intensity ($p = 0.293$). The same pattern was found for pN (increasing with time: $p < 0.001$ and decreasing with light intensity, $p = 0.004$).
- 24:0 (light: dark): pC and pN were significantly affected by time ($p < 0.001$), whereas light intensity only affected pN ($p = 0.034$), but not pC ($p = 0.071$).

Initial pC and pN values were highest at the light-dark cycle = 8:16 (Fig. 1). During permanent light supply highest pC values were observed after 5 days (light: dark = 24:0). After 10 days, pC decreased to a minimum in the treatments with permanent light, but at day 15 an increase of pC was noticed under permanent light. For pN the same trend was observed.

Under continuous **dark conditions** specimens of foraminifera showed increasing carbon uptake (pC) ($p = 0.002$) and nitrogen uptake (pN) ($p < 0.001$) with time.

Carbon uptake (pC) at **50 µmol photons m^{-2} s^{-1}** increased with time ($p = 0.003$), but did not depend on the light: dark cycle ($p = 0.180$). Highest pC was observed at 50 µmol photons m^{-2} s^{-1} at day 1 for light: dark cycles = 8:16 and 16: 8, respectively. At day 1, under permanent light, there was no difference in food uptake between all light exposed foraminifera. From day 1 to 5, an increase of food uptake was observed for the 8:16 and 16:8 light cycles. After day 5, a permanent increase of pC (except light cycle 24:0) was observed and pN showed an overall increase across all light: dark regimes. pN at this light intensity increased generally with time ($p < 0.001$) and with light cycle ($p = 0.023$). An extreme increase of pN between day 10 and day 15 (+300%) was observed for the light cycle 16:8.

At **100 µmol photons m^{-2} s^{-1}**, no significant changes in pC with time ($p = 0.118$) and light cycle ($p = 0.558$) were found. Based on the mean values, the carbon uptake at 8:16 light: dark cycle decreases from day 1 to day 10. After day 10 an increase of food uptake was recorded. In contrast, pC uptake at 16:8 (light: dark) was almost constant throughout the experiment. pC values of the permanent light treatment resembled those of the 50 µmol photons m^{-2} s^{-1} treatment. For pN there was no effect of light regime ($p = 0.160$), but a strong positive correlation with incubation time was found ($p < 0.001$). Based on the mean values of the nitrogen uptake, pN increased with time for all light regimes. Only for the permanent light regime we found a marked increase in pN from day 10 to 15 (+400%).

At **200 µmol photons m^{-2} s^{-1}**, pC was not affected by time ($p = 0.524$) or light regime ($p = 0.378$). The temporal trend was similar to that at 100 µmol photons m^{-2} s^{-1}, though with lower variation. Only for the 16:8 light cycle, a strong decrease of pC was observed after 1 and 10 days, which was not seen in the 100 µmol photons m^{-2} s^{-1} experiments. pN was significantly increasing with time ($p < 0.001$), but not affected by light regime ($p = 0.268$). Further, pN showed the same pattern as the 100 µmol photons m^{-2} s^{-1} experiments. Also for the 16:8 light cycle, pN decreased and was lowest compared to all other light regimes.

The initial isotopic ratios of carbon and nitrogen (expressed as $\delta^{13}C$ and $\delta^{15}N$ values) are compared with the isotopic signatures at the end of the experiments (Fig. 2). The isotopic value of the food source (*P. tricornutum*) is much higher (value) than that of the foraminifera before the beginning of culturing (value).

The isotopic ratios after 15 days in every treatment were significantly higher, indicating food uptake in all treatments (Fig. 2). A statistical evaluation of the 15 day-data (Table 2) within each light regime is presented in Table 3. pC was not significantly affected by light regime ($p = 0.899$) and light intensity ($p = 0.081$). pN was significantly different between light cycles ($p = 0.041$) and was decreasing with increasing light intensity ($p = 0.010$). Under the light-dark cycle 8:16, the ratios clustered independently of light intensity and overlapped with the values from continuous dark treatments. In contrast, the values of the 16:8 h incubation experiments showed a linear increase of heavy C and N isotopes with decreasing irradiance. The uptake at the incubation at permanent light showed no clear trend. In incubations with 200 µmol photons m^{-2} s^{-1}, $\delta^{13}C$ values increased, but $\delta^{15}N$ values remained constant (Fig. 2). In contrast, the 100 µmol photons m^{-2} s^{-1} samples showed a shift in $\delta^{15}N$, but not in $\delta^{13}C$ signatures. This increase of $\delta^{15}N$ correlated with light intensity. Foraminifera incubated at 50 µmol photons m^{-2} s^{-1} exhibited increases in $\delta^{15}N$ and $\delta^{13}C$ values.

The cytoplasmic foraminiferal C:N ratios in the dark incubations were almost stable and were in the range of 6 – 8 (Fig. 3). For the light regimes of 8:16 h and 16:8 h, no clear trend between light intensity and

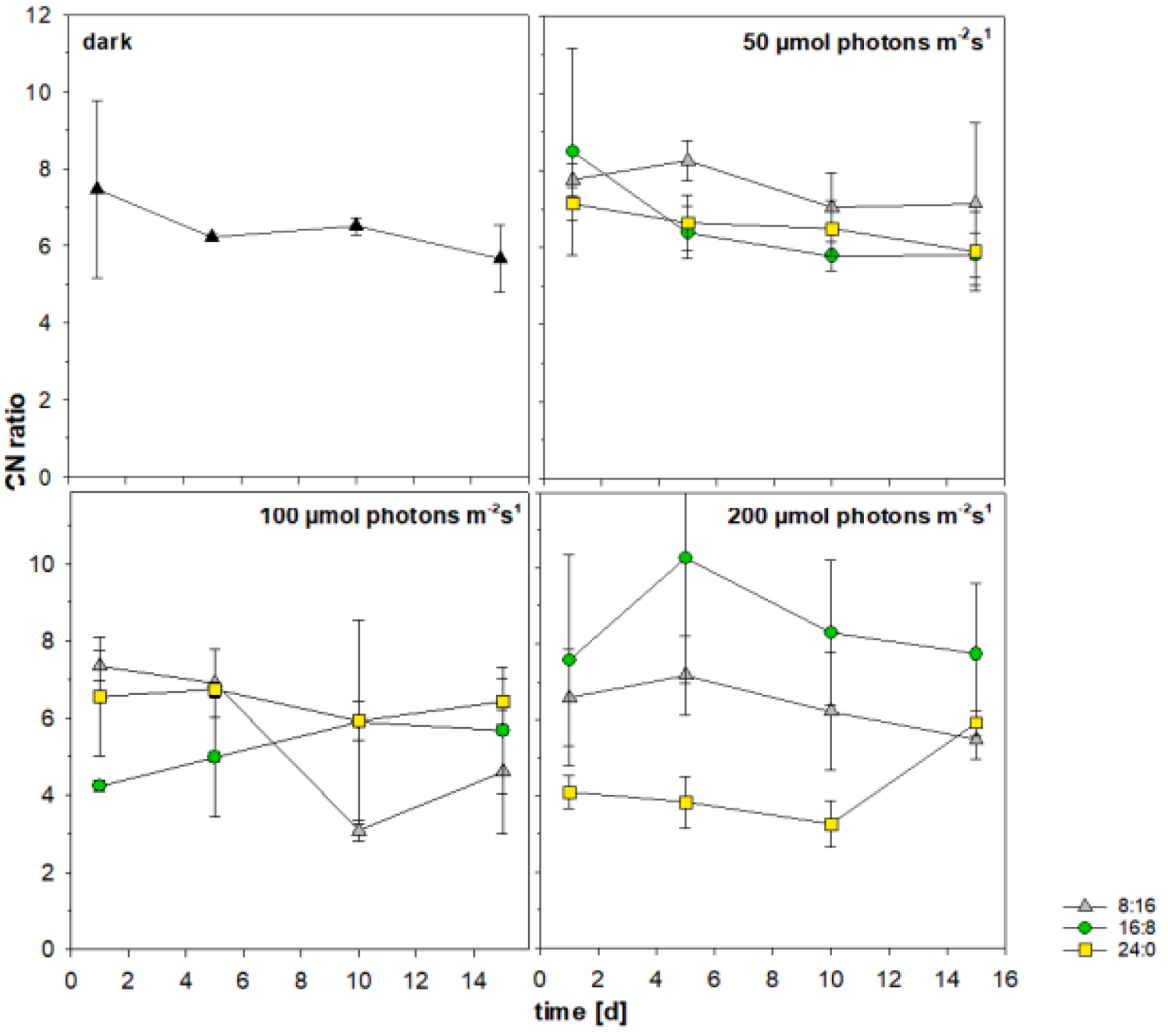

Fig. 3. Changes in cytoplasmic C:N ratios of *C. selseyense* with time. The title of the plots represents the light: dark cycle in hours and in the legend the irradiance supply in µmol photons $m^{-2}\,s^{-1}$ is given.

cytoplasmic C:N ratio was observed. During the incubation at permanent light, the cytoplasmic C:N ratio remained relatively stable at lower irradiances (50 and 100 µmol photons $m^{-2}\,s^{-1}$) over time, while at the highest level of 200 µmol photons $m^{-2}\,s^{-1}$ the ratio was also stable (C:$N = 7$) until day 10, until when it increased and equalled other treatments.

4. Discussion

4.1. Food uptake of C. selseyense at different light regimes

The food uptake of foraminifera depends on several physical parameters, such as salinity [44] and temperature [10], as these parameters influence the feeding activity of foraminifera (e.g., [45]). However, not only environmental parameters are important controls of the food uptake of foraminifera, but also the type of food (Lee at al., [14, 32]), and food quality and quantity [46]. Most of these experiments were done under laboratory conditions, but not infrequently, an indication of the light availability is missing. Our results show, that it is essential to perform such experiments under a homogeneous and well-defined light supply and further report this values in the manuscript.

Light intensity has been shown to be a key parameter for the biogeographic and habitat distribution of foraminifera (e.g., [33, 34]). Foraminifera either live on sediment surfaces (epifaunal) or in sediments (infaunal). Both, foraminiferal C and N uptake were strongly affected by light intensity (Table 1). This demonstrates that light intensity not only affects depth distribution but also directly affect the foraminiferal food uptake. Many fully heterotrophic taxa are also found in the dark deep-sea, where no sunlight reaches the seafloor [35]. These foraminifera depend only indirectly on the light supply, due to their feeding on photoautotrophic algae living in the euphotic zone (phytodetritus) (e.g., [36]). Therefore, it can be postulated that light have an impact on all living foraminifera either directly or indirectly. Although irradiance is very low in the natural environment of *C. selseyense* (e.g., [37]), our experiments revealed that this taxon is able to deal with higher light intensities. Kitazato et al. [38] found that foraminifera have higher reproduction rates in spring and summer than in winter, largely depending on seasonal changes in temperature, light supply, food availability and/or biotic interactions. One possible trigger for this seasonal pattern in foraminiferal activity could be changes in irradiance. Our experiments showed that food uptake slightly increased with longer light periods (Fig. 1). This suits well to the observation of Kitazato et al. [38], where the activity of foraminifera is seasonally triggered by physical parameters like the light supply.

Furthermore, our experiments indicate that the highest food uptake occurred at intermediate light intensities, i.e., 50 µmol photons $m^{-2}\,s^{-1}$. We, however, cannot conclude that this amount of light is reaching the cytoplasm as light transmission through the test walls is not known yet. Summarizing, the factor light is highly important for the activity of foraminifera and needs to be considered in future studies.

4.2. Foraminiferal adaption to high light conditions

It is important to define the optimal light conditions for foraminifera, so this knowledge can be employed in future culturing studies. As highest food ingestion rates were observed at 50 μmol photons m^{-2} s^{-1} and a light-dark rhythm of 16:8 h, these conditions may represent "optimal conditions" for the feeding activity of *C. selseyense*. At high light conditions (200 μmol photons m^{-2} s^{-1}) the foraminifera showed decreasing isotopic incorporation rates, indicating negative effects of excess light.

An interesting finding was the difference in C and N uptake rates of *C. selseyense* depending on light intensity (cite Fig. 1, 3 and Table 1). Some foraminifera are able to produce lipid droplets (e.g., [47]), which are mainly composed of triacylglycerol and contain generally a higher amount of C than of N and can probably influence the cytoplasmic C:N ratio in foraminifera. We observed changes in the C:N ratio based on a shift in the irradiance supply. These change in the C:N ratio can probably lead to a production of lipid droplets or stress proteins. To clarify this aspect in detail, further transmission electron microscopy studies are necessary. The accumulation of lipid droplets [47–49] seems to be a response to stressful conditions [49]. The offered light intensities of 100 and 200 μmols m^{-2} s^{-1} were high in comparison to natural habitat conditions of *C. selseyense*, which may have led to unfavourable and stressful conditions. We assume that foraminifera produce more lipid droplets under higher light conditions, which can cause a light scattering effect and therefore probably lower the light intensity entering the foraminiferal cell. In that way, the lipid droplets may act as a protection against increasing light intensities.

Another hypothesis describes the presence of opsins in foraminifera. Some eukaryotic protozoa, are thought to have the ability to produce such light-sensitive proteins [39]. Since *C. selseyense* obviously responds to light, but has no photosymbionts or kleptoplasts, the response to different light cycles could indicate that foraminifera can also produce such proteins. So far, nothing can be found in the literature on this topic, but this study can be a first starting point for further investigations in this direction.

4.3. Daily light-dark regime effects on the food uptake of C. selseyense

Our experiment indicates that there was no significant difference (except of maximum pN occurring at 50 μmol photons m^{-2} s^{-1}) in food uptake with changes in the light-dark cycle (Table 2). Based on our observations, we assumed that the activity of *C. selseyense* increases with increasing light intensity.

Lintner et al. [40] found a higher food uptake of *C. selseyense* under light conditions compared to darkness. This pattern was, however, not observed in this experiment, which might be explained by foraminiferal activity being affected by season. The foraminifera in the study of Lintner et al. [40] were sampled in late autumn and might have reached a "stationary phase", due to the lower natural temperature (seasonal cycle) compared to foraminifera collected for this study, i.e., in early summer.

In summary, light is a potential trigger affecting food incorporation in fully heterotrophic Foraminifera species (here: *C. selseyense*), which needs to be considered in more detail in future nutritional studies with foraminifera. Exposing foraminiferal specimens to different light intensities can lead to marked changes in the pattern of food uptake, whereas a change in the light-dark regime did not affect food uptake to that extent here.

Declaration of Competing Interests

None

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