Effect of the silica content of diatom prey on the production, decomposition and sinking of fecal pellets of the copepod *Calanus sinicus*

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Abstract

The effects of changing the amount of silica in the cell wall of diatom prey, on the production, decomposition rate and sinking velocity of fecal pellets of the calanoid copepod, *Calanus sinicus*, were examined. Using different light intensities to control the growth of the diatom *Thalassiosira weissflogii* also led to the accumulation of different amounts of biogenic silica. Copepods were then fed with either low (~1600 cells L^{-1}) or high (~8000 cells L^{-1}) concentrations of this diatom. Copepods fed on a high concentration of diatoms with high-silica content, exhibited a lower grazing rate and lower fecal pellet production rate than those fed on a high concentration of diatoms with low-silica content. However, there was no difference in either the grazing or fecal pellet production rates at low prey concentrations with high- or low-silica content. The size of the fecal pellets produced was only affected by the prey concentration, and not by the silica content of prey. In addition, the degradation rate of the fecal pellets was much higher for copepods fed a low-silica diet than for those fed on a high-silica diet. Significantly lower densities and sinking rates only occurred in the fecal pellets of copepods fed a low-silica diet and a low prey concentration. Calculating the L-ratio (the ratio of degradation rate:sinking rate) for each group indicated that the fecal pellets produced by copepods fed on highly silicified diatoms are likely to transport both biogenic silica and organic carbon to the deep layer; whereas those produced following the consumption of low-silica diatoms are likely to decompose in the mixing layer.

Introduction

In the marine environment, zooplankton fecal pellets constitute a main vehicle for transporting biogenic elements to the sediments, although a substantial proportion of this flux is recycled or repackaged in the water column by microbial decomposition and zooplankton coprophagy (Turner, 2002; 2015). Diatoms are among the most abundant phytoplankton, and they represent a main component in the diet of zooplankton in marine environments. Studies show that zooplankton with a diatom diet usually produce fecal pellets that sink faster than those on other diets (Feinberg and Dam, 1998). Dagg et al. (2003) reported that the contribution of fecal pellets to the flux of particulate organic carbon (POC) and biogenic silica (bSi) is higher during the spring diatom bloom than during the summer within the Antarctic Polar Front region. Similarly, Goldthwait and Steinberg (2008) reported an increase in mesozooplankton biomass and fecal production and flux inside cyclonic and mode-water eddies. However, González et al. (2007) reported a negative correlation between the vertical carbon flux of diatoms and the production of fecal material in a time-series study in the upwelling waters off Chile.

The quantity and characteristics of the fecal pellets produced by zooplankton depend on several factors. The pellet production rate is reported to be affected by the rate of ingestion and assimilation efficiency (Butler and Dam, 1994; Besiktepe and Dam, 2002). It has also been demonstrated that the type of diet can affect the characteristics of the fecal pellets produced; including size, density and sinking rates (e.g., Feinberg and Dam, 1998 and ref. therein). In addition, the decomposition rate of pellets varies with water temperature, as well as with both microbial and metazoan activity (Poulsen and Iversen, 2008; Svensen et al., 2012). Factors that contribute to the sinking velocity of the pellets include size, density and shape, all of which can vary dramatically both among different zooplankton species and within the same

zooplankton species feeding on different types of prey (Fowler and Small, 1972; Turner, 1977; Feinberg and Dam, 1998). Turbulence in the water column, the presence or absence of a peritrophic membrane, and the production of microbial gas within a peritrophic membrane might also affect the sinking rate of pellets (Honjo and Roman, 1978; Bathmann et al., 1987). Indeed, the sinking rate and decomposition rate are the two most important parameters used, to determine whether a pellet will or will not be successfully transported into deeper water before its contents are degraded. For example, a slowly-sinking pellet is more likely to decompose and become part of the recycled materials before it exits the euphotic zone (Dagg and Walser, 1986).

The cell wall (frustrule) of diatoms is composed of two silicate shells, which are believed to act as a defense mechanism to prevent ingestion by grazers (Pondaven et al., 2007); thus different levels of silicification of the frustrule might affect the grazing rate of copepods (Friedrichs et al., 2013, Liu et al., in revision). The silica content of the cell wall of diatoms is not only species-specific, but it is also affected by environmental parameters such as light, temperature, salinity, pH, nutrients and trace metals (Martin-Jézéquel et al., 2000 and ref. therein; Claquin et al., 2002; Vrieling et al., 2007; Herve et al., 2012; Liu et al., in revision). Although the frustule has no nutritional value for zooplankton, it is thought to provide ballast, which is especially advantageous when the fecal pellets are sinking. Hence, pellets with a high diatom biomass generally exhibit higher levels of export of POC (Armstrong et al., 2002; François et al., 2002; Klaas and Archer, 2002). Thus, the content of the zooplankton diet (and therefore the type and concentration of ballast minerals ingested) might strongly affect the sinking velocity of the fecal pellets produced, and hence the vertical flux of biogenic silica and carbon.

Most of the studies describing the production rates and characteristics of copepod fecal pellets have focused on aspects such as food types (Feinberg and Dam,

1998), or the different periods of phytoplankton blooms (Butler and Dam, 1994). There are currently no reports that describe the effect of the silica content of diatoms on the production, degradation and sinking of fecal pellets. Liu et al. (2016) recently demonstrated that the diatom *Thalassiosira weissflogii*, when grown at different light levels, contain varying amounts of silica, and that the small calanoid copepod *Parvocalanus crassirostris*, when fed on diatoms containing high levels of silica exhibited a reduced feeding rate, and stagnant growth as well as low egg production and hatching success. In this study we used the same diatom species with different silica content as prey to study the characteristics of the fecal pellets produced by the herbivorous copepod, *Calanus sinicus*.

Materials and Methods

Copepod and prey culture conditions. The herbivorous copepod *Calanus sinicus* was collected from the coastal waters around Hong Kong in February 2013. Copepods were maintained on a 14 h light:10 h dark cycle at 23.5°C in 2 L glass containers with 0.2 μm-filtered seawater. The copepods were fed a mixed algal diet consisting of *Rhodomonas* sp. and *Thalassiosira weissflogii* at a concentration of ~5000 cells L^{-1}; this food suspension was supplied to the cultures twice a week and the whole culture seawater was replaced every week. The copepods were maintained for more than one month prior to the start of the experiment to ensure that all the adults were grown in approximately the same conditions and were of approximately the same age.

The diatom, *T. weissflogii*, was maintained in exponential growth in f/2 medium (Guillard, 1975), under light intensities of either 15 μmol photons s^{-1} m^{-2} or 200 μmol photons s^{-1} m^{-2} to generate cells with different cellular silica contents (Liu et al., 2016). The diatom cultures were transferred every 4 or 8 days for the high- and low-light

batches, respectively. After two transfers the amount of biogenic silica in the diatom cells was measured using a modified version of the method described by Paasche (1980), following the procedures described more recently by Grasshoff et al. (1999). Cells were collected on a 1 μm polycarbonate filter (47mm diameter) and washed with 10 ml autoclaved seawater and 0.01M HCl during filtration to remove the intercellular silicate pools. The folded filter was immediately placed into a 15 ml polypropylene tube and stored at −80°C. Hydrolysis was carried out using 4ml of 5% NaOH, digested at 85°C for 2 hours. After cooling, 0.72 ml of 1.0 M HCl was added to each tube, lowering the pH to 3-4. Silicic acid concentration of samples was determined colourmetrically, through the formation of blue coloured silicocomplexes.

Experimental design. Active adult female *Calanus sinicus* with intact appendages were selected and starved for 24 hours before an experiment. A total of seven experiments was conducted to determine fecal pellet production, degradation and sinking, and in each experiment these parameters were measured both at low and high food concentrations, and at high and low levels of silica contained in the diatom prey (Table 1). In each experiment, the copepods were fed with the same species of diatom (i.e., *T. weissflogii*), at either ca. 1600 cells L^{-1} (low concentration), or ca. 8000 cell L^{-1} (high concentration), the latter being above the food saturation level according to Frost (1972). The abundance and volume of diatoms were measured (triplicate subsamples) using a Beckman Coulter Z2 Particle Counter and Size Analyzer.

In the fecal pellet production experiments, five replicate bottles containing one copepod per bottle, and two control bottles without a grazer, were used. All the bottles were filled with 100 ml freshly-prepared media consisting of 0.2 μm prefiltered seawater and suspensions of the respective prey for each treatment. All incubations

were conducted at 23.5°C and in the dark for 24 hours. At the end of the incubation period, a 2 ml sample was collected from each bottle and fixed with acid Lugol's at a final concentration of 2%, for subsequent diatom quantification. The remaining water was collected in a 50 ml polypropylene tube and fixed with glutaraldehyde at a final concentration of 1%, for further quantification of the fecal pellets.

In order to obtain fresh pellets for the degradation experiments, two plastic beakers were prepared for the high- and low-silica content prey. Each beaker contained 7-8 copepods and 700 ml culture medium, prepared as described for the production experiments. After 12 hours of incubation (except for experiment # 3, which was incubated for 18 hours), the medium was sieved through a 40 μm mesh to collect the fecal pellets and then rinsed with autoclaved 0.22 μm filtered seawater. At least 20 intact fecal pellets were selected using a glass Pasteur pipette under a stereomicroscope and poured into a 250 ml polycarbonate bottle containing 200 ml of 2 μm pre-filtered sea water taken from the field. The number of replicate bottles and the incubation period of each experiment are show in Table 2. All the bottles were put on a roller at 0.4 rpm in the dark at 23.5°C and then at the end of the respective incubation times, the whole water of each bottle was collected in a plastic bottle and fixed with glutaraldehyde at a final concentration of 1% for further fecal pellet analysis.

Experiments to estimate the fecal pellet sinking rate were conducted by obtaining fecal pellets using the degradation experiment procedure (described above) but with an incubation time of 24 hours. After collecting all the fecal pellets from the beakers, 50 intact pellets were selected and suspended in 260 ml 0.2 μm prefiltered autoclaved seawater. The fecal pellet sinking rate was measured using a SETCOL chamber (49 cm height, 2.6 cm inner diameter) made by 4 mm Plexiglas (Bienfang, 1981), filled with well-mixed pellet-containing seawater. The chamber was allowed to settle for 6

min, and then the whole column of water was collected from outflow tubes in a top-to-bottom order. The water was collected in a plastic bottle and fixed with glutaraldehyde as described above, for subsequent fecal pellet analysis.

Determining the number and size of fecal pellets. The water samples containing the fecal pellets in the 50-ml polypropylene tubes were allowed to settle for 24 hours. The upper water was then removed smoothly and the remainder was poured into the well of a 6-well plate and the number of pellets was counted using an inverted microscope (Olympus IX51) at 100× magnification. Only intact fecal pellets and fragments with end points were counted. The total number of fecal pellets was then calculated to include all of the intact fecal pellets plus half of the pellet fragments. Images of at least 30 intact fecal pellets were acquired with a CCD camera (Model 4.2, Diagnostic Instrument Inc., USA), after which the length and width of each fecal pellet was measured and the volume was calculated making the assumption that they are cylindrical in shape.

Calculating the fecal pellet degradation rate. The rate of degradation of the fecal pellets was calculated from the loss of fecal pellet equation, described by:

$$N_t = N_0 e^{-rt}$$

where N is the total number of fecal pellets in the incubation bottle at the beginning (N_0) and end of the experiment (N_t); t is the incubation time (in days); and r is the degradation rate (d^{-1}). The degradation rate estimated in this study only considered the effect of microbial organisms and assumed that the loss rate was exponential.

Calculating the fecal pellet sinking velocity. The rate that fecal pellets sank was calculated from the formula reported by Bienfang et al. (1982), which was originally used to measure the average sinking rate of phytoplankton. Thus:

$$S = \frac{N_S}{N_T} \times \frac{L}{t}$$

where S is the average sinking velocity; L is the height of the sinking column; t is the duration of the trial; N_T is the total number of fecal pellets within the settling water volume; and Ns is the total number of fecal pellets that settled during the trial time.

In addition, the density of the fecal pellets was calculated using the semi-empirical equation deduced by Komar (1980), as follows:

$$w_s = 0.079 \frac{1}{\mu} (\rho_s - \rho) g L^2 \left(\frac{L}{D} \right)^{-1.664}$$

where w_s is the sinking velocity of the fecal pellets; μ and ρ are the fluid viscosity and density, respectively; L and D are the length and diameter of the fecal pellets, respectively, assuming they are in the cylindrical shape; g is the acceleration of gravity; and ρ_s is the density of fecal pellet.

205 **Results**

206 **Grazing response**

207 The cellular silica content of first and second generation *T. weissflogii* when
208 cultured at high and low light intensities is shown in Fig. 1. After two transfers the
209 cellular biogenic silica content was significantly different (t-test, $p<0.05$; Fig. 1) when
210 comparing the high-light and low-light culture conditions. The silica content of high-
211 and low-silica diatoms used in all the experiments was consistent and the differences
212 between the two treatments were all statistically significant (Table 1). Other cellular
213 parameters, such as cell size and carbon and nitrogen contents, were also measured
214 for selected samples (data not shown), and the results were consistent with those
215 reported in a previous study (Liu et al., 2016), which showed no significant difference
216 between the two types of prey.

217 The grazing response of *C. sinicus* to diatoms with different silica contents
218 showed similar patterns between high (ca. 8000 cells ml^{-1}) and low (ca. 1600 cells
219 ml^{-1}) prey concentration (Fig. 2). At high concentrations of prey, *C. sinicus* grazed the
220 diatoms with low cellular silica content two times faster than when they had a high
221 silica content (t-test, $p<0.05$). The same trend was also observed at low concentrations
222 of the prey, although in this case the difference was not statistically significant. In
223 addition, the rate of clearance was significantly higher for the low-silica prey than for
224 the high-silica prey at both low and high prey concentrations (t-test, $p<0.05$). These
225 results indicate that the silica content of diatoms can affect the grazing activity of
226 copepods.

227

228 **Fecal pellet production**

229 The rate of fecal pellet production varied both with the silica content and the
230 concentration of the prey (Fig. 3A). At a high prey concentration, *C. sinicus* that were

fed on low-silica prey produced significantly higher amounts of fecal pellets (192±32 FP ind^{-1} d^{-1}) than those fed on high-silica prey (113±47 FP ind^{-1} d^{-1}, $p<0.05$); which corresponds well with the rate of ingestion (Fig. 2A and 3A). At a low prey concentration, however, the production of fecal pellets by *C. sinicus* fed with the low and high-silica prey was not significantly different (Fig. 3A). In addition, the size of the fecal pellets was only affected by the concentration of the prey, and not by the silica content of the prey (Fig. 3B). Thus, the fecal pellets produced in the high concentration of prey groups had a mean length and width of 582.4±98.7 μm and 72.5±4.5 μm, respectively, which are significantly larger than the size of those produced in the low concentration of prey groups, which had an average length and width of 352.4±54.7 μm and 59.6±6.8 μm, respectively (ANOVA, $p<0.05$).

Fecal pellet degradation rate and sinking rate

The degradation rate of fecal pellets was significantly different when the copepods fed on diatoms with different silica content (Table 2). The degradation rate of the fecal pellets produced from the low-silica prey was approximately 4-5-fold higher than that of the pellets generated from the high-silica prey, irrespective of the prey concentration or the period of degradation incubation. In addition, the degradation rate of the fecal pellets from low prey concentration was significantly higher than ones from high prey concentration after an incubation period of 24 hr ($p <0.05$, ANOVA). Furthermore, the degradation rate obtained following 48 h incubation was significantly higher than that following just 24 h incubation (only high prey concentration experiments) for both the high ($p <0.05$, t-test) and low ($p< 0.01$, t-test) silica prey (Table 2), indicating an acceleration of degradation in the second day of incubation.

The sinking rate of fecal pellets was also different for the high and low prey

257 concentrations (Fig. 4). At a high concentration of prey, the sinking rates of the pellets
258 produced by the high- and low-silica prey (i.e., 3.05 and 3.13 cm min^{-1}, respectively),
259 were not significantly different. However, at a low prey concentration, the sinking
260 rate of pellets from the high-silica-content prey (i.e., 2.59 cm min^{-1}) was significantly
261 greater (t-test, $p<0.01$) than that of pellets from the low-silica-content prey (i.e., 0.53
262 cm min^{-1}). The average density of the fecal pellets was calculated as being
263 1.093-1.095 g cm^{-3} at the high prey concentration, and 1.035-1.097 g cm^{-3} at the low
264 prey concentration. The variation in the calculated density of fecal pellets is consistent
265 with the pattern of sinking rate, with the lowest density occurred in fecal pellets from
266 low-silica prey at the low prey concentration (Fig. 4).
267
268 **Discussion**
269 The grazing activity of copepods varies not only with the concentration of the
270 prey but also with the nutritional quality of the prey. In our study, the grazing and
271 clearance rates determined with varying food concentrations followed a similar trend
272 to that described in the literature (e.g., Frost, 1972). In addition, the grazing activity
273 was affected by the cellular silica content of the prey, as has been observed with other
274 copepod species (Liu et al., 2016). Silicification has been suggested to be one of the
275 strategies that is used by diatoms to protect them from ingestion by grazers (Pondaven
276 et al., 2007). Friedrichs et al. (2013) examined the mechanical strength of the frustules
277 of three diatom species and measured the feeding efficiency of copepods on these
278 diatoms. Their results showed that the diatom species with the more weakly-silicified
279 frustules and the highest growth rate was the least stable and was fed upon the most,
280 whereas the species with the most complex frustule exhibited the greatest stability and
281 was fed upon the least. Within the same species of diatom, different growth rates have
282 resulted in different amounts of silica in the frustule (Claquin et al., 2002). This

283 results in higher copepod ingestion and clearance rates for diatoms with a low silica
284 content when compared with those for diatoms with a higher silica content (Liu et al.,
285 2016). The results obtained in the current study are consistent with those reported by
286 Friedrichs et al. (2013) and Liu et al. (2016).
287 Previous studies indicate that while there is a linear relationship between the
288 ingestion rate and the total number of fecal pellets produced per unit time (Ayukai and
289 Nishizawa, 1986; Ayukai, 1990), there is a high level of variation among different
290 diets (Båamstedt et al., 1999 and references therein; Besiktepe and Dam, 2002). In
291 addition, the size of fecal pellets increases as the concentration of the food increases,
292 such that they reach a maximum size when the concentration of food is above the
293 saturation level (Dagg and Walser, 1986; Butler and Dam, 1994). Our results
294 confirmed these previous findings and demonstrated that the size of fecal pellets
295 produced was only affected by the concentration of prey, and fecal pellets did not
296 show any significant size differences when comparing prey of high and low cellular
297 silica content. Butler and Dam (1994) reported that when sufficient food was
298 available, the size of the fecal pellets varied with the nutritional quality (e.g., the C:N
299 ratio) of the prey. Since diatoms with different silica content (generated by varying the
300 light intensity) do not differ in their cellular C:N ratio (Claquin et al., 2002; Liu et al.,
301 2016), these ratios did not affect the size of the pellets produced.
302 The degradation rate and sinking velocity of the fecal pellets are highly
303 dependent on the characteristics of the pellets, which are in turn affected by the
304 quality and quantity of the food ingested (Feinberg and Dam, 1998; Turner, 2002;
305 2015 and references therein). For example, it is known that the decomposition rate of
306 the fecal pellets is affected by diet, pellet size and the producer of the pellets (e.g.,
307 Shek and Liu, 2010), but no research have addressed the degradation rates of fecal
308 pellets produced by prey under different stoichiometric conditions. Hansen et al.

309 (1996) estimated the degradation rate of fecal pellets produced from diets of
310 *Thalassiosira weissflogii*, a diatom; *Rhodomonas baltica*, a nanoflagellate; or
311 *Heterocapsa triquetra*, a dinoflagellate. Fecal pellets produced from a diet of the
312 diatom species presented the slowest rate of degradation when compared with those
313 produced from diets of the nanoflagellate or dinoflagellate species. Similarly, Olesen
314 et al. (2005) compared the degradation rate of fecal pellets produced on a diet of the
315 diatom, *Skeletonema costatum*, or the nanoflagellate, *Rhodomonas salina*, and
316 reported a similar trend but higher degradation rates than Hansen et al. (1996). The
317 relationship between the surface:volume ratio and the degradation rate of fecal pellets
318 was used to explain the variation in the degradation rate of pellets produced with
319 different diets. Our results (Table 2) were higher than those reported by Hansen et al.
320 (1996), which were 0.024 d^{-1} for *T. weissflogii*, but our results showed a similar trend
321 to those summarized by Olesen et al. (2005) (dashed line in Fig. 5), in that there was
322 an increase in the degradation rate with the increase in fecal pellet surface:volume
323 ratio, although the degradation rates that we measured, exceeded the predicted rates in
324 most cases, particularly for fecal pellets produced with low-Si diatom prey (Fig. 5).
325 The generally higher rates in our study might be caused by the higher temperature that
326 we used when compared with the previous studies (i.e., 23.5°C in our study *versus* 17°
327 C and 18°C in Olesen et al., 2005 and Hansen et al., 1996, respectively), but the
328 differences in predator and prey quality, particular the cellular Si content in this study,
329 cannot be ignored.
330 The sinking rate of fecal pellets is usually considered to be related to their size
331 and density, which is in turn dependent on the concentration and composition of the
332 prey (Bienfang, 1980; Urban et al., 1993; Feinberg and Dam, 1998). We also
333 demonstrated that fecal pellet size, sinking rate and density were correlated with the
334 concentration of prey (Fig. 3B, 4), especially in the low-silica diatom prey treatment.

335 Using the ratio of ingestion rate : fecal pellet production rate ratio as an index to
336 compare the diatom content per fecal pellet, no differences were found in pellets
337 produced from diets of the same silica content (Fig. 6), indicating that prey
338 concentration does not affect the package content of the fecal pellets. On the other
339 hand, copepods were shown to pack fewer hard-shelled (i.e., high-Si) diatoms into
340 each fecal pellet in comparison to the soft-shelled (i.e., low-Si) diatoms, although
341 these data were not significantly different statistically (Fig. 6).

342 The fecal pellets of copepods are formed in the midgut surrounded by a
343 peritrophic membrane, which is believed to protect the gut wall from the sharp edges
344 of the prey's cell wall. Moreover, the different sizes of fecal pellets with similar prey
345 content per fecal pellet are thought to result from the decreasing gut passage time with
346 the increasing of food concentration. A high prey concentration results in the food
347 passing through the gut more quickly and results in incomplete digestion, whereas a
348 low prey concentration allows the food to be kept in the intestinal tract for a longer
349 time and therefore digestion is relatively more complete. We showed that the silica
350 content of the diatom cell wall determines the density and sinking rate of the fecal
351 pellets when the prey concentration was low due to complete digestion. In addition,
352 we showed that only the low concentration of low-Si prey group, resulted in a
353 significantly lower fecal pellet density and sinking rate. In previous studies, the
354 sinking rate and density of the fecal pellets of *Calanus* were shown to be 70-171 m
355 day^{-1} and 1.07-1.17 g cm^{-3}, respectively (Bienfang, 1980; Urban et al., 1993), which
356 are considerably higher than our results (Fig. 4). We suggest that these differences
357 might be caused by the differences in methodology used (Griffin, 2000).

358 To compare the combined effects of sinking and degradation rates for each
359 treatment, the reciprocal length scale, or L-ratio, which is the fraction of pellet
360 degradation per unit length traveled, was calculated (Feinberg and Dam, 1998). The

product of the L-ratio multiplied by the depth of the mixed layer can then be used to provide the degree of degradation of a pellet within this layer. The results from such calculations suggest that some diets might result in pellets that are substantially recycled within the epipelagic layer whereas others result in pellets that are exported out of the mixed layer in a relatively non-degraded manner. It should be pointed out, however, that the degradation rates we calculated are likely to be highly underestimated due to the absence of zooplankton activities. For example, it has been reported that copepod ingestion of entire fecal pellets (i.e., coprophagy) or only partial break down of fecal pellets might dramatically reduce the overall downward transport of fecal material and thus increase its retention in the epipelagic layer (Lampitt et al., 1990; Gonzalez and Smetacek, 1994; Svensen et al., 2012). For the same reason, plus the absence of turbulence in our experimental set-up, our sinking rate measurements are likely to be overestimated. Nevertheless, the L-ratio provides a relative indicator of the export efficiency of the fecal pellets produced on diatom diets of different silica content and can be used for a comparison with copepod fecal pellets produced with other diets. Our results also show that pellets produced from high silica content diatoms are more likely to sink out of the mixed layer before being degraded, when compared with pellets from low silica content diatoms. On the other hand, fecal pellets produced from a low concentration of prey with low-Si content are the most likely to be degraded in the mixed layer (Table 3). Our results suggest that the grazing activity of copepods might result in organic matter being mostly recycled in the mixed layer during the fast-growth period of diatoms (e.g., at the beginning of the bloom), whereas it could accelerate the export of POC to the deep ocean by producing fast-sinking fecal pellets during the slow-growth period of diatoms (e.g., during the senescent stage of the diatom bloom).

In conclusion, the silica content of the cell wall of diatoms can affect the grazing

activity of copepods and influence the rates of production, decomposition and sinking of their fecal pellets. Our findings suggest that it is not only the nutritional quality, but also the digestion process of copepods that can result in the different characteristics of the pellets produced. In addition, it is a combination of both degradation and sinking rates, (which are affected by the abundance and cellular silica content of the diatom prey among other physicochemical factors), that determines the efficiency of the downward export of biogenic silica and organic carbon by fecal pellets.

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530 an important role of external ionic strength. PNAS, 104, 10441-10446, 2007.
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532

533 Table 1. Summary of the concentration and cellular silica content of the diatom prey

534 in each experiment.

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Expt.	Measurements	[Prey]	Silica level	Initial prey density (cells mL^{-1})	Cellular silica (pg SiO_2 $cell^{-1}$)
1		High	High	8194 ± 166.9	55.7 ± 1.7
	Fecal pellet	High	Low	7976 ± 8.5	38.2 ± 1.4
2	production	Low	High	1640 ± 28.3	51.7 ± 1.9
		Low	Low	1490 ± 84.9	31.4 ± 6.6
3		High	High	8194 ± 166.9	55.7 ± 1.7
		High	Low	7976 ± 8.5	38.2 ± 1.4
4	Fecal pellet	High	High	7499 ± 63.6	58.9 ± 2.4
	degradation*	High	Low	7344 ± 169.7	33.4 ± 4.3
5		Low	High	1640 ± 28.3	51.7 ± 1.9
		Low	Low	1490 ± 84.9	31.4 ± 6.6
6		High	High	8114 ± 138.0	56.5 ± 5.9
	Fecal pellet	High	Low	7904 ± 124.7	27.0 ± 0.6
7	sinking	Low	High	1790 ± 48.1	52.1 ± 1.3
		Low	Low	1545 ± 75.0	30.3 ± 3.1

536 The incubation time of the 3 fecal pellet degradation experiments can be found in

537 Table 3.

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Table 2. Degradation rate of the fecal pellets produced by *C. sinicus* after they were fed on diatoms with different silica content.

Prey concentration	Incubation period	Silicon status of prey	n	Degradation rate (day^{-1})
High	48 hr	HSi	3	0.21±0.15
		LSi	3	0.91±0.17
High	24 hr	HSi	4	0.03±0.04
		LSi	4	0.15±0.02
Low	24 hr	HSi	3	0.08±0.04
		LSi	2	0.38±0.03

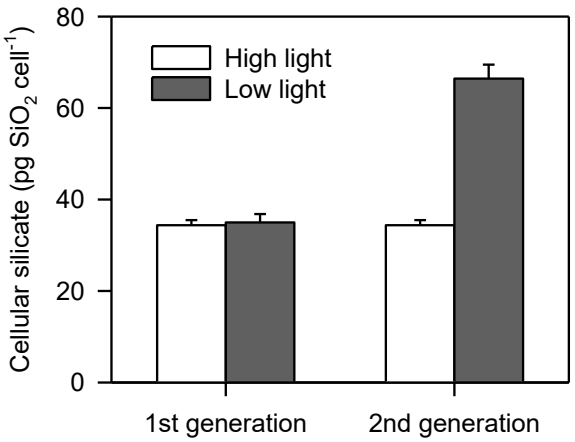
HSi: high silica content, LSi: low silica content.

Table 3. The L-ratio (m^{-1}), determined as the mean degradation rate constant (t^{-1}), divided by the mean sinking rate (m d^{-1}), for each treatment.

Prey silica content	High food concentration	Low food concentration
High Si	3.91×10^{-4}	7.56×10^{-4}
Low Si	1.09×10^{-3}	1.65×10^{-2}

558 Fig. 1. The cellular silica content of *T. weissflogii* grown under different light

559 intensities. The error bars show one standard deviation (n=3).



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562 Fig. 2. Grazing rate (A) and clearance rate (B) of *C. sinicus* fed on diatoms with
563 different silica content. HSi and LSi are high and low silica diatom prey, respectively.
564 The error bars show one standard deviation (n=5).

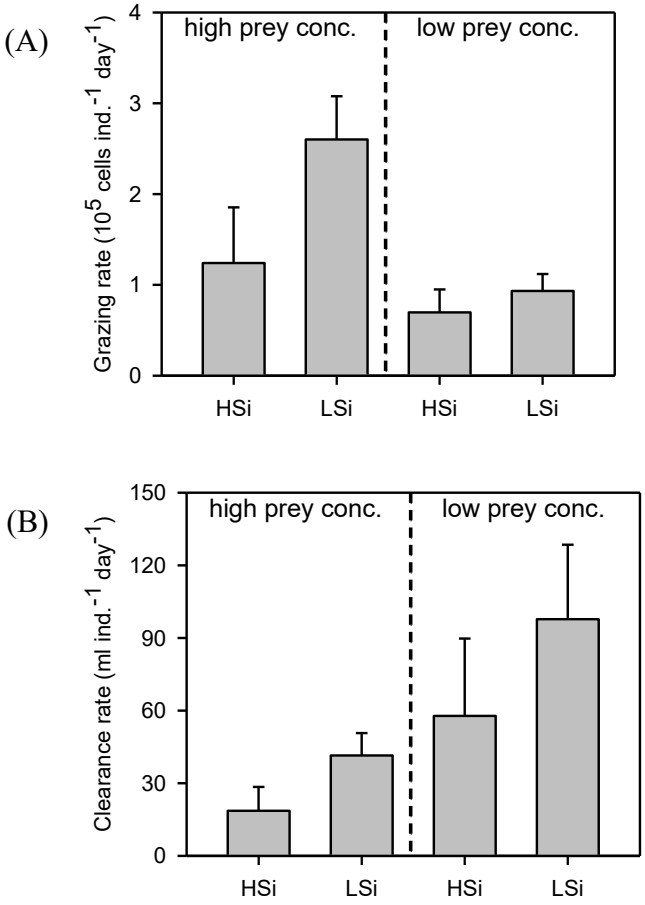

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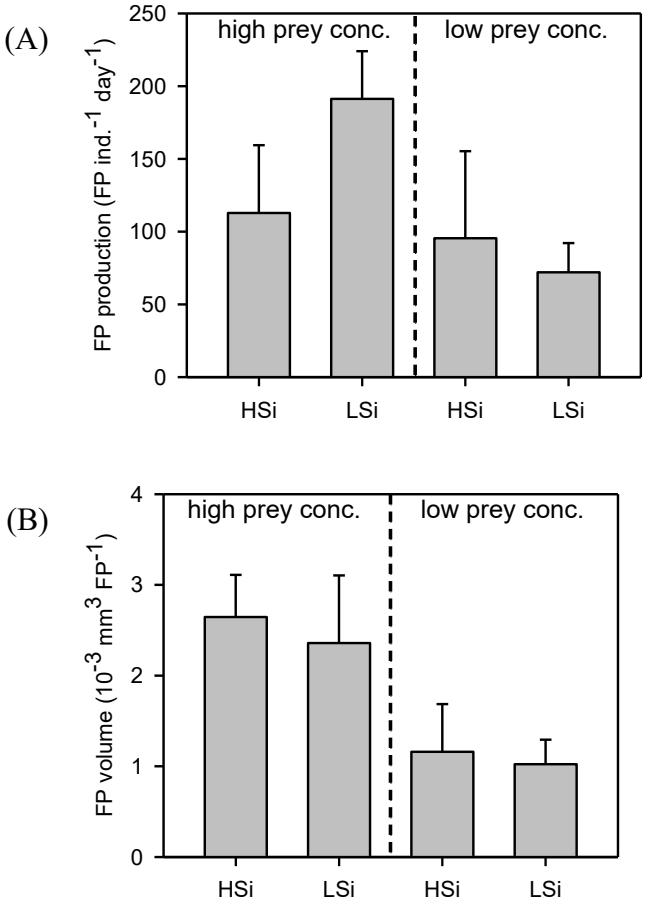
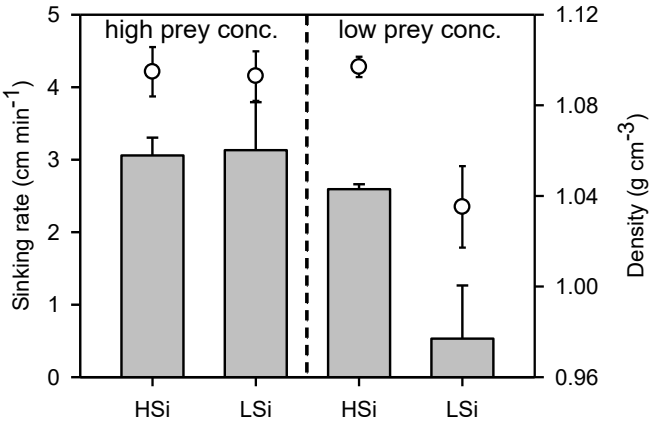
Fig. 3. The rate of fecal pellet production (A), and the average volume of each fecal pellet (B), produced by *C. sinicus*. HSi and LSi indicate high and low silica diatom prey, respectively. The error bars show one standard deviation (n = 5).



573 Fig. 4. The sinking rate (bars) and calculated density (open dots) of the fecal pellets
574 generated by *C. sinicus* produced following each treatment. HSi and LSi are high and
575 silica diatom prey, respectively. The errors bar show one standard deviation (n=3).



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582 Fig. 5. The relationship between degradation rates and surface:volume ratio of fecal
583 pellets from different experimental treatments. HSi and LSi are high and low silica
584 content diatoms, respectively; high and low prey are high and low prey concentrations,
585 respectively; 48 hr and 24 hr are the incubation periods used for the degradation
586 experiments. The error bars show ±1 standard deviation and the dashed line shows the
587 relationship curve generalized by Olesen et al. (2005).
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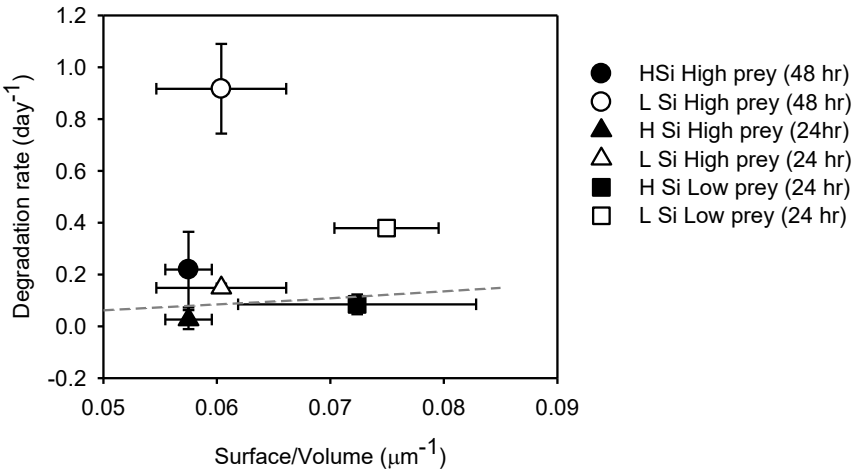

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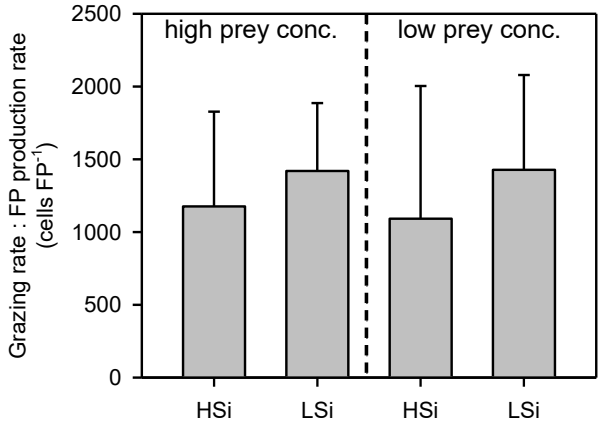
592 Fig. 6. The grazing rate: fecal pellet production rate ratio of each treatment. HSi and
593 LSi are the high and low silica diatom prey, respectively. The error bars show one
594 standard deviation.



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