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1 **Silicified cell walls as a defensive trait in diatoms**

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6 ABSTRACT

7 Diatoms contribute near half of the marine primary production. These microalgae differ from  
8 other phytoplankton groups in having a silicified cell wall, which is the strongest known  
9 biological material relative to its density. While it has been suggested that a siliceous wall may  
10 have evolved as a mechanical protection against grazing, empirical evidence of its defensive  
11 role is limited. Here, we experimentally demonstrate that grazing by adult copepods and  
12 nauplii on diatoms is near inversely proportional to their silica content, both within and among  
13 diatom species. While a 6-fold increase in silica content leads to a 4-fold decrease in copepod  
14 grazing, silicification provides no protection against protozoan grazers that directly engulf their  
15 prey. We also found that the wall provides limited protection to cells ingested by copepods,  
16 since <1% of consumed cells were alive in the faecal pellets. Moreover, silica deposition in  
17 diatoms decreases with increasing growth rates, suggesting a possible cost of defence.  
18 Overall, our results demonstrate that thickening of silica walls is an effective defence strategy  
19 against copepods. This suggests that the plasticity of silicification in diatoms may have  
20 evolved as a response to copepod grazing pressure, whose specialized tools to break  
21 silicified walls have co-evolved with diatoms.

22 Keywords: silica wall, diatoms, traits and trade-offs, mechanical defence, zooplankton grazing

## 23 INTRODUCTION

24 Higher plants have a wide range of morphological and chemical defensive traits to avoid or  
25 reduce consumption by herbivores [1]. Similarly, unicellular organisms of the pelagic realm  
26 have evolved a stunning array of defences, including escape behaviours, toxin production,  
27 and morphological traits, such as colony formation and strengthened cell walls [2]. In many  
28 cases, these traits are inducible, meaning that they are activated or intensified in response to  
29 the presence of grazers or grazer cues, which strongly supports their defensive role [3–6].  
30 However, often the benefits to the defended organism remain somewhat speculative, and  
31 quantitative estimates of the trade-offs are rare [2]. This also applies to the role of silicified cell  
32 walls in diatoms, whose protective function together with a number of other adaptive roles  
33 have been suggested. For example, the siliceous wall may provide necessary support for the  
34 large vacuole [7–9], enhance nutrient uptake [10], facilitate light harvesting [11], and/or  
35 protect the cell against UV radiation [12]. Hence, the silica wall may not necessarily only, or  
36 mainly, represent an adaptation to reduce herbivory, as is the case in higher plants [13], and  
37 the scarcity of experimental evidence for its defensive role in diatoms makes ample room for  
38 speculations.

39 The grazers of diatoms are diverse and include both metazoans (e.g., copepods and  
40 euphausiids) and protozoans (e.g., ciliates and heterotrophic dinoflagellates) [7,14,15].  
41 Copepods are key grazers in the ocean since they account for approximately 80% of the  
42 mesozooplankton biomass [16]. Calanoid copepods bear powerful mandibles lined with silica-  
43 reinforced teeth, well suited to crack diatom shells [17]. Although diatoms that are smaller  
44 than the copepod mouth may be directly ingested and survive the passage through the

45 copepod guts [18–20], it has been suggested that the copepods taste and break diatoms  
46 before ingestion [21]. However, in some cases, the siliceous wall is able to withstand the  
47 crushing forces exerted by the copepod mandibles [21–23]. Planktonic protozoans, such as  
48 heterotrophic dinoflagellates and large ciliates are another major group of grazers in the  
49 ocean [14]. Although the silica content in diatoms can affect the palatability and digestibility to  
50 protozoan grazers [24], little is known about the potential defensive role of silicified walls  
51 against these grazers.

52 While all diatoms have silicified cell walls, there is a large variation in silica content among  
53 and even within species [25–30]. This variation in the silica content may imply different levels  
54 of mechanical strength and consequently susceptibility to copepod grazing. Although some  
55 studies have suggested that diatoms with a weakly silicified frustule experience higher  
56 grazing mortality [23,30], the quantitative relationship between silica content and predation  
57 mortality remains unknown. On the other hand, the silica shell is probably of little  
58 consequence to heterotrophic dinoflagellates and large ciliates [14] – thus far, very little is  
59 known about the potential defensive role of silicified walls against protozoan grazers.

60 According to optimal defence theory, the advantage of a defence mechanism must come at  
61 some cost. Since diatoms have evolved an obligate requirement for dissolved silica, low  
62 concentrations of silicon in the environment will limit their growth, which can be regarded as a  
63 potential cost of silicification [9,31]. Furthermore, silicon deposition depends on the growth  
64 rate of diatoms. Many diatom species do not store sufficient amounts of silicon for the  
65 formation of a new valve and must, therefore, harvest most of the required quantities  
66 immediately before the cell division [25]. Consequently, fast growth will lead to a reduced

67 period available for silicon uptake, which will result in lighter silicification of the walls, even  
68 under non-limiting silicon conditions [32]. Therefore, if increased silicification in response to  
69 grazer cues requires reduced growth, this may represent a direct cost of defence. Other costs  
70 appear to be less important, and include biochemical costs related to the uptake and  
71 deposition of dissolved silica [33], and the costs associated with the potential losses due to  
72 elevated sinking rates of heavy silicified cells [2,34].

73 Here, we experimentally investigated how different degrees of silicification within and among  
74 diatom species affect predation by different types of planktonic grazer: adult copepods,  
75 copepod nauplii, and heterotrophic dinoflagellates that engulf their prey. Moreover, we  
76 examined whether the survival of diatoms during the gut passage of copepods is related to  
77 the silica content of the cells. Finally, we assessed the potential cost of diatom silicification in  
78 terms of growth reduction.

## 79 MATERIALS AND METHODS

### 80 *Organisms and culture conditions*

81 The diatom species used as prey were *Amphiprora paludosa* (CCMP125), *Cyclotella cryptica*  
82 (CCMP331), *Cyclotella meneghiniana* (CCMP336), *Navicula incerta* (CCMP542), *Nitzschia*  
83 *laevis* (CCMP559), *Thalassiosira guillardii* (CCMP988), and *Thalassiosira weissflogii*  
84 (unknown strain, UNK). The first six species were obtained from the NCMA at Bigelow  
85 Laboratory, Maine, while *T. weissflogii* was obtained from DTU Aqua, Denmark. The species  
86 are all non-chain forming, have no spines or other cell structures, are non-toxic and of similar  
87 size to minimize other effects than silica content on grazing (Table 1). We used different  
88 levels of light to acquire different degrees of silicification within a species, as silica contents in

89 cells increase under conditions of light limitation [35]. Specifically, each of the seven  
90 monocultures was split into two subcultures that were placed under low ( $15 \mu\text{mol photons m}^{-2}$   
91  $\text{s}^{-1}$ ) or high ( $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) light conditions (14:10 L:D cycle). All subcultures were  
92 maintained at a temperature of  $15^\circ\text{C}$  in B1 medium with silica [36], and grown until the  
93 stationary phase, where the cells contained maximum concentrations of biogenic silica.

94 We also measured grazing on various species of nanoflagellates (non-silicified prey) in the  
95 experiments with copepod nauplii and heterotrophic dinoflagellates to separate size- and  
96 silica effects on grazing rates. The nanoflagellate species used as prey were *Heterosigma*  
97 *akashiwo* (UNK), *Isochrysis galbana* (UNK), *Rhodomonas salina* (UNK), and *Tetraselmis* sp.  
98 (Table 1). All species were obtained from DTU Aqua (Denmark) and maintained in cultures in  
99 B1 medium.

100 Grazers were adults and nauplii of the copepod *Temora longicornis* (ca. 1 mm cephalothorax  
101 length and 0.3 mm body length, respectively) isolated from the Danish coastal waters and  
102 maintained in continuous laboratory cultures at  $15\text{-}18^\circ\text{C}$  and a salinity of 32 psu in the dark,  
103 on a mixed diet of phytoflagellates and diatoms for several generations. To obtain nauplii  
104 cohorts of *T. longicornis*, adults were separated from the stock culture with a  $200\text{-}\mu\text{m}$ -mesh  
105 sieve and placed in a new tank. After 48 h, the adults were removed and newly produced  
106 eggs were transferred to a new tank with food *ad libitum* for 4 days until nauplii stages NIII-IV  
107 were obtained. The dinoflagellate *Oxyrrhis marina* (ESD  $17 \mu\text{m}$ ) was obtained from DTU Aqua  
108 (Denmark) and maintained in culture on a diet of the cryptophyte *Rhodomonas salina* for  
109 many generations.

110 New diatom batch cultures were established from inoculums of the above subcultures at initial  
111 densities of  $16 (\pm 6) \times 10^6 \mu\text{m}^3 \text{mL}^{-1}$  and maintained as described above. The growth rates,  
112 cellular silica contents, and copepod or dinoflagellate grazing on the diatoms were monitored  
113 every second day for several days. Because silica contents in diatoms scale approximately  
114 with their volume (Figure 1A), we normalized cellular silica content with cell volume and  
115 computed the daily relative change in silica content as

$$116 \quad \Delta Si = \frac{\ln(Si_1) - \ln(Si_0)}{t_1 - t_0}, \quad (1)$$

117 where  $Si_0$  and  $Si_1$  are biogenic silica concentrations ( $\text{fmol Si } \mu\text{m}^{-3}$ ) at times  $t_0$  and  $t_1$ .

#### 118 *Copepod and nauplii grazing experiments*

119 Every second day (days 1, 3, 5, 7, and 9), ingestion rates of *Temora longicornis* adult  
120 copepods and nauplii on diatoms were measured. To separate the size- and silica effects on  
121 grazing, the ingestion rates of nauplii were additionally measured on small non-silicified  
122 nanoflagellates for 6 days (days 1, 3, and 5) (Table 1). Prey suspensions at concentrations of  
123  $3.5 \times 10^6$  and  $5 \times 10^5 \mu\text{m}^3 \text{mL}^{-1}$ , respectively, were prepared in B1+Si medium. For each  
124 diatom species and treatment (low and high light), the prey suspension was added to nine 68-  
125 mL glass bottles: triplicate initials, triplicate controls (without grazers), and triplicate grazing  
126 bottles (with grazers). From each initial bottle, a subsample of 5-mL was removed for cell  
127 enumeration and fixed with acidified Lugol's solution; a subsample of 10-mL was withdrawn  
128 for the biogenic silica analysis and treated as described below; and a subsample of 15-mL for  
129 measurement of the cell volume. On the experimental days, 15-copepods or 40-nauplii that  
130 had starved for 24 h were added to each grazing bottle, and control and grazing bottles were

131 then mounted on a slowly rotating (0.5 rpm) plankton wheel (diameter of 37 cm) for 24 h in  
132 darkness and at a temperature of 15°C. At the end of the incubation, subsamples of 5-mL for  
133 cell enumeration were fixed with acidified Lugol's solution. The number of copepods or nauplii  
134 was counted and their ingestion rates calculated according to [37].

135 The remaining volume from the grazing bottles with adult copepods was dispensed into  
136 culture plates, and approximately 30-faecal pellets from each bottle picked under a dissecting  
137 microscope. Faecal pellets were immediately examined under a fluorescence microscope  
138 (Olympus, blue filter, magnification 200x), where the number of viable cells per faecal pellet  
139 was counted. Afterwards, the remaining contents were fixed with acidified Lugol's solution,  
140 and the number of faecal pellets determined. Survival of diatoms during gut passage was  
141 calculated as a fraction of ingested diatoms that survived gut passage.

#### 142 *Dinoflagellate grazing experiment*

143 Predation by the dinoflagellate *Oxyrrhis marina* was measured on 4 diatom species every  
144 second day for 10 days (days 1, 3, 5, 7, and 9) and on nanoflagellates for 4 days (days 1 and  
145 3). Diatom and nanoflagellate prey suspensions at concentrations of  $1 \times 10^6 \mu\text{m}^3 \text{mL}^{-1}$  were  
146 prepared in B1+Si and B1 medium, respectively. The prey suspensions were then dispensed  
147 in twelve 68-mL glass bottles: six controls and six grazing bottles with 100-dinoflagellates mL<sup>-1</sup>.  
148 Three bottles from each treatment were immediately harvested for determination of initial  
149 prey and grazer concentrations as well as biogenic silica content in diatoms. All the other  
150 bottles were mounted on a plankton wheel and harvested at the end of the 24 h incubation.  
151 The ingestion rates of dinoflagellates were calculated according to [37,38].

#### 152 *Biogenic silica analysis*

153 The total cellular silica content (biogenic silica) was determined according to [39]. Briefly, 10-  
154 mL subsamples were filtered on polycarbonate filters with a pore size of 3- $\mu\text{m}$ , and washed  
155 twice with acidic MilliQ water (pH of MilliQ was adjusted to 5.0 using concentrated  
156 hydrochloric acid to minimize dissolution of silica). Filters were then placed in 50-mL sterile  
157 polyethylene tubes, dried at 65°C for 90 minutes, and stored at -20°C until analysis.  
158 Dissolution of biogenic silica was later achieved by adding 15-mL of 0.5% (w/v) sodium  
159 carbonate solution to the tubes and heating to 85°C for 90 minutes. When cool, the pH was  
160 adjusted to 5.0–6.0 by adding concentrated sulfuric acid. The reactive silicate concentration  
161 was determined according to the molybdate method of [40]) using a SmartChem 200 wet  
162 chemistry analyser (Unity Scientific, MA).

### 163 *Statistical analysis*

164 R-package *lmerTest* [41] was used to perform a linear mixed effects analysis on the  
165 relationship between ingestion rates, silica content, and size, with species as random effects.  
166 The Akaike Information Criterion (AIC) was used to select the best model. The model was  
167 validated by visual inspection of residual plots.

## 168 RESULTS

### 169 *Growth and biogenic silica content*

170 Our data together with the data compiled from the literature show that the silica content is  
171 near proportional to the cell volume (Figure 1A). Hence, we normalized cellular silica content  
172 (i.e.,  $\text{pmol cell}^{-1}$ ) with cell volumes (i.e.,  $\text{pmol } \mu\text{m}^{-3}$ ).

173 Diatoms cultured at low light intensities experienced slower growth compared to those  
174 cultured under high light conditions (Figure S1). The silica contents of all diatom species  
175 decreased during the development of the cultures, and were in most cases higher in light-  
176 limited than in light-saturated cells (Figure S2). The silica content of cells was not related to  
177 their growth rate (Figure S3A) because cultures never achieved steady state, but the rate of  
178 change in silica content declined with the growth rate of diatoms (Figure 1B). Overall, we  
179 obtained diatoms with a 6-fold variation in silica content among species, and a nearly 2-fold  
180 variation within species.

#### 181 *Grazing on diatoms with different degrees of silicification*

182 Ingestion rates of the adult copepod *Temora longicornis* decreased with increasing silica  
183 content of diatoms when compared across species, and varied approximately inversely with  
184 the silica content (hyperbolic relation) (Figure 2A). A hyperbolic relationship implies that  
185 grazing mortality initially decreases rapidly with increasing silica content but as silica content  
186 increases further the mortality reduction diminishes. Overall, a 6-fold variation in silica content  
187 from 1 to 6 fmol Si  $\mu\text{m}^{-3}$  lead to a 4-fold decrease in grazing mortality. The same pattern of  
188 reduced grazing mortality with increasing silica content was also found within all species and  
189 was significant in 4 out of 7 cases.

190 Cell size was a confounding factor since ingestion rates also varied with prey size, although  
191 much less than with silica content (Figure 2B). Among the tested models for the ingestion  
192 rates, the AIC indicated that the best fit was with silica content per volume as a fixed factor,  
193 and diatom species as a random effect (Table 2).

194 Similarly, ingestion rates of the copepod nauplii decreased with increasing silica content of  
195 diatoms both within and among the species (Figure 2C). Overall, less than a 3-fold increase in  
196 silica content led to almost negligible ingestion rates. Moreover, prey size variation within the  
197 examined range did not affect the ingestion rates of the nauplii (Figure 2D).

198 Grazing of the dinoflagellate *Oxyrrhis marina* on diatoms and flagellates were unaffected by  
199 silica contents, and was similar between the two prey groups (non-silicified nanoflagellates vs.  
200 diatoms) (Figure 2E). On the other hand, the grazing rates significantly varied with prey size  
201 (Figure 2F).

#### 202 *Survivorship of ingested diatoms*

203 A small fraction of the ingested diatoms survived gut passage intact (up to approx. 0.5%);  
204 however, survivorship of ingested diatoms was independent of biogenic silica content (Figure  
205 3A) and/or size of diatoms (Figure 3B).

## 206 DISCUSSION

#### 207 *Protective value of silicified cell walls*

208 Here we have shown that the siliceous shell provides partial protection against copepod  
209 grazing, and that the protective value increases with the silica content of the cells (Figure 2A  
210 and 2C). The relations found here are, of course, specific to the grazers used, and smaller or  
211 larger copepod species may be more or less constrained by the silica shell than found here –  
212 just as the small nauplii are more impacted by the shell thickening than the larger adults are.  
213 The fact that very few cells pass the gut intact suggests that even small cells are broken  
214 during or after ingestion, and this is consistent with mechanical protection. This may also

215 explain why the less powerful copepod nauplii, in particular, were unable to consume heavy  
216 silicified cells. Note that the differences in silicification among species can be higher than  
217 those obtained in our experiments [25], and therefore expected differences in predation  
218 mortality from copepods can be even more drastic than observed here. Similar protective  
219 value of silicification may be expected against euphausiids, which too need to crush diatoms  
220 before ingestion.

221 Other major metazoan grazers of diatoms in the ocean are tunicates. Tunicates are  
222 considered unselective feeders that ingest all prey within their food size spectrum. However,  
223 unlike copepods, consumed diatoms are not broken during ingestion and digestion, and thus  
224 the silica frustule will allow higher survivorship in tunicate faecal pellets [45].

225 This dependency of copepod grazing mortality on silica content is consistent with  
226 experimental measurements as well as finite element method (FEM) simulations,  
227 demonstrating that the mechanical strength of diatom shells increases with increasing silica  
228 content [23,29,46,47]. It is also consistent with scaling arguments (see below) that both the  
229 fracture strength and the critical buckling load of a diatom shell increase approximately with  
230 the cell wall thickness raised to the power of 3. This scaling additionally explains, in a general  
231 sense, why the advantage of further thickening decreases as the silica content increases: the  
232 un-breakability of cells increases rapidly with their silica content.

233 While the diatom wall reduces copepod grazing mortality in our experiments, the wall seems  
234 to provide no protection against the protistan grazer *Oxyrrhis marina* (Figure 2E).  
235 Phagotrophic dinoflagellates display a variety of feeding mechanisms, such as direct  
236 engulfment of prey, pallium feeding, and tube-feeding mechanisms [48], but regardless of the

237 strategy, the ability of dinoflagellates to utilize a specific prey is governed mainly by the size  
238 and shape of the prey [49]. For pallium feeders and those that engulf their prey, such as  
239 *Oxyrrhis marina*, crushing resistance of the silicified cell walls is of no importance, as these  
240 organisms digest the cell contents without damaging the walls [8]. It has been speculated that  
241 the wall may increase the prey handling time and hence decrease feeding rate [7], but we did  
242 not find any significant effects. On the other hand, a silicified wall may be an obstacle to tube-  
243 feeding dinoflagellates that pierce the prey by means of a peduncle and suck out its contents,  
244 and therefore it is not surprising that these dinoflagellates predominantly feed on naked cells  
245 [22,50]. However, there are some parasitoid peduncle-feeding dinoflagellates that  
246 successfully feed on diatoms, although their host range is generally restricted to closely  
247 related species [50], possibly indicating co-evolution of silica walls and attack strategies  
248 based on the mode of accessing the frustule [22].

249 Thus, the defensive role of a silicified cell wall in diatoms appears to primarily provide partial  
250 protection against grazers that crush the cells. Below we discuss how the mechanical  
251 properties of silica shells vary with cell size and silica content in the context of copepod  
252 predation. We next discuss the cost of silicification and explore the trade-off function, i.e., the  
253 relation between the costs and benefits of the siliceous shell.

#### 254 *Size scaling of the mechanical properties of the diatom shell*

255 The material properties of the diatom shell are unique: it has the highest mechanical strength  
256 relative to its density among any known biological material, and for diatom shells this ratio is  
257 close to the theoretical maximum [51]. In addition to several other potential functions of the  
258 shell – to support the vacuole [52], enhance nutrient uptake rate [10], and facilitate light

259 harvesting [53,54] –, its outstanding strength strongly suggests that it also has a protective  
260 and defensive function, as demonstrated here. The silica content of diatoms scales  
261 approximately with cell volume (Figure 1A), demonstrating that the thickness of the shell  
262 increases with cell size. As rationalized below, the observed scaling is further evidence that  
263 the shell mainly serves a protective function. We can explore the scaling by asking how the  
264 silica content must increase with cell size to be able to resist the same force before it cracks  
265 or buckles. For this crude analysis we ignore structural differences in the shell among  
266 species.

267 For a thin-walled structure (shell thickness  $h$ , size  $L$ , and surface area  $A \approx L^2$ ), the silica wall  
268 mass  $W$  scales with its volume as

$$269 \quad W \approx L^2 h. \quad (2)$$

270 Several criteria may be derived for the mechanical strength, such as maximal (fracture)  
271 stress, or the critical buckling load. Assume first that failure is due to fracture. Consider a ring  
272 of radius  $R$  and cross section  $h \times h$  that is subject to a centrally directed force  $F$ , and apply the  
273 Bernoulli-Euler beam equation. The ring would experience a maximum moment  $M = RF/\pi$   
274 and maximal stress  $\sigma = 6RF/\pi h^3$ , implying for  $R \approx L$  that  $h \approx L^{1/3}$ . If this relation, on grounds of  
275 dimensional analysis, also applies to a sphere, spheroid, or cylinder, then Eq. (2) leads to the  
276 scaling law

$$277 \quad W \approx L^{2.33}. \quad (3)$$

278 With similar arguments, the exact same scaling arises if one considers a plate that is subject  
279 to a central force applied by a bar, maybe better resembling the mandible acting on a diatom

280 cell. Next, consider instead that failure is due to buckling. The buckling load  $P$  for a column  
281 (cross section  $h \times h$  and length  $L$ ) is  $P = \pi^2 EI / 4L^2$ , where  $E$  is Young's modulus, and the  
282 moment of inertia  $I$  scales as  $I \approx h^4$  (as derived by Euler in 1755 [55]). This implies  $I/L^2 \approx h^4/L^2$   
283  $\approx$  constant or  $h \approx L^{1/2}$ . Attempting to approach the morphology of a diatom shell, assume the  
284 column has a cross-section  $h \times b$  with  $b \gg h$ , i.e., a plate of thickness  $h$ , then the buckling  
285 load would scale as  $P \approx h^3 b / L^2 \approx h^3$  just as suggested by the FEM simulation of [47]. For this  
286 case, using the criterion  $P \approx h^3 b / L^2 \approx$  constant, i.e.,  $h \approx L^{2/3}$ , Eq. (2) now leads to the scaling  
287 law

$$288 \quad W \approx L^{2.67} \approx V^{0.87}, \quad (3)$$

289 where  $V$  is the cell volume (assumed proportional to the cube of linear dimension of the cell,  
290  $L^3$ ). Incidentally, this is identical to the observed size scaling of silica content in diatoms  
291 (Figure 1A). Both arguments, therefore, suggest that shell thickness should increase with size  
292 to retain constant resistance to external forces, thus further supporting the adaptive value of  
293 the shell as a defensive structure.

#### 294 *Trade-offs*

295 While our experiments demonstrate the benefits of the silica wall in terms of reduced  
296 predation mortality, the actual magnitude of the benefit of course depends linearly on the  
297 concentration of grazers. However, there may be costs of the defence. These costs are not  
298 immediately obvious from our experiments, but may nevertheless be explored from available  
299 information. The biochemical costs of forming a siliceous wall seem to be negligibly small; in  
300 fact, building a silicified cell wall appears to be much cheaper than the formation of ordinary  
301 cell walls [33]. There is evidence that shell thickening can be induced by grazer cues [5], and

302 that thickening of the silica wall may require reduced cell division rate, suggesting a possible  
303 trade-off. This is because the incorporation of silica is linked to the duration of the cell wall  
304 synthesis phase in the cell division cycle. Since slow growth prolongs this phase, this will lead  
305 to higher incorporation of silica in the frustule [32]. As a consequence, silica content in  
306 diatoms will decline with growth rate under non-limiting silica conditions, as demonstrated  
307 repeatedly [32,35,39,56]. If the silica deposition rate was constant and independent of cell  
308 division rate, the silica content at steady state would vary inversely with growth rate (i.e.,  
309 growth rate raised to the power of -1). Because the mortality rate due to copepod grazing in  
310 our experiments varies approximately inversely with silica content, this would imply a linear  
311 relationship between growth rate and grazing mortality; i.e., suggesting a linear trade-off.  
312 However, data from the literature suggest that silica incorporation rate does depend on cell  
313 division rate and that the steady state silica content rather varies with the cell division rate  
314 raised to a power of  $b$  that is smaller than -1 (Figure 4). From the five experiments reported in  
315 Figure 4, an average slope of -0.54 is estimated. This, in turn, implies that the grazing  
316 mortality increases with the growth rate raised to the power of 0.43 (when  $IR \sim Si^{0.79}$  from  
317 Table 2). This suggests that as growth decreases, the reduction in predation mortality  
318 accelerates.

319 While the above demonstrates how shell thickness is a 'passive' function of cell division rate,  
320 there is evidence for one species, *Thalassiosira weissflogii*, that shell thickening may be  
321 induced by grazer cues [5]. If this observation can be generalized, it remains to be  
322 demonstrated whether and to what extent induced shell thickening requires a reduced growth  
323 rate, as suggested by the above, or whether the thickening is the result of an increased rate

324 of silica incorporation in the frustule. However, inducible defences suggest that the defence is  
325 costly [57], and those costs must eventually lead to a reduced cell division rate. More  
326 experiments are required to clarify this and to quantify the trade-off function between the  
327 benefits and costs of induced thickening of cell walls in diatoms.

### 328 *Survival of diatoms during gut passage*

329 Previous studies reported intact diatoms in copepod faecal pellets, suggesting that the silica  
330 wall may enable viable gut passage of ingested diatoms [18,19]. Our experiments show,  
331 however, that only a minute fraction of the cells survive and that the survival is independent of  
332 the silica content in the cells (Figure 3A). However, the number of live cells may have been  
333 underestimated in our study, as some viable cells are not auto-fluorescent [58]. The few cells  
334 that do survive gut passage may sink to the seafloor together with the faecal pellet and thus  
335 be removed from the pelagic population. Sinking and overwintering in the sediment are an  
336 integral part of the diatom life-cycle strategy [34], and sedimented cells contribute to  
337 reseedling the pelagic the following year when brought back to the surface by upwelling or  
338 vertical mixing [9].

### 339 *Concluding remarks*

340 Diatoms are the major contributors to phytoplankton blooms because their mortality rates are  
341 lower than those of other groups with similar growth rates, making them central in aquatic  
342 ecosystems as well as the global carbon cycle [46]. But what makes diatoms unique are their  
343 silicified cell walls, whose mechanical strength relative to density is the highest among any  
344 known biological material [51]. Our results demonstrate that the silicified cell wall truly is an  
345 effective morphological defence mechanism against copepods, whose opal teeth have likely

346 co-evolved with their prey, but ineffective against protozoans with direct engulfment as  
347 feeding mechanisms. However, a specific trait does not need to perform perfectly to be  
348 maintained in a population and if the grazing mortality rate is decreased by even a small  
349 factor, then as long as this benefit offsets the costs of employing the trait, it can be retained  
350 [8].

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511 Figure 1: (A) log-log relationship between biogenic silica content ( $\text{pmol cell}^{-1}$ ) and cell volume  
512 ( $\mu\text{m}^3$ ). The regression line is  $\log(\text{Si}) = 0.87 \log(V) - 2.89$  [ $r^2=0.84$ ;  $P<0.05$ ]. Data are from this  
513 study (average silica content and cell volume of diatoms grown under high light conditions)  
514 and [25,39,42–44]; and (B) relationship between relative change in biogenic silica content  
515 ( $\text{SiR}$ ;  $\text{d}^{-1}$ ) and growth rate ( $\text{GR}$ ;  $\text{d}^{-1}$ ). The regression line (with 95 % confidence limits) is  $\text{SiR} =$   
516  $-0.29\text{GR} + 0.02$  [ $r^2=0.6$ ;  $P<0.05$ ]. Details about experimental organisms are provided in Table  
517 1. HL – high light conditions; LL – low light conditions

518 Figure 2: (A) log-log relationship between copepod ingestion rate ( $\text{IR}$ ;  $\text{cell volume copepod}^{-1}$   
519  $\text{h}^{-1}$ ) and biogenic silica content ( $\text{Si}$ ;  $\text{fmol } \mu\text{m}^{-3}$ ); and (B) between copepod ingestion rate and  
520 cell size ( $\text{size}$ ;  $\mu\text{m}$ ). See Table 2 for the model selection. (C) Relationship between nauplii  
521 ingestion rate ( $\text{IR}$ ;  $\text{cell volume nauplius}^{-1} \text{ h}^{-1}$ ) and biogenic silica content ( $\text{Si}$ ;  $\text{fmol } \mu\text{m}^{-3}$ ). A  
522 nonlinear regression was fitted to the silica range:  $\text{IR} = 19360 \times \exp(-0.81\text{Si})$  [ $r^2=0.71$ ;  
523  $P<0.05$ ]; long-dashed lines are 95% confidence intervals; and (D) relationship between nauplii  
524 ingestion rate and cell size ( $\text{size}$ ;  $\mu\text{m}$ ) ( $P>0.05$ ). (E) Relationship between dinoflagellate  
525 ingestion rate ( $\text{IR}$ ;  $\text{cell volume dinoflagellate}^{-1} \text{ h}^{-1}$ ) and biogenic silica content ( $\text{fmol } \mu\text{m}^{-3}$ )  
526 ( $P>0.05$ ); and (F) between dinoflagellate ingestion rate and prey size ( $\text{size}$ ;  $\mu\text{m}$ ). A log-normal  
527 distribution was fitted to the prey size spectrum:  $\text{IR} = 309.1 \times \exp[-0.5 \times ((\log(\text{size})-7.7)/2.9)^2]$   
528 [ $r^2=0.55$ ;  $P<0.05$ ]; long-dashed lines are 95% confidence intervals. Details about experimental  
529 organisms are provided in Table 1. HL – high light conditions; LL – low light conditions

530 Figure 3: (A) Relationship between fraction of viable cells in copepod faecal pellets  
531 ( $\text{Survivors}$ ; %) and biogenic silica content of ingested cells ( $\text{fmol } \mu\text{m}^{-3}$ ) ( $P>0.05$ ); and (B)

532 between viable cells and size of diatoms ( $\mu\text{m}$ ) ( $P>0.05$ ). Details about experimental  
533 organisms are provided in Table 1. HL – high light conditions; LL – low light conditions

534 Figure 4: Steady state silica content in diatoms as a function of cell division rate for diatoms  
535 that are limited by factors other than silica (nitrogen  $N$ , phosphorus  $P$ , light  $L$ , or temperature  
536  $T$ , as indicated in the legend). Data obtained from [32,35,39,56]. A fitted mixed linear model  
537 with one slope and random intercepts was fitted to the log-log relationships between silica  
538 content and growth rate. The estimated slope is -0.54 [-0.36, -0.72]

539 Table 1: Functional group of phytoplankton, prey species, abbreviated species name, cell size  
540 expressed as Equivalent Spherical Diameter (ESD) and longest dimension, and type of  
541 grazer (adult copepods, nauplii NIII-IV, and dinoflagellates). ESD was measured with a  
542 Multisizer 3 Coulter Counter and the longest dimension of pennate diatoms with an optical  
543 microscope (Leica, magnification 200 $\times$ ; measured number of cells >130)

544 Table 2: Model comparisons of ingestion rates  $\log(IR)$ , where cell size  $\log(size)$ , silica content  
545 per volume  $\log(Si)$ , or silica content per volume and cell size  $\log(Si)+\log(size)$  are fixed  
546 factors, and species is a random effect. Selected model is presented in bold. \*  $P<0.05$ ; \*\*  
547  $P<0.01$ ; and \*\*\*  $P<0.001$