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Is chain length in phytoplankton regulated to evade predation?

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Formation of cell chains in phytoplankton is ecologically important, but no single factor driving the evolution of chain formation has been identified. Chain length in the diatom *Skeletonema marinoi* declines in response to grazer cues, which reduces grazing losses in simple laboratory incubations. Here we explore a more ecologically relevant scenario with fluctuating populations of different sized grazers, and test whether chain-length plasticity provides a selective advantage by lower grazing mortality. We used a model with empirical grazer densities, the effect of grazer cues on chain length, and size selective grazing rates. Finally we compared the model outcome with *Skeletonema* chain length and copepod biomass in the field. Low copepod densities induced chain-length reduction in *Skeletonema*, showing that the signaling system is sensitive enough to operate in nature. The model shows that reducing chain length in response to copepod cues reduces annual grazing losses by 31 and 36% compared with fixed traits with either single cells or long chains, respectively. The field measurements agree well with chain length being regulated by grazer abundances. We conclude that chain-length plasticity is a selective trait, and suggest that grazer regime could be an evolutionary driver of chain formation in phytoplankton.

KEYWORDS: diatom; plankton; grazing; plasticity; colony

INTRODUCTION

Phytoplankton account for half of the global organic production (Field *et al.*, 1998). Yet, we know strikingly little about many of these microscopic organisms. Our understanding of their functional morphology is rudimentary compared with that of higher plants. As an example, many dominating phytoplankton taxa form long chains of interlinked daughter cells. Chain formation is particularly common in non-motile organisms like diatoms and cyanobacteria, but it occurs also among motile phytoplankters like the

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swimming chains of the dinoflagellate *Alexandrium* spp. The evolutionary rationale behind chain formation in phytoplankton is poorly understood, and has historically been viewed from a resource-driven, bottom-up, perspective. Earlier work mainly viewed chain formation as an adaptation to optimize sinking rate to enhance nutrient absorption by maintaining steep concentration gradients in turbulent conditions (Smayda, 1970). In his seminal paper "On the life-forms of phytoplankton as survival alternatives in an unstable environment", Ramon Margalef concluded that "nutrient supply and turbulence are the most important factors shaping phytoplankton through evolution, and the only reason for proceeding to a functional interpretation of their morphology" (Margalef, 1978). This was an influential synthesis, but since then additional aspects of phytoplankton evolution have been revealed. Most important is the emergent view that phytoplankton–zooplankton interactions may drive the evolution of many adaptive traits. Grazing pressure can be extreme in plankton communities, and the upper water column is cleared on a daily to weekly basis (Lampert *et al.*, 1986). The most important grazers are microzooplankton, mainly ciliates and heterotrophic dinoflagellates, followed by mesozooplankton, mainly copepods, cladocerans and meroplanktonic larvae. Together they consume more than 90% of the primary production (Calbet, 2001; Calbet and Landry, 2004). Moreover, grazing is not indiscriminate. The grazers clear food items at different rates, depending on the size, shape and chemical composition of the food (Verity and Smetacek, 1996).

Micro- and mesozooplankton differ in feeding strategies and food preferences. Copepods often graze selectively on the large particles (Paffenhöfer, 1971; Hansen *et al.*, 1994). The considerably smaller ciliates and heterotrophic dinoflagellates generally feed on a lower prey size spectrum (O'Connors *et al.*, 1980; Hansen *et al.*, 1994). Some dinoflagellates like those feeding with a large pseudopodial pallium, and the largest *Gyrodinium* spp., may have an exceptionally broad prey size spectrum (Hansen and Calado, 1999; Naustvoll, 2000; Buck *et al.*, 2005). However, most of the non-pallium or peduncle feeding dinoflagellates are limited to small phytoplankton, or chains up to 4 cells chain^{-1} (Du Yoo *et al.*, 2009). Thus, micro- and mesozooplankton in general graze on different size ranges of phytoplankton and represent contrasting selection pressure on prey size.

When abundant, mesozooplankton graze down microzooplankton, giving rise to cascading effects in the food web (Calbet and Saiz, 2005). When microzooplankton are suppressed, smaller phytoplankton are released from grazing pressure while larger phytoplankton become more exposed to grazing. This fluctuation in grazing regime may manifest in higher abundance of smaller phytoplankton at times of intense mesozooplankton grazing (Olson *et al.*, 2006). Despite the established role of grazing in trophic transfer, research on plankton communities has mainly focused on bottom-up factors like nutrient dynamics, light and turbulence (Verity and Smetacek, 1996). However, recent studies show that some phytoplankton are capable of adjusting their phenotype to reduce predation from the prevailing grazer community (Long *et al.*, 2007; Selander *et al.*, 2011; Bergkvist *et al.*, 2012). As an example, chain-forming diatoms sense chemical cues from copepod grazers and respond by splitting up chains into single cells or shorter chains, thus reducing grazing rate more than 10-fold compared with longer chains (Bergkvist *et al.*, 2012). Chains exposed to the scent of caged copepods without direct physical contact with the diatoms still responded by splitting up chains, which proves the chemical nature of the interaction.

Despite the many laboratory studies indicating that such grazer-induced responses may be influential in modulating trophic interactions (Hessen and van Donk, 1993; Long *et al.*, 2007; Selander *et al.*, 2011) there has been no analysis of their ecological relevance under field conditions. Here, we test the hypothesis that phytoplankton chain formation and chain-length plasticity has an adaptive function in reducing grazing mortality. We model the effect of chain-length plasticity on grazing mortality in *Skeletonema marinoi* over a temperate year based on empirical data on grazer densities, chain length and grazing rates. We compare the model predictions with field data to verify our findings.

METHOD

Sensitivity to grazer cues

To model the effect of grazer-induced chain-length plasticity, it is necessary to know at what concentration of copepods the chains respond by splitting up into shorter units. We cultured the diatom *S. marinoi* (strain isolated from the west coast of Sweden), hereafter named *Skeletonema*, in IMR/2 medium with silicate (Eppley *et al.*, 1967) at 15°C with 12:12 h light:dark cycles. The cultures were grown in sealed bottles on a revolving plankton wheel (0.2 rpm) for 3 days prior to the experiment to form natural chains in exponential phase. Female *Acartia tonsa* copepods (from culture in Kristineberg, Sweden, prosome length mean ± SD: 743 ± 29 μm) were used to test the sensitivity to grazer cues. *Acartia tonsa* occur in the study area even if *A. clausi* is the more common *Acartia* species.

We incubated *Skeletonema* in 620 mL bottles at ≈2×10^4 cells mL^{-1} with triplicates of 0, 1, 3 and 6 *A. tonsa* females in 48 h. Using live copepods was necessary to measure a realistic effect of grazer abundance, as extracts

from grazers may degrade rapidly and the use of cages may prevent mixing and thus reduce the signal strength. Grazing was always below 10% in these experiments and consequently confounding effects from selective grazing were not influential. The bottles were placed on a plankton wheel (0.2 rpm) at 15°C and the light:dark cycle was set to 14:10 h to mimic natural conditions more closely. After incubation we counted the chain length (cells chain^{-1}) in >100 chains from each replicate under an inverted microscope. Differences in chain length were tested with a two-way ANOVA, with proportion of chains in 5 length classes as response and number of copepods and length class as factors. We used Tukey HSD post hoc test to determine significant difference between treatments within each length class.

Grazing rates

We tested the ability of micro- and mesozooplankton to graze on *Skeletonema* with and without induced chain length reduction. We used field caught copepods of common grazers rather than lab cultures to increase the ecological relevance. The copepod *Acartia clausi* was sampled with a WP-2 plankton net (200 μm mesh size) from 30 to 0 m depth in Kosterfjord, Sweden. *Acartia clausi* were kept in 15°C and fed *Rhodomonas salina* prior to the experiments. The *Skeletonema* cultures were prepared by incubation in f/2 medium (Guillard and Ryther, 1962) and either three female *A. clausi* or without grazers in 620 mL bottles over 3 days. The chain lengths in the induced and natural cultures were counted under an inverted microscope. The use of different media in the induction and grazing experiments should not confound the experiments as cultures were kept in exponential phase in excess nutrients and only differences between cultures in the same nutrient regime (media) were considered.

The natural (7.1 ± 5.1 mean ± SD cells chain^{-1}) and induced (1.5 ± 1.0 mean ± SD cells chain^{-1}) cultures were incubated in 320 mL bottles in five concentrations corresponding to 20, 50, 100 and 500 μg C L^{-1} (Strathmann, 1967). For each culture and concentration, seven replicates were without grazers and seven replicates supplemented with eight *A. clausi* females. We added 0.2 mL f/2 medium to each bottle. The bottles were incubated on a revolving plankton wheel (0.2 rpm) at 15°C in darkness for 12 h. This incubation time should be short enough to largely avoid chemically induced effect on chain length (J. Bergkvist, unpublished data). Grazing by *A. clausi* on long and short chains was calculated from measured *Skeletonema* biovolume with a Beckman Coulter Multisizer III. Using biovolume we avoid a biased estimate due to different particle size in the cultures (Kim and Menden-Deuer, 2013). Clearance rate (F, mL ind.$^{-1}$ day^{-1}) was calculated with a modified equation from Frost (Frost, 1972):

$$F = \frac{V}{N \cdot t} \ln\left(\frac{v_1}{v_0}\right)$$

where V is incubation volume, N is number of grazers, t is incubation time, and v_0 and v_1 is the total biovolume of *Skeletonema* (μm^3 mL^{-1}) after incubation with and without copepods.

Difference in clearance on long and short chains was tested with a paired t-test of the average grazing rates from grazer-induced and control treatments for each level of food concentration ($N = 5$).

The spectrum of microzoolankton predators on *Skeletonema* is very broad. We included one common ciliate species, *Strobilidium spiralis*, as a representative of the important grazer guild of microzooplankton to serve as a contrast to mesozooplankton grazers like copepods. We acknowledge that other microzooplankton, e.g. heterotrophic dinoflagellates, may affect *Skeletonema* differently. The ubiquitous oligotrich ciliate *Strobilidium spiralis* was collected with a bucket from surface water in the Kosterfjord. Shortly after sampling 4 replicates of 10 individual ciliates were incubated in 4 mL micro-wells (Nunc) with *Skeletonema* in chains of 1–4 cells at a concentration 20 000 cells mL^{-1}. After 15 min, grazing was terminated by adding 0.2 mL of 4% formalin. We then picked out the ciliates individually onto a microscope slide for inspection under an inverted epifluorescence microscope. Ingested cells of *Skeletonema* were counted and sized, and we measured the clearance rate (μL h^{-1} ind.$^{-1}$).

Model of grazing on *Skeletonema* with induced chain-length reduction

We explored how a plastic change in *Skeletonema* chain length may affect fitness in the field with a model of grazing by copepods and microzooplankton, here represented by oligotrich ciliates. Specifically, we tested the hypothesis that a plastic strain of *Skeletonema* would benefit from lower predation mortality over a typical annual cycle of predator abundance compared to a strain with a fixed chain length. The model first considers how the distribution of chain length changes with copepod abundance. From the empirical data on chain-length distribution in Fig. 1 we fitted logarithmic ($y = a \cdot \ln(x) + b$) or power ($y = a \cdot x^b$) regressions between copepod abundance and the proportion of each of the five chain-length classes, the choice of a logarithmic curve fit was made based on the best prediction (r^2). Secondly, the model calculates the weighted sum of predation risk (day^{-1}) from copepod and ciliate clearance for each of the chain-length classes. The clearance rate per

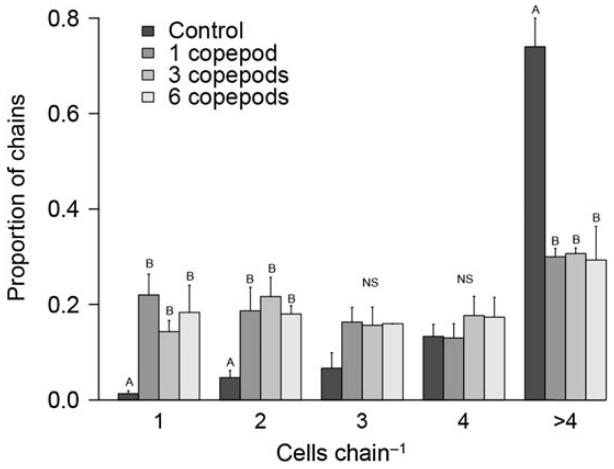

Fig. 1. Proportions of chain lengths (mean ± SD) in *Skeletonema* cultures after 48 h incubation with 0, 1, 3 or 6 female *Acartia tonsa* in 620 mL bottles. The different letters indicate significant differences between treatments (Two-way ANOVA, $P < 0.05$).

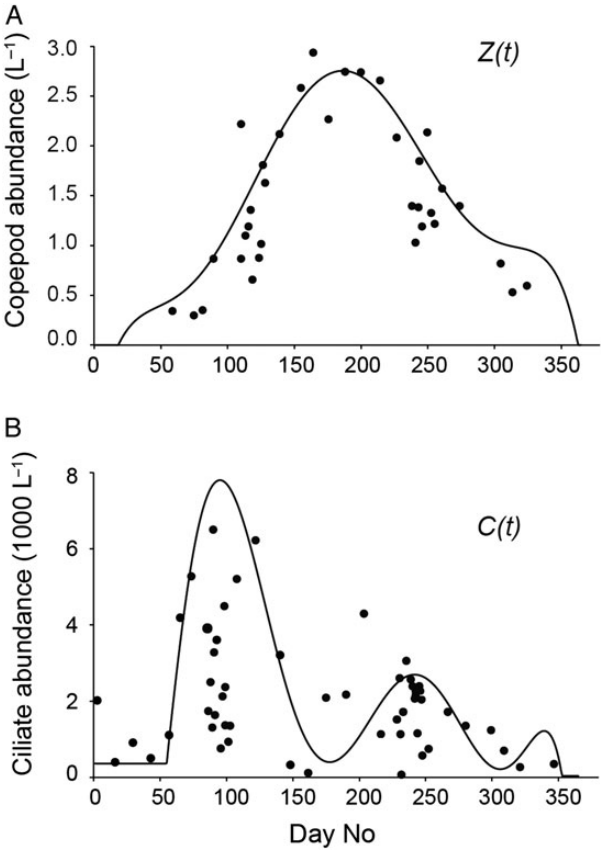

Fig. 2. (**A**) Grazer densities over an annual cycle in the Kattegat Sea. Copepod abundances calculated from Kiørboe & Nielsen (Kiørboe & Nielsen, 1994), the curve is a polynomial fit to cube-splined data, described by: $Z(t) = -0.085x^6 + 3.058x^5 - 40.78x^4 + 245.1x^3 - 661.5x^2 + 880.9x - 336.6$. (**B**) Ciliate abundances calculated from Nielsen & Kiørboe (Nielsen & Kiørboe, 1994), the curve is a polynomial fit to cube-splined data described by: $C(t) = -0.002x^7 + 0.0918x^6 - 1.688x^5 - 15.90x^4 - 80.68x^3 + 210.1x^2 - 237.1x + 79.40$.

copepod (represented by *Acartia tonsa*) in mL day^{-1} on *Skeletonema* for the five chain size classes was obtained from Fig. 7 in Bergkvist *et al.* (Bergkvist *et al.*, 2012). Clearance rate in mL day^{-1} per ciliate was derived from experimental data in Jonsson (Jonsson, 1986) for the oligotrich ciliate *Strombidium* cf. *reticulatum*. This ciliate represents a typical size of planktonic ciliates in the north-east Atlantic (Günther *et al.*, 2012). A quadratic polynomic relation between clearance (mL day^{-1}) to prey size was fitted to the data (24·max [0,-0.000147·(chain length·4.64)^2 + 0.0016·(chain length·4.64) − 0.0017]), where 4.64 represents the equivalent spherical cell size (μm) of single cell *Skeletonema*. Finally, the model was forced by field data on planktonic copepods (Kiørboe and Nielsen, 1994) and ciliates (Nielsen and Kiørboe, 1994) covering a full year in the Kattegat Sea. We first smoothed the original data using a cubic spline and then interpolated data for all 365 days during a year, which resulted in an envelope containing most field measurements. From each interpolated series we then fitted a high-order polynomic function (6 terms for the copepods and 7 terms for the ciliates; polyfit in Matlab (The MathWorks, Inc., 2013) that was used to force the grazing on *Skeletonema* in the models ($z[t]$ and $c[t]$ in Fig. 2). For the conversion of biomass to concentration (ind. L^{-1}) we assumed that a ciliate with a cell volume of 20 000 μm^3 contained 0.0028 μg C (Putt and Stoecker, 1989), and that a typical copepod (*A. tonsa*) contained 6.7 μg C (Durbin *et al.*, 1990). For the copepod data (Kiørboe and Nielsen, 1994), we used a depth of 28 m to convert depth-integrated data to concentration.

We finally evaluated the predation risk for *Skeletonema* by calculating the distribution of *Skeletonema* chain size as a function of chain reduction due to the ambient concentration of copepods ($z[t]$), and calculated the summed clearance rates by copepods and ciliates. The plastic strain of *Skeletonema* with the ability to change chain length was compared with non-plastic, fixed strains with chain lengths of either more than 5 cells or single cells, respectively. Predation risk is expressed as the volume cleared of prey per time per volume (day^{-1}).

Field verification

Plankton samples from the Swedish west coast were provided by the Swedish Metrological and Hydrological Institute, from their monthly monitoring program in 2011 and 2012 (SMHI, 2014). The phytoplankton samples were taken by slowly lowering a hose into the water to 20 m depth. The hose was subsequently sealed, hauled on board and emptied into a bucket before a well-

mixed sample was taken and preserved with Lugol's solution. We let the Lugol samples settle in Utermöhl chambers or 12-well multidishes (Nunc) and counted the number of cells per chain in *Skeletonema marinoi* under an inverted microscope.

Zooplankton monitoring data were also provided for the sampling sites, based on vertical hauls of a WP-2 plankton net (200 μm mesh size) from 25 to 0 m. The prosome length, stage and species of the sampled copepods were translated to biomass (μg DW L^{-1}), by specific length–weight relationships (Supplementary data, Table SI). We plotted the *Skeletonema* chain length and copepod biomass measurements aggregated per month to explore the seasonal patterns. Using the average values per month we tested the relationship between *Skeletonema* chain length and the copepod biomass (log-transformed) with a linear regression model in R (R Core Team, 2014).

RESULTS

Chain-length reduction was induced by all densities of copepods used in the grazer sensitivity experiment (Fig. 1). The proportion of chains with one and two cells increased and the proportion of chains longer than four cells decreased dramatically with copepods present compared to the control (two-way ANOVA with Tukey HSD: $P < 0.05$). The response was the same with 2, 5, or 10 copepods L^{-1} ($P > 0.05$).

We found that the copepods and ciliates grazed selectively on different chain lengths of *Skeletonema* (Fig. 3A and B). The clearance rate by copepods was higher on the culture with longer chains (chain length 7.1 ± 5 cells $chain^{-1}$, mean ± SD) than in the culture with induced chain-length reduction (chain length 1.5 ± 1 cells $chain^{-1}$, mean ± SD) over all food concentrations (one-sided paired *t*-test: $P = 0.02$). Clearly, the difference was strongest at the lowest food concentration, with a clearance rate of 22.6 ± 6.4 mL $ind.^{-1}$ day^{-1} (mean ± SE) on long chains versus 1.7 ± 4.5 mL $ind.^{-1}$ day^{-1} (mean ± SE) on short chains. However, variation was high within each concentration. The ciliate *S. spiralis* consumed single cells of *Skeletonema* and did not ingest any chains. The clearance rate was 0.39 ± 0.26 μL $ind.^{-1}$ h^{-1} (mean ± SD), corresponding to an ingestion rate of 7.8 ± 5.2 cells $ind.^{-1}$ h^{-1}.

The modeled predation risk, expressed as potential clearance by copepods and ciliates over a temperate year, showed that the plastic strain was on average the least susceptible when compared with strains with a fixed size, either as single cells or as chains longer than five cells (Fig. 4). The grazing risk for single cells peaked in early spring coinciding with high numbers of microzooplankton grazers, whereas the long chains are exposed to high grazing risk in summer when copepods are more abundant. Over the whole annual cycle the predation risk was 31% higher for the single-cell strain and 36% higher for the long chains compared with the plastic strain (Fig. 4B).

The total copepod biomass in the field showed a clear seasonal pattern. The average copepod biomass per month increased from 8.2 μg DW L^{-1} in winter to a peak in late spring with 168.8 μg DW L^{-1}, and then decreased again during autumn to 19.4 μg DW L^{-1} (Fig. 5A). The

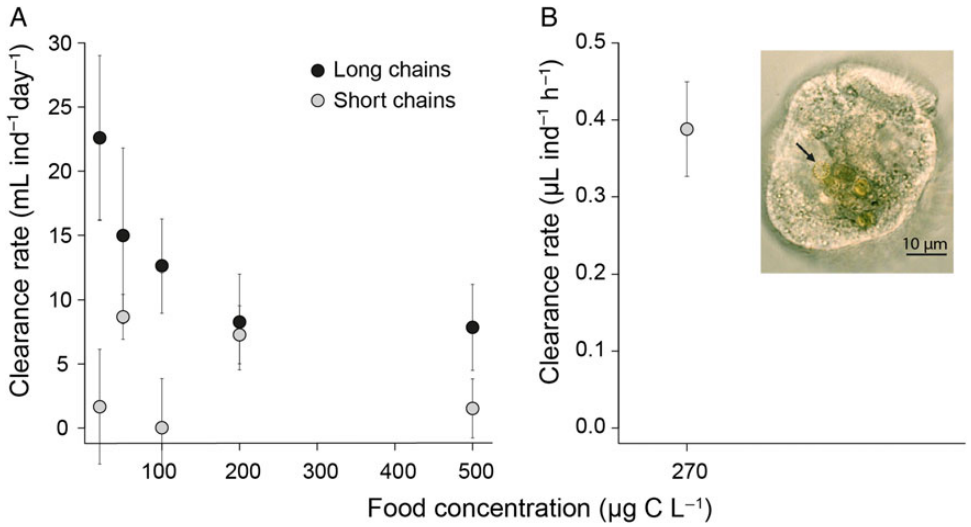

Fig. 3. Copepod and ciliate grazing rates. (**A**) Clearance by the copepod *Acartia clausi* on cultures of *Skeletonema* with long chains (filled symbols) and with grazer-induced short chains (open symbols) (mean ± SE). A paired *t*-test of the mean values for each food concentration shows higher clearance rate on the culture of long chains ($P = 0.025$). (**B**) Clearance rate by the ciliate *Strobilidium spiralis*, which only ingested single cells (open circle) when offered a *Skeletonema* culture of mixed chain lengths. After incubation the ingested *Skeletonema* cells were visible inside the ciliate (arrow).

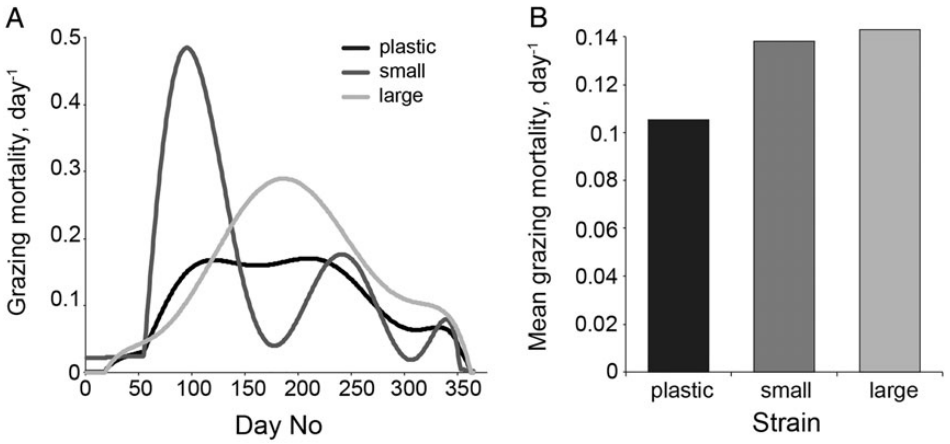

Fig. 4. Modeled predation risk on three fictive strains of *Skeletonema* with different inherent chain-length strategies. The small strain only forms single cells, the large strain only chains longer than five cells and chain length of the plastic strain responds to copepod cues according to Fig. 1. (**A**) The predation risk is modeled over an annual cycle, and is expressed as the total clearance rate per volume (d^{-1}) by copepods and ciliates. (**B**) Predation risk summed over the year for the three strains. The strains with single cells and long chains have 31 and 36% higher average grazing risk over the year than the strain with grazer-induced plasticity.

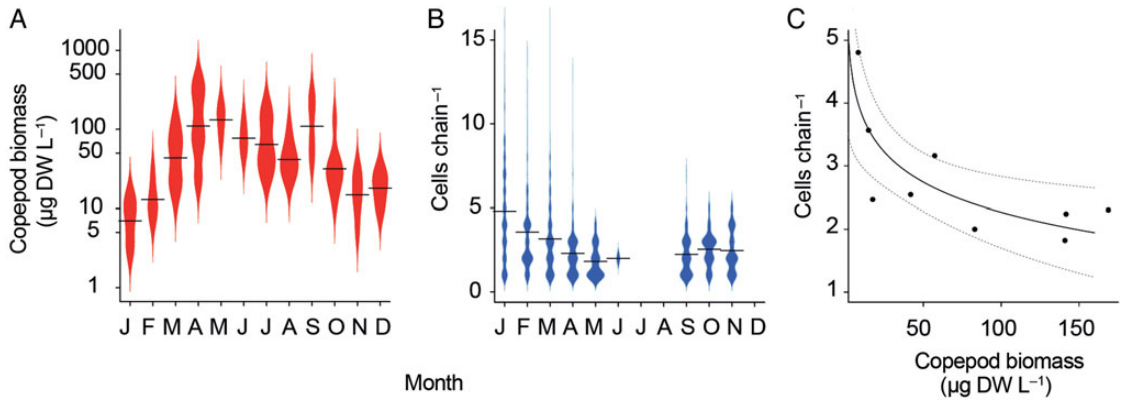

Fig. 5. Field measurements from plankton samples taken in 2011 and 2012 by SMHI (SMHI, 2014) on the Swedish west coast. The measurements are aggregated per month. (**A**) Total copepod biomass (on log scale). The horizontal lines are the geometric mean values per month and the shapes show the distribution of measured biomass values. (**B**) *Skeletonema* chain lengths. The horizontal lines are mean chain lengths per month and the shapes show the distribution of measured chain lengths. (**C**) The monthly mean *Skeletonema* chain length as a function of monthly mean copepod biomass in the field. Linear model fit: $Y = 5.59 - 0.76 \cdot \log X$, $R^2 = 0.67$, $N = 9$. The dotted lines are the 95% confidence interval of the fitted line.

Skeletonema chain lengths in the field varied from 1 to 16 cells chain^{-1}. The average chain lengths per month also indicated a seasonal pattern with chain length decreasing during spring to a local minimum in summer and a slight increase again during autumn (Fig. 5B). Field concentrations of *Skeletonema* ranged from absence to extreme bloom densities and unfortunately, *Skeletonema* was not found in the samples from July, August and December. Over the year, the patterns in copepod biomass and *Skeletonema* chain length were reflected in a significant negative correlation between mean chain length and mean copepod biomass per month, ($R^2 = 0.67$, $P = 0.0042$, Fig. 5C).

DISCUSSION

The two major groups of grazers on phytoplankton, micro- and mesozooplankton are functionally different, and select for contrasting prey size. Since mesozooplankton also graze efficiently on microzooplankton, the two grazer groups tend to be negatively correlated in time. Our model of grazing risk shows that this heterogeneity in selection pressures favors the plastic chain-length strategy, allowing *Skeletonema* to optimize resistance against both groups of grazers. This is in line with theory on the evolution of phenotypic plasticity where an inducible defense improves fitness when predator threat is variable

and unpredictable (Tollrian and Harvell, 1999). The modeled grazing risk for different chain-length strategies shows how *Skeletonema* with induced chain-length plasticity cuts mortality peaks during the annual cycle of predator diversity. Long chain length in early spring reduces grazing from microzooplankton, and the induced response to increasing copepod abundance protects from mesozooplankton grazing in summer. In the pelagic environment, where predation is thought to be the main mortality factor, decreased losses to grazing contribute directly to fitness. The predation on *Skeletonema* in this study was low on average, but fluctuated up to 0.5 day^{-1}. With the rapid turnover in marine plankton more than 30% reduced mortality gained with chain-length plasticity is enough to be influential. From an evolutionary point of view, a mutant that reduces its predation mortality may still increase in frequency and ultimately replace the wild type even if growth rate is significantly higher than predation rate.

Chain length in *Skeletonema* was remarkably sensitive to copepod chemical cues. The threshold copepod concentration inducing response in the experiment is comparable with natural spring conditions. As the copepods were in direct contact with the *Skeletonema* in our experiment, selective grazing and mechanical fragmentation could as well have contributed to the shorter chain length (Fig. 1). However, there are strong indications that the shift in the chain length distribution was primarily a response to chemical cues from the grazers and that fragmentation and grazing contribute only marginally to this response. Firstly, copepod chemical cues alone have already been shown to trigger chain fragmentation in *Skeletonema* (Bergkvist *et al.*, 2012). Secondly, the same study showed that the maximum clearance rate for a copepod in similar concentration of *Skeletonema* is around 100 mL day^{-1} $ind.^{-1}$. Thus, given that the bottles held 620 mL a single copepod could not have cleared more than one-third of the volume during the incubation time. The cell concentrations after incubation with and without grazers indicated that grazers removed $<10\%$ of the cells compared with controls. Finally, the effects of direct contact with grazers would almost certainly have resulted in a dose-dependent type of response as more copepods would encounter more chains. In contrast, the uniform response found in our induction experiment may well be induced by chemical grazer signal. This response indicates that the threshold bulk concentration is reached at low copepod abundance, suggesting a strong potential for grazers to induce chain reduction also in the wild.

The copepod grazing rates are in line with earlier findings where grazing rates increase with particle size (Paffenhöfer, 1971; Wilson, 1973; Bergkvist, 2012). The consumption of single cells of *Skeletonema* confirms that ciliates can exert a strong grazing pressure on single celled *Skeletonema* when they are abundant, and that chain formation provides a size refuge from these grazers.

The negative correlation of chain length and copepod biomass in the field is also consistent with the hypothesis that grazer cues regulate *Skeletonema* chain length. The seasonal variation in copepod biomass corresponds to the range within which the grazer-induced response can be expected, based on our experiment (Fig. 1). However, experimental results rarely translate directly to the complex and dynamic marine environment. For instance, the chain length in *Skeletonema* also follows other marked changes over the year in the temperate pelagic such as variation in nutrients and temperature that may contribute (Takabayashi *et al.*, 2006). Yet, the most rapid decline in chain length (Fig. 5B) coincides with high nutrient levels and increasing temperature, which is the opposite of what would be expected if chain reduction was driven by nutrients and temperature.

Measurements of diatom chain lengths from the field are rare. Interestingly however, Turner *et al.* (Turner *et al.*, 1983) also observed that *Skeletonema* chain length was negatively correlated with mesozooplankton abundance over a year, in the Pectonic Bay estuary, New York. Landeira *et al.* (Landeira *et al.*, 2014) found a reduction in diatom chain length in a tidal front from spring tide to neap tide. The authors suggested that adaptation to nutrient acquisition in low turbulence and nutrient conditions explained the shorter chains during neap tide. Zooplankton measurements from the same cruise (Schultes *et al.*, 2013) showed that zooplankton was not numerically more abundant during neap tide. However, there was a significant increase in large copepods (*Calanus* sp.) from spring tide to neap tide, which possibly caused the increase in the total zooplankton biomass (mg C m^{-2}). This increase of larger copepods may thus have contributed to the observed reduction in diatom chain length.

Skeletonema is not the only phytoplankton with grazer-induced morphological plasticity. Species from a variety of taxa adjust chain or colony size adaptively to grazer cues in the laboratory. Grazers may trigger colony formation making prey exceed the critical size for capture and ingestion, as in the dinoflagellate *Cochlodinium ploykrikoides* (Jiang *et al.*, 2010) and the green alga *Scenedesmus subspicatus* (Hessen and van Donk, 1993). In contrast, grazers feeding on larger prey may induce splitting-up of chains, as in, for example, *Skeletonema* (Bergkvist *et al.*, 2012) and *Alexandrium tamarese* (Selander *et al.*, 2011). An extreme example is *Phaeocystis globosa* which can change from single cells to very large colonies induced by specific cues from both micro- and mesozooplankton, thereby reducing grazing by both of them (Long *et al.*, 2007). The splitting up of chains in the presence of copepods has also been reported long

before the effect of grazer chemical cues was discovered. Deason (Deason, 1980) and O'Connors *et al.* (O'Connors *et al.*, 1980) found that grazers modified diatom chain length by grazing activity, but this could potentially also result from induced splitting of chains. The prevalence across taxa and the effective grazer protection strongly indicate that the size plasticity is an adaptive mechanism that may contribute to the evolution of colony size and plasticity in phytoplankton.

There are several hypotheses on the adaptive value of chain formation. In particular, much effort has been devoted to determine the benefit of chains in resource acquisition and buoyancy. Chain formation might increase the particle motion relative to ambient water, and thereby enhance diffusive and advective nutrient flux. Smayda (Smayda, 1970) suggested that chain formation gives an advantage of increased nutrient transport by higher sinking rates. Musielak *et al.* (Musielak *et al.*, 2009) showed how chains benefit from higher nutrient acquisition due to small scale turbulence, however, only in nutrient limited (summer) conditions. Nutrient uptake by chains compared with single cells was found in another study to be higher only in nutrient replete, typical spring conditions (Arin *et al.*, 2002). Also, chains might increase local nutrient depletion, and thus limit nutrient uptake (Pahlow, 1997). Apparently, the resource advantage in chain formation depends on the local turbulence and nutrient conditions. However, turbulence does not only transport nutrients, it also increase the encounter rate with grazers (Rothschild and Osborn, 1988), implying that turbulence might shape chain formation also via top-down processes. Experiments also show that turbulence may enhance chain formation in dinoflagellates (Sullivan *et al.*, 2003) although the effect sizes are lower and less consistent compared with the effect of grazers cues (Selander *et al.*, 2011). Factorial experiments with both turbulence and grazing would be helpful to resolve the relative importance of these factors. Maintenance of suspension is another potential benefit of *Skeletonema* chains, which despite higher total mass sink slower than single cells (Smayda and Boleyn, 1966). In contrast, dead and stressed chains sink faster than individual cells (Waite *et al.*, 1997), indicating that suspension depends more on physiological state than on chain length. Besides, light absorption is higher for single cells than cells in a chain. Theory predicts that inducible defenses should come with a cost to be favored in front of constitutional defenses (Tollrian and Harvell, 1999). It is not clear from the current study whether the short or long chained morphologies are to be considered the defended one as both are favored but by different grazer guilds. In addition, we were not able to estimate any costs of the chain-length plasticity here, but the chemical alarm signals from copepods, copepodamides, have been identified (Selander *et al.*, 2015). Provided that the same compounds induce chain splitting in diatoms, copepodamides without copepods could be used to estimate any costs associated with the morphs without confounding effects of grazing.

So far, resource acquisition has not successfully explained the adaptive significance of chain formation. Clearly though, chain length is constrained by growth (Takabayashi *et al.*, 2006). Hence resources, which were not measured in our study, may account for a considerable variation in chain length. In fact, other processes such as direct effects of selective grazing may also lead to the seasonal pattern that we found in the field. Still, the consistent results from our laboratory experiments, model predictions and field observations of *Skeletonema*, as well as support from the theoretical and empirical literature underline the selective advantage of chain-length plasticity in a variable grazer regime. This further suggests that grazing mortality may be instrumental in driving the evolution of chain formation and the plasticity in chain length.

Insight in the morphological adaptations in phytoplankton is crucial for understanding pelagic processes. Chain- and colony formation determines the length of the food web (Stibor *et al.*, 2004), sinking rates (Smetacek, 1985), and thus influences major fluxes of energy and elements in the oceans. Grazer regulation of colony formation opens up a new perspective compared with the traditional view of a resource-driven evolution of phytoplankton diversity, and highlights the role of top-down mechanisms in shaping plankton communities.

SUPPLEMENTARY DATA

Supplementary data can be found online at (http://plankt.oxfordjournals.org).

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