Chain length in phytoplankton is regulated to evade predation

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Abstract

Many dominating phytoplankton form chains of attached cells. Chain length strongly influences how the organism interacts with its environment, but the factors driving the evolution of chain formation and chain length plasticity are not entirely clear. We tested the hypothesis that chain formation in diatoms is a grazer avoidance strategy. We modelled the effect of chain length plasticity on grazing mortality in *Skeletonema marinoi* over a temperate year, based on empirical data on grazer densities, induced chain length plasticity, and grazing rates. The predicted optimal chain length strategy was compared with field data of *S. marinoi* and copepod biomass. We found that low copepod densities, corresponding to spring conditions in the field, induced chain length reduction in *S. marinoi*. Modelled grazing risk over an annual cycle showed that fixed traits with either single cells or long chains have respectively 31 % and 36 % higher mortality than *S. marinoi* with grazer induced chain length plasticity. Field measurements of chain length and grazer abundances also agree well with chain length plasticity as a grazer defense strategy. We conclude that grazer regime could be a major driving force in the evolution of chain length plasticity in phytoplankton organisms.
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 and our understanding of their functional morphology is rudimentary compared to that of higher plants. 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 long 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 (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 new 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 and Landry, 2004; Calbet, 2001). Moreover, grazing is not indiscriminate. The grazers clear food items at different rates, depending e.g. 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 (Hansen et al., 1994; Paffenhöfer, 1971). The considerably smaller microzooplankton, mainly 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 sp, 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 is manifested in higher abundance of smaller phytoplankton at times of intense mesozooplankton grazing (Olson et al., 2006). It is surprising that such a strong and discriminant mortality factor as grazing has not been more fully considered to explain the evolution of adaptive traits in phytoplankton. 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 to longer chains (Bergkvist et al., 2012).

Despite the many laboratory studies (Hessen and van Donk 1993; Long et al. 2007; Selander et al. 2011) indicating that such grazer induced responses may be influential in modulating trophic interactions there is no analysis of the ecological relevance under field conditions. Here, we test the hypothesis that phytoplankton chain formation and chain length plasticity has evolved to reduce 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, induced chain length plasticity, 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 *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. 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.

We incubated *Skeletonema* in 620 mL bottles at \( \approx 2 \times 10^4 \) 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. The bottles were placed on the plankton wheel (0.2 rpm) at 15°C and 14:8 h light:dark cycles. 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. The copepod *Acartia clausii* was sampled with a WP-2 plankton net (200 µm mesh size) from 30-0 m depth in the Kosterfjord, Sweden. *A.clausii* were kept in 15°C and fed *Rhodomonas salina* prior to the experiments. The *Skeletonema* cultures were prepared by incubation with f/2 medium (Guillard and Ryther, 1962) and either 3 female *A.clausii* 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 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 5 concentrations corresponding to 20, 50, 100 and 500 µg C l\(^{-1}\) (Strathmann, 1967). For each culture and concentration, 7 replicates were without grazers and 7 replicates supplemented with 8 *A. clausii* females. We added 0.2 mL F/2 medium to each bottle. The bottles were incubated on a revolving plankton wheel (0.2 rpm) in 15 °C in darkness for 12 hours. This incubation time should be short enough to largely avoid chemically induced effect on chain length (J. Bergkvist, unpublished data). Grazing by *A. clausii* 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 (\(F, \text{ml ind}^{-1} \text{day}^{-1}\)) was calculated with a modified equation by Frost (Frost, 1972):

\[
F = \frac{V}{N} t \ln \left( \frac{v_f}{v_0} \right)
\]

where \(V\) is incubation volume, \(N\) is number of grazers, \(t\) is incubation time, and \(v_0\) and \(v_f\) is the total biovolume of *Skeletonema* (µm\(^3\) ml\(^{-1}\)) after incubation with and without copepods. Differences in clearance on long and short chains was tested with a paired \(t\)-test of the average grazing rates form grazer induced and control treatments for each level of food concentration (\(N=5\)).

Microzooplankton, represented by 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 concentration 20.000 cells ml\(^{-1}\). After 15 minutes the grazing was terminated by adding 0.2 mL 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. 2 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. Secondly, the model calculates the weighted sum of predation risk (\( d^{-1} \)) from copepod and ciliate clearance for each of the chain-length classes. The clearance rate per copepod (represented by Acartia tonsa) in ml d\(^{-1}\) on Skeletonema for the five chain size classes were obtained from Fig. 7 in Bergkvist et al (2012). Clearance rate in ml d\(^{-1}\) per ciliate was derived from experimental data in 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 polynomial relation between clearance (ml d\(^{-1}\)) to prey size was fitted to the data (24·max\([0,-0.000147\cdot(\text{chain length} \cdot 4.64)^2+0.0016\cdot(\text{chain length} \cdot 4.64) - 0.0017]\)), 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. From each interpolated series we then fitted a high-order polynomial function (6 terms for the copepods and 7 terms for the ciliates; polyfit in Matlab (Matworks.Inc 2013)) that was used to force the grazing on Skeletonema in the models (\( z[t] \) and \( c[t] \) in Fig. 1). For the conversion of biomass to concentration (ind l\(^{-1}\)) we assumed that a ciliate with a cell volume of 20000 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. The concentration was further divided by 2, as to not overestimate the inductive effect of the copepod community in the field.
Figure 1. A: Grazer densities over an annual cycle in the Kattegat Sea. Copepod abundances calculated from 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 (1994), the curve is a polynomial fit to cube-splined data described by:

$$C(t) = -0.002x^7 + 0.0918x^6 - 1.688x^5 - 15.90x^4 - 80.68x^3 + 210.1x^2 - 237.1x + 79.40$$

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 size was compared to 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 ($d^{-1}$).

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 meters 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 was also provided for the sampling sites, based on vertical hauls of a WP-2 plankton net (200 µm mesh size) from 25-0 m. Information on prosome
length, stage, and species of the sampled copepods was translated to biomass (µg DW l⁻¹), by specific length-weight relationships (Supplementary material, Table 1). 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 2). The proportion of chains with 1 and 2 cells increased and the proportion of chains longer than 4 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).

![Figure 2. Proportions of chain lengths (mean ± SD) in *Skeletonema* cultures after 48 h incubation with *Acartia tonsa* in densities corresponding to 0, 2, 5, and 10 copepods l⁻¹, *significant differences between control and copepod treatments (Two-way ANOVA, *P* < 0.05). NS indicate no significant difference between any of the groups.](image)

We found that the copepods and ciliates grazed selectively on different chain lengths of *Skeletonema* (Fig. 3 A 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 on the lowest food concentration, with a clearance rate of 22.6 ± 6.4 ml ind⁻¹ d⁻¹ (mean ± SE) on long chains versus 1.7 ± 4.5 ml ind⁻¹ d⁻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.
Figure 3. Copepod and ciliate grazing rates. A: Clearance by the copepod *Acartia clausii* on cultures of *Skeletonema* with long chains (green symbols) and with grazer induced short chains (red 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 (red circle) when offered a *Skeletonema* culture of mixed chain lengths. After incubation the ingested *Skeletonema* cells were visible inside the ciliate (Arrows).

The model of predation risk, expressed as potential clearance by copepods ciliates over a temperate year, showed that the plastic strain was on average the least susceptible when compared to strains with a fixed size, either as single cells or as chains longer than 5 cells (Fig. 4). The grazing risk for single cells peaked in early spring, whereas the long chains are exposed to high grazing risk in summer. Over the whole annual cycle the predation risk was 31% higher for the single-cell strain and 36% higher for the long chains compared to the plastic strain (Fig. 4B).
Figure 4. Modelled predation risk on 3 fictive strains of *Skeletonema* with different inherent chain length strategies. The small strain only forms single cells, the large strain only chains longer than 5 cells and chain length of the plastic strain responds to copepod cues according to Fig. 2. A: The predation risk is modelled 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.

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 *Skeletonema* chain lengths in the field varied from 1 – 16 cells chain⁻¹. The average chain lengths per month also indicated a seasonal pattern with chain length decreasing during spring and slightly increasing during autumn (Fig. 5B). 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 was reflected in a significant negative correlation between mean chain length and mean copepod biomass per month (cells chain⁻¹ = 5.59 – 0.76 × log (copepod µg l⁻¹), $R^2 = 0.71, P = 0.0082$, Fig. 5C).

**Discussion**

Two major groups of grazers on phytoplankton are functionally different, and select for contrasting traits in the prey. Since mesozooplankton also graze efficiently on microzooplankton, the relative abundance of the two grazer groups fluctuates in time. Our model of grazing risk showed that this heterogeneity in opposing selection pressures
favors the plastic chain length strategy, allowing the diatom *Skeletonema* to optimize defense 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 modelled 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. Hence, the chain length plasticity gives a fitness advantage.

Chain length in *Skeletonema* is remarkably sensitive to copepod chemical cues. The threshold copepod concentration inducing response in the experiment is comparable to natural spring conditions. The almost identical chain length responses with different copepod concentrations suggest that the observed effect primarily reflects induced response to grazer cues. Size selective grazing or physical disintegration of chains by copepods would have resulted increased effect size with increasing copepod density. The high sensitivity to grazer chemical cues shows a strong potential for grazers to induce chain reduction also in the wild.

![Figure 5](image.png)

*Figure 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: $Y = 5.44 - 0.69 \cdot \log X$, $R^2 = 0.65$, $N = 9$. The dotted lines are 95% confidence interval of the fitted line.
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, together with small heterotrophic dinoflagellates can make 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 in line 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.2). 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. (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. (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 plasticity of morphology. 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.,...
and the green alga *Desmodesmus subspicatus* (Hessen and van Donk, 1993). In contrast, grazers feeding on larger prey may induce splitting-up of chains, as in e.g. *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 (1980) and 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 by the size adjustments indicate that the response is an adaptive mechanism selected for by the strong grazing pressure on 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 (1970) suggested that chain formation gives an advantage of increased nutrient transport by higher sinking rates. 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 in chains compared to 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 the 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 to 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.
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.

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 considerable variation in chain length. In fact, other processes such as direct effects of selective grazing, may also lead to the seasonal pattern we found in the field. Still, the consistent support from our laboratory experiments, model predictions, and field observations of Skeletonema, as well as support from the theoretical and empirical literature, indicate that grazer induced chain length plasticity is functional in the field. This further suggests that grazing mortality could 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 carbon and energy fluxes in the oceans. Also, chain length is important for the life cycle and bloom dynamics of the diatoms (Smetacek, 1985), and the plasticity in chain length has evolutionary implications for specialization and adaptations in phytoplankton (Agrawal, 2001). Grazer regulation of colony formation opens up a new perspective compared to the traditional view of a strictly resource driven evolution of phytoplankton diversity, and highlights the role of top-down mechanisms in shaping plankton communities.

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References


Supplementary table

**Table S1.** The length-weight relationships for copepod species and stages, used to convert data of abundance (ind L⁻¹) and prosome length (pl) of copepods to biomass (µg dry weight L⁻¹). The conversion formula is $\log_{10}(\mu g \text{ DW ind}^{-1}) = b \log_{10}(pl) - a$.

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<td>(Hirche and Mumm 1992)</td>
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<td>7.682</td>
<td>(Hay et al. 1991)</td>
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<td>(Satapoomin 1999)</td>
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<td>(Uye 1982)</td>
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<td>(Hay et al. 1991)</td>
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<td>5.567</td>
<td>(Hay et al. 1991)</td>
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References in supplementary table


