

## The influence of diatom abundance on the egg production rate of *Calanus helgolandicus* in the English Channel

**Abstract**—Weekly measurements of *Calanus helgolandicus* egg production rates and phytoplankton species identification and abundance have been carried out at a station in the English Channel for a period of 7 yr. These data have been analyzed to investigate the effects of the microplankton composition on egg production rates with special emphasis on groups where nutritional value is controversial, such as diatoms and the Prymnesiophyte, *Phaeocystis pouchetii*. Diatoms did not show any negative effect on the egg production rate. *Phaeocystis pouchetii* also showed no negative effects, whereas in contrast, a dinoflagellate, *Gyrodinium aureolum*, clearly showed a negative effect. We conclude that diatoms do not have a negative effect on egg production rates at the concentrations found in the field and for these natural species assemblages, and that when such an effect exists, as is the case with *G. aureolum*, the effect can be clearly identified.

In recent years the composition of the diet has been identified as an important factor for the productivity of copepods (Kleppel 1993). This is particularly important in the case of bloom periods, when one group of phytoplankton tends to greatly dominate the food available, and secondary productivity may be directly affected by their nutritional quality. This is the case for example for blooms of *Phaeocystis pouchetii*, diatom-dominated spring blooms, or dinoflagellate red tides. The case of diatoms is of particular interest because their traditional role in marine ecosystems has recently been challenged (Ianora et al. 1999). Recent laboratory studies have shown that diatom extracts or unialgal diatom diets may have negative effects on copepod egg production and egg hatching success. For example Ban et al. (1997) found that from 37 combinations of copepod and diatom species, 25 reduced egg production rate, and 29 hatching success. However, it is still unclear whether this is due to toxicity or to a low nutritional value (Jónasdóttir et al. 1998; Míralto et al. 1999). This is an important difference because if diatoms are toxic one should expect a decrease in egg production when diatom concentrations increase in the field, whereas in the case of low nutritional value, copepods could complement their diet with other components of the microplankton while still profiting from diatoms as a carbon source.

The oceanographic community has been reluctant to accept a possible toxic effect of diatoms because, in field studies, per capita egg production generally increases during the spring diatom bloom (see Niehoff et al. [1999] for a recent example). However, to our knowledge there are no field studies where a possible deleterious effect has been considered and the data analyzed to check it. The results providing evidence for deleterious effects are all from laboratory experiments, usually with somewhat high food concentrations and monospecific diets.

The Plymouth Marine Laboratory has maintained a time series at a station in the English Channel (Sta. L4, 50°15'N,

4°13'W) where egg production (and occasionally hatching success) of *Calanus helgolandicus* and microplankton composition have been surveyed weekly for the last 7 yr. The objective of this study is to analyze these data putting a special emphasis on checking whether diatoms or other microplankton groups show deleterious effects on the egg production of *C. helgolandicus* under the range of natural field conditions observed over the 7 yr.

Weekly samples were collected between February (October for microplankton) 1992 and December 1998 from a 50-m-deep coastal station (Sta. L4: 50°15'N, 4°13'W) about 10 km off Plymouth in the English Channel.

Animals for egg production experiments were collected by slow and short duration oblique tows using a 500- $\mu\text{m}$  WP2 net. All samples were transported back to the laboratory within 2 h of collection. Samples for *C. helgolandicus* abundance were collected in vertical tows, from bottom to the surface, with a 200- $\mu\text{m}$  WP2 net.

Egg production was measured by placing five females in 2-L glass beakers filled with GF/A filtered seawater. Each beaker contained a plexiglass egg production tube (500  $\mu\text{m}$  mesh) to avoid egg cannibalism. Five replicates were incubated for 24 h in filtered seawater at environmental temperature. Hatching success was only measured during 1994 and the results have been published by Pond et al. (1996). From the egg production experiments 100 eggs were randomly selected and placed individually into the 1.5-ml wells of a multiwell tray filled with filtered seawater. Trays were monitored daily to check egg viability.

Water samples for microplankton species identification and carbon estimation were collected at 10 m depth, preserved with 1% final concentration Lugol's iodine solution, and a separate sample was fixed with neutral formalin for determination of coccolithophorids (Holligan and Harbour 1977). One hundred-milliliter subsamples were settled (Utermöhl) and counted with an inverted microscope. Phytoplankton carbon biomass was estimated from cell volume according to Strathmann (1967) and using a factor of 0.21 pg C  $\mu\text{m}^{-3}$  (Ohman and Runge 1994) for ciliates. Heterotrophic dinoflagellates were separated from autotrophic forms according to taxonomical considerations (Lessard and Swift 1986).

Figure 1 presents the time series of egg production and microplankton abundance. Some of the peaks in egg production coincided with peaks in diatom abundance but this relation was not general (see detailed examples for the spring bloom in Fig. 2). However, no depression of egg production was observed during the periods when diatoms were abundant. In contrast, a sharp decline in egg production was observed during the characteristic summer *Gyrodinium aureolum* blooms (Fig. 1). Other peaks in egg production coincided with the abundance of flagellates and ciliates (Fig.

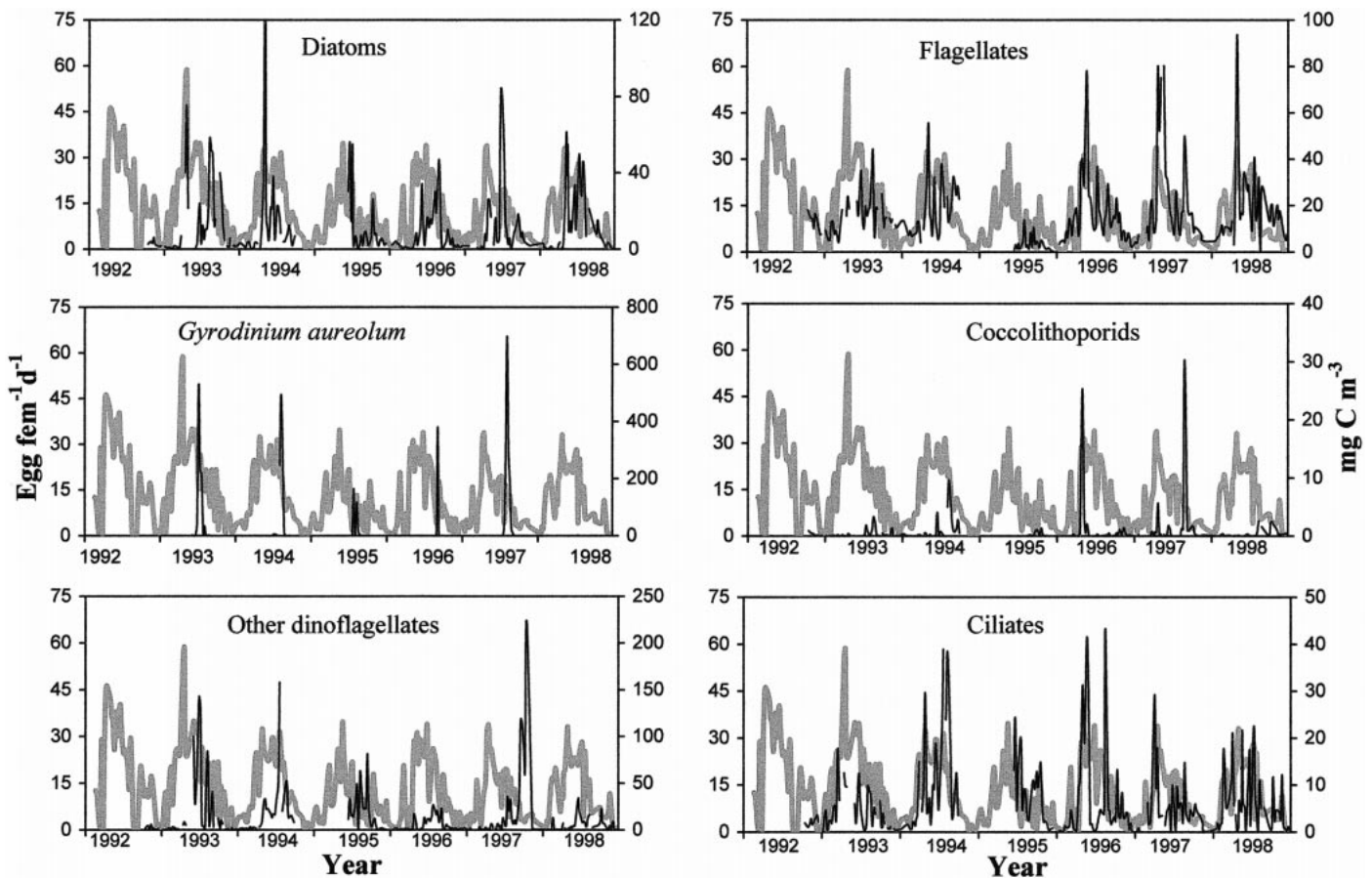


Fig. 1. Time series of the per capita egg production (gray line) and carbon biomass of microplankton components (black line) from 1992 to 1998.

1). An interesting feature is the difference in the spring bloom composition between years. In 1993, 1994, and 1998 the spring bloom was dominated by diatoms, representing from 61 to 78% of the total microplanktonic carbon (Fig. 2; Table 1). In contrast, in 1996 and 1997 diatoms only represented 14–28% of the total microplanktonic carbon, with flagellates and ciliates forming the bulk of the biomass (Table 1). However, in terms of total carbon there were no significant differences (Fig. 2).

To consider possible deleterious effects in relation to other food sources we have employed a similar approach to that proposed by Jónasdóttir et al. (1998). In this approach the egg production is plotted against the percentage in the diet of the food suspected to be deleterious (toxic or nutritionally insufficient) in relation to a reference food. If the suspected food is not deleterious one should expect the slope to be 0, whereas if negative effects occur a negative slope should be observed with increasing proportion in the diet of the bad food. The distribution of the points should be different depending on whether the food is toxic or only nutritionally insufficient (see Jónasdóttir et al. [1998] for details).

To achieve this with our data we have used a multiple regression approach where we have included the amount of microplanktonic carbon and the percentage of the phytoplankton group whose effect we wanted to investigate as

explanatory factors. Results are presented in Table 2 and graphically represented in Figs. 3 and 4. In Fig. 4 the residuals of the microplankton–egg production regression presented in Fig. 3 have been plotted against the percentage of each microplankton group considered in relation to the total microplanktonic carbon. Therefore the approach is basically the same as that of Jónasdóttir et al. (1998), the effect of a component in the diet being considered in relation to the rest of the microplankton assemblage as a reference food. The results should be interpreted in the same way, if the component considered has a negative effect the residuals would tend to be negative when the component percentage in the diet increases.

The percentage of diatoms did not show any negative effect; on the contrary, the effect is positive and significant (Table 2). This means that when diatoms are abundant, the egg production rate is higher than expected from the food concentration. Other groups that showed a positive significant relation were ciliates and *P. pouchetii* (Table 2). The percentage of dinoflagellates and flagellates did not have a significant effect, whereas the percentage of *G. aureolum* showed a negative and significant effect (Table 2). Coccolithophorids generally represented too low a percentage of the total microplanktonic carbon for the analysis to be reliable.

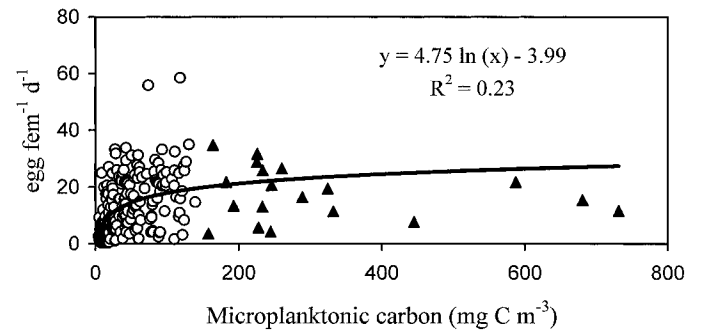
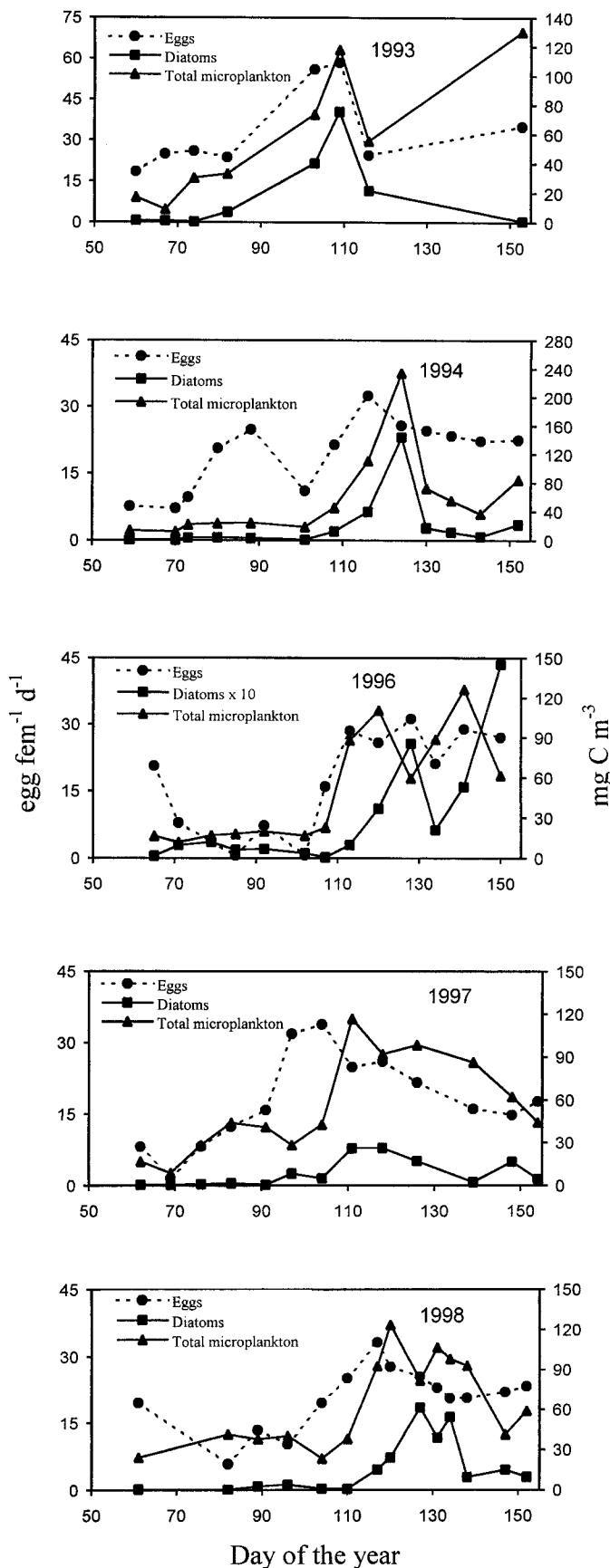


Fig. 3. Egg production rates versus microplankton concentration. Circles for values under 150 mg m<sup>-3</sup> and triangles for values over 150 mg m<sup>-3</sup>, generally corresponding to *G. aureolum* blooms.

These results are supported by forward stepwise regression analysis of the group-specific concentration data, where flagellates and ciliates appear to play the most important role explaining egg production, but the role of diatoms is positive and significant (Table 3).

Our results indicate that, at least at the natural concentrations found off Plymouth, diatoms did not have any negative effect on the egg production rate of *C. helgolandicus* and confirm previous results at the same station where a positive relationship between egg production and diatom concentration was found (Pond et al. 1996; Irigoien et al. 2000). If toxic, a negative relationship between diatom abundance and egg production rate, as in the case of *G. aureolum*, was to be expected and this was not found in the analysis of the 7-yr time series. A possibility is that *C. helgolandicus* avoided the diatoms. However, studies on selective feeding performed at the same station during some of the years considered in this study indicate that *C. helgolandicus* showed a slight preference for diatoms over other phytoplankters (Irigoien et al. 2000).

Ban et al. (1997) find that, in 25 out of 37 cases, a diatom diet reduced the egg production rate. In other cases, even with the same diatom species, it has been found that egg production rates increased with the diatom concentration, whereas the hatching success decreased (Chaudron et al. 1996; Miralto et al. 1999; Starr et al. 1999). Therefore the hatching success could play a negative role even if the egg production rate increases with a diatom diet. Off Plymouth the hatching success was measured weekly during 1994, one of the years having dominance of diatoms during the spring bloom: hatching rates showed a slight drop during the spring diatom bloom, but the decrease is far from being dramatic, and similar drops were found throughout the year without a relation with the diatom blooms (Fig. 5; Pond et al. 1996). Another field study in the English Channel also showed no apparent relationship between diatom occurrence and hatch-

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Fig. 2. Examples of the evolution of the per capita egg production, diatom concentration, and total microplankton concentration during the different spring blooms. For the year 1996 the diatom concentration is multiplied by 10 for better representation.

Table 1. Species composition in terms of carbon of the spring diatom maximum and summer dinoflagellate maximum at station L4 from 1993 to 1998.

Year	Spring bloom		Summer bloom	
1993	Date	19 Apr		05 Jul
	Species	% diatoms	Species	% dinoflagellates
	<i>Chaetoceros socialis</i>	96.52	<i>G. aureolum</i>	97.5
	Group	% of microplankton		% of microplankton
1994	Date	04 May		08 Aug
	Species	% diatoms	Species	% dinoflagellates
	<i>Rhizosolenia delicatula</i>	68.39	<i>G. aureolum</i>	96
	<i>Guinardia flaccida</i>	20.59		
1995	Date			22 Jul
	Species	% diatoms	Species	% dinoflagellates
	No data	—	<i>G. aureolum</i>	94.7
	Group		% microplankton	
1996	Date	07 May		02 Sep
	Species	% diatoms	Species	% dinoflagellates
	<i>Chaetoceros decipiens</i>	45.94	<i>G. aureolum</i>	96.7
	<i>Chaetoceros densus</i>	17.04		
1997	Date	28 Apr		04 Aug
	Species	% diatoms	Species	% dinoflagellates
	<i>Rhizosolenia delicatula</i>	64.46	<i>G. aureolum</i>	99
	<i>Coscinodiscus concinnus</i>	9.97		
1998	Date	07 May		
	Species	% diatoms	Species	% dinoflagellates
	<i>Detonula pumila</i>	56.63	No. dinoflagellate bloom	—
	<i>Rhizosolenia shrubsolei</i>	15.49		
	<i>Rhizosolenia delicatula</i>	10.47		
	<i>Lauderia annulata</i>	5.62		
	<i>Chaetoceros socialis</i>	4.67		
	<i>Thalassiosira rotula</i>	2.80		
	Group		% microplankton	
	Diatoms	77.60	No dinoflagellate bloom	—

Table 2. Results from the stepwise forward multiple regression analyses using total microplankton (tot micro) concentration and the percentage of the different groups as explanatory factors for egg production. N.S., not significant.

Regression	Factor	$r^2$	$4P$	$n$
EPR = 4.9ln(tot micro) + 0.1% diatoms - 6.6	Tot micro	0.22	<0.01	229
	% diatoms	0.04	<0.01	
EPR = 5.7ln(tot micro) - 0.12% <i>G. aureolum</i> - 8.5	Tot micro	0.18	<0.01	229
	% <i>G. aureolum</i>	0.07	<0.01	
EPR = 5.1ln(tot micro) - 0.03% dinoflagellates - 4.7	Tot micro	0.23	<0.01	229
	% dinoflagellates	—	N.S.	
EPR = 4.4ln(tot micro) + 0.25% <i>P. pouchetii</i> - 3.4	Tot micro	0.22	<0.01	229
	% <i>P. pouchetii</i>	0.05	<0.01	
EPR = 4.5ln(tot micro) - 0.02% flagellates - 2.3	Tot micro	0.23	<0.01	229
	% flagellates	—	N.S.	
EPR = 4.9ln(tot micro) - 0.2% coccolithophorids - 4.5	Tot micro	0.22	<0.01	229
	% coccolithophorids	—	N.S.	
EPR = 5.2ln(tot micro) + 0.13% ciliates - 7.6	Tot micro	0.22	<0.02	229
	% ciliates	0.03	<0.01	

ing success with high values of hatching success from March to April, when diatoms formed the highest proportion of the phytoplankton biomass, 60–70% (Laabir et al. 1995, 1998).

The *C. helgolandicus* abundance combined with the interannual differences in the spring bloom composition also provide another way of checking the effect of diatoms on hatching rates and population dynamics. If diatoms significantly reduced the hatching success of *Calanus* eggs, thereby seriously impacting recruitment of future copepod generations, one would expect the abundance of *Calanus* for the period immediately after the bloom and also for the rest of the year to be significantly lower for the years when the spring bloom was dominated by diatoms compared to the years when it was dominated by flagellates and ciliates. In Table 4 we present the average abundance of *C. helgolandicus* for the period April to the end of June and also for the complete year compared to the diatom abundance and percentage of diatoms in the microplankton during the spring. We find that the highest abundance for both the period April–June and a complete year was found in 1993, a year with the spring bloom strongly dominated by diatoms. However, the lowest abundance also corresponds to a year with a diatom spring bloom, whereas the years without diatoms during the spring bloom, 1996 and 1997, showed values in between 1993 and 1994. These results suggest that if the diet composition plays an important role in the produc-

tivity of the copepods, its role is not determinant for the recruitment and final abundance.

Whether diatoms are nutritionally insufficient is more difficult to elucidate from field data because the components of the diet possibly lacking in diatoms are probably available from other sources even if they are scarce, and in that case no negative effect would be detected. In addition diatom blooms are more diverse and less dominant in terms of total carbon than dinoflagellate red tides. As an example, at L4 during the period of maximum diatom concentration, one to seven species of diatoms were necessary to represent 95% of the total diatom carbon and diatoms represented from 14 to 78% of the total microplanktonic carbon (Table 1). On the other hand, during the *G. aureolum* bloom, this single species always represented more than 95% of the dinoflagellate carbon and from 78 to 96% of the total microplanktonic carbon (Table 1). In any case, no sign of nutritional deficiency was found even when diatoms represented up to 80% of the microplanktonic carbon.

Another possibility is that the species of diatoms found off Plymouth were not toxic or nutritionally deficient. Recent studies have shown that not all diatoms have a deleterious effect, some being an excellent food both in terms of egg production and hatching (Starr et al. 1999). The closest species, of the same genus, to those used by Ban et al. (1997) is *Chaetoceros socialis*, which in our case is the one pro-

Table 3. Results from the stepwise forward multiple regression analysis between the egg production and the different group concentrations performed with raw data and with data transformed as  $\ln(x + 1)$ . Flagellate concentration includes *P. pouchetii*.

Regression	Group	$r^2$	$P$	$n$
EPR = 0.21Flag + 0.38Cil + 0.15Diat + 5.99	Flagellates	0.17	<0.01	229
	Ciliates	0.1	<0.01	
	Diatoms	0.06	<0.01	
$\ln(\text{EPR} + 1) = 0.34 \ln(\text{Cil} + 1) + 0.38 \ln(\text{Flag} + 1) + 0.16 \ln(\text{Diat} + 1) - 0.24 \ln(\text{Cocc} + 1) + 0.61$	Ciliates	0.21	<0.01	229
	Flagellates	0.12	<0.01	
	Diatoms	0.04	<0.01	
	Coccolithophorids	0.01	<0.01	

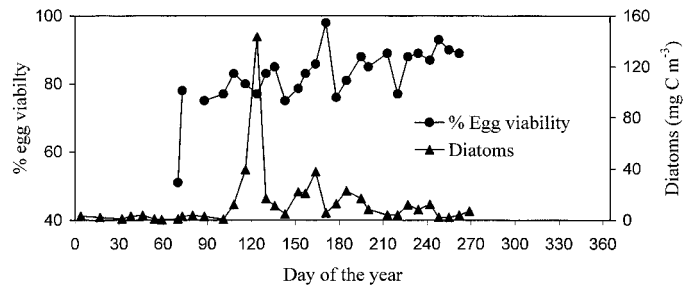
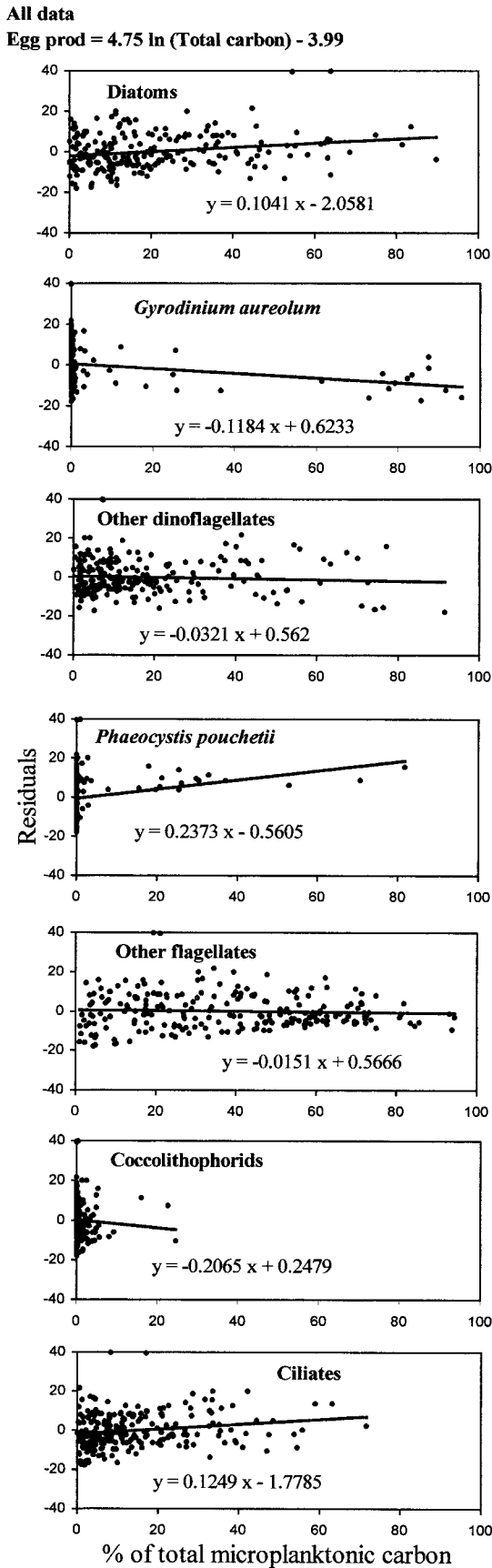


Fig. 5. Percentage of the egg viability and diatom concentration during 1994. Redrawn from data in Pond et al. (1996).

ducing a nearly monospecific bloom but also the one where the positive link between diatoms and egg production is the most evident (Fig. 3, 1993). However, this fact is of limited significance because even for the same species contradictory effects have been reported (see the different effects of *Skeletonema costatum* in Ban et al. [1997] or compare with Starr et al. [1999] for *S. costatum* and Koski et al. [1998] for *Thalassiosira weissflogii*). In any case, this indicates that to understand the possible effects of diatoms on copepod production it is necessary to look at field data because the laboratory strains are not necessarily representative of the most abundant species in the field but of those easiest to cultivate, and also because the strains maintained in the laboratory may have nutritional characteristics different from the species in the field.

The case of *G. aureolum* clearly shows that when negative effects are present these can be identified in the field. *G. aureolum* blooms have been reported as a cause for fish mortality even if it is unclear if the alga is toxic (Bullock et al. 1985; Partensky et al. 1989) or the mortality is due to excreted mucilage (Partensky et al. 1989). In laboratory studies with *C. helgolandicus* the use of *G. aureolum* as a diet does not increase mortality but reduces the egg production to zero (Gill and Harris 1987). The fact that in the field the egg production never falls to zero, even when *G. aureolum* represents 95% of the microplankton, suggests nutritional deficiency rather than toxicity (see also discussion in Irigoien et al. [2000]).

If diatoms do not show negative effects on egg production, they also do not appear to be the most important factor influencing egg production of *C. helgolandicus* off Plymouth. The multiple regression analysis indicates that the pair flagellates-ciliates are the most important nutritional factor explaining egg production. This agrees with the results from other authors where the importance of the microbial loop and the role of the microzooplankton in coastal water has been recognized (Kleppel 1993; Ohman and Runge 1994).

In conclusion our study shows that, at the concentration and species composition found off Plymouth, diatoms did

Fig. 4. Residuals of the regression showed in Fig. 3 versus the percentage of the different groups with respect to the total microplankton.

Table 4. Year-to-year variations in the abundance of *C. helgolandicus* and diatoms at station L4.

	Average April–June		Annual average		Average April–May		Spring maximum	
	Individ- uals m <sup>-3</sup>	SE	Individ- uals m <sup>-3</sup>	SE	mg C m <sup>-3</sup>	%	mg C m <sup>-3</sup>	%
1993	147.4	8.8	110.0	25.1	32.1	45.7	75.2	63.9
1994	56.0	14.2	34.2	14.7	32.7	40	143.6	61.5
1996	90.3	20.3	45.7	10.6	4	6	8.5	14.3
1997	69.9	20.6	41.2	8.6	12.6	17.8	26.1	28.5

not have any negative effect on egg production rate and apparently neither on egg viability or *C. helgolandicus* abundance. A nutritional deficiency in diatoms cannot completely be discarded; however, this is very unlikely to play any role limiting secondary production in the field because other species, phytoplankton groups, or microzooplankton are generally present at concentrations high enough to complement the diet with the potentially deficient components.

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