Effects of diatom diets on the reproduction of the planktonic copepod *Calanus finmarchicus*

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The potential for an adverse influence of diatom diets on the reproductive success of the planktonic copepod *Calanus finmarchicus* was investigated experimentally under laboratory conditions. A monospecific diet of the common diatom *Thalassiosira nordenskioldii* significantly reduced the viability of *Calanus* eggs, which either failed to hatch or hatched into deformed nauplii. The production of nonviable eggs increased with increasing *Thalassiosira* concentration and was proportional to the female ingestion rate. At a cell concentration of 10^4 ml^{-1} (typical bloom concentration in the St. Lawrence Estuary), the proportion of nonviable eggs was as high as 83 % of the total daily production. Nonviable egg production was also induced by a diatom of the genus *Navicula*, but not by two other diatoms, *Skeletonema costatum* and *Chaetoceros debilis*. Among non-diatom diets, maternal feeding on a dinoflagellate (*Prorocentrum micans*) and two flagellates (*Isochrysis galbana*, *Pavlova lutheri*) at food-saturated conditions resulted in the production of normal eggs, more than 70 % of which hatched into healthy nauplii. The hatching success of eggs was independent of the daily egg production rate, as only three of the algal species (*T. nordenskioldii*, *S. costatum*, and *P. micans)* supported maximum egg production at superabundant food concentrations. A failure of embryonic development also occurred when females were exposed to a diversified diet composed of *Thalassiosira*, *Chaetoceros* and *Skeletonema*, even though *T. nordenskioldii* contributed less than 60 % of total ingested carbon. We conclude from these experiments that extended feeding on certain extremely common diatom species, by themselves and apparently also in mixtures where they predominate, could have a negative impact on *C. finmarchicus* recruitment rates.

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INTRODUCTION

A target species of GLOBEC (Global Ocean Ecosystem Dynamics) programs in the North Atlantic Ocean is the planktonic copepod *Calanus finmarchicus*, a predominant constituent of northern subtropic mesozooplankton communities. Recruitment into *Calanus* populations is a key biological process in coupled physical-biological models that are fundamental to the GLOBEC approach. Recruitment comprises not only the female egg production rate, but also the mortality in the egg and early naupliar stages. Understanding the factors influencing the latter is of particular importance, as mortality in the early life stages of planktonic copepods is variable and frequently extremely high, contributing in some instances to over 90 % of the total loss in egg output (e.g. Kiørboe & Nielsen 1994; Peterson & Kimmerer 1994).

Recently, attention has focused on the role of maternal diet as a factor influencing the survival of embryonic and early naupliar stages in copepod populations. In the past, many field studies have reported that fecundity in marine suspension-feeding copepods is largely governed by fluctuations in the availability of phytoplankton food, particularly diatoms (e.g. Marshall & Orr 1955; Kiørboe & Nielsen 1994; Plourde & Runge 1993). It is well known that diatoms are prominent in copepod diets, especially in productive (e.g. upwelling) ecosystems and during the phytoplankton blooms in temperate and high latitudes (e.g. Marshall & Orr 1955; Urban & al. 1992). The linkage between diatom outbursts and productivity of copepods is widely recognized as characteristic of productive pelagic food webs (Cushing 1989; Legendre 1990; Mann 1993). However, the significance of diatoms as a high quality food source for copepod reproduction has recently been questioned (Kleppel & al. 1991; Ianora & Poulet 1993; Jónasdóttir 1994; Poulet & al. 1994, 1995; Miralto & al. 1995; Laabir & al. 1995; Ianora & al. 1995, 1996; Uye 1996; Chaudron & al. 1996; Ban & al. 1997). For example,

new studies have reported that several diatom species can induce up to 100 % egg mortality, due to inhibition of egg development, either when fed to female copepods at high food concentrations or when freshly spawned eggs were exposed to diatom extracts. On the basis of these results, it has been hypothesized that diatoms either contain chemical compounds that inhibit embryogenesis (e.g. Poulet & al. 1994, 1995; Ianora & al. 1996; Ban & al. 1997), or are deficient in certain nutritional components necessary for early development (e.g. Jónasdóttir & Kiørboe 1996).

Here, we investigate the potential for an adverse influence of diatom diets on the reproductive success of *C. finmarchicus*. The diatoms selected for our study are among the most common species in spring blooms in waters of the northwest Atlantic. We compared, in laboratory experiments, the effect of various diatom diets, whether as single species or in a mixture, on egg production rate as well as egg hatching success. A dinoflagellate and two flagellate diets were used as controls. The results obtained are interpreted with regard to the role of diatoms in recruitment cycles in *Calanus* populations.

MATERIAL AND METHODS

The experiments were conducted with *Calanus finmarchicus* females collected on 20-22 June (first experimental series) and 5-7 August (second experimental series), 1995, at a station located off Rimouski in the lower St. Lawrence Estuary (48°40'N, 68°35'W). Zooplankton was collected with a 1-meter diameter, 333-µm mesh size net towed vertically from 250 m to the surface. The net contents were immediately transferred into 4-liter glass jars filled with surface seawater and transported in an insulated box to the shore laboratory, where healthy females were sorted out with the aid of a dissecting microscope. Prior to the start of experiments, the females were acclimated for 5-7 d to the experimental conditions (14 h light : 10 h dark cycle; 5-6 °C, and 28-30 PSU) in 4-liter containers (40-50 ind. container^{-1}) filled with 0.2-µm filtered seawater without addition of food. This acclimation period was designed to exclude any influence of previous feeding history on experimental variables.

The reproductive response of *C. finmarchicus* to various food treatments was quantified by incubating females in 1-liter egg separation containers, as described in Runge (1985). The containers consisted of Plexiglas cylinders with a 571-µm mesh size nitex screen cemented to the bottom and immersed in 2-liter glass beakers containing 1.5 l of rearing medium. The screen separated eggs and fecal pellets from the adult copepods in order to minimize cannibalism and coprophagy. There were 4 replicate containers (7 females per container) per food treatment, and experiments lasted 23 days. Each day, the females were transferred to new containers with fresh medium and egg production was recorded by counting all eggs present. In order to assess egg viability, eggs from each container were gently transferred with a micropipette to Erlenmeyer flasks containing 125 ml of filtered sea water (0.2 µm) and incubated for 72 h. Estimates of egg mortality were obtained from the proportion of unhatched eggs and unhealthy (i.e. dead or deformed) nauplii at the end of the incubation period relative to initial number of eggs.

Phytoplankton species used in the experiments comprised: (1) four diatoms, *Thalassiosira nordenskioldii* (22-24 µm in length and 14-16 µm in width; mean cell volume: 4064 µm^3), *Chaetoceros debilis* (4-10 µm in length and 4-10 µm in width; mean individual cell volume: 269 µm^3, usually connected in chains of > 30 µm in length), *Navicula* sp. (8-10 µm in length and 4-5 µm in width; mean cell volume: 177 µm^3) and *Skeletonema costatum* (4-9 µm in length and 4-9 µm in width; mean individual cell volume: 215 µm^3, usually connected in chains of > 30 µm in length); (2) the dinoflagellate *Prorocentrum micans* (30-50 µm in length and 14-25 µm in width; mean cell volume: 27 980 µm^3) and (3) the flagellates *Isochrysis galbana* (5-6 µm in length and 2-4 µm in width; mean cell volume: 39 µm^3) and *Pavlova lutheri* (6-8 µm in length and 3-4 µm in width; mean cell volume: 67 µm^3). Except for *Navicula*, which was isolated from the lower St. Lawrence Estuary on 20 May, 1995, the algae were obtained from culture collections at the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME (Clone designations: CCMP995 for *T. nordenskioldii*; CCMP172 for *C. debilis*; CCMP780 for *S. costatum*; CCMP693 for *P. micans*; CCM1325 for *P. lutheri*; CCM1323 for *I. galbana*). Each algal species was cultured in 19-liter batch cultures using natural seawater (filtered at 0.2 µm) enriched with f/2 medium (Guillard & Ryther 1962). Cultures were maintained under a 16-hour light : 8-hour dark cycle (fluorescent cool white lighting) at a temperature of 15 to 16 °C and a salinity of 28 to 30 PSU. Air filtered at 0.2 µm was continuously bubbled through the cultures to supply CO_2 and maintain algae in suspension. Each algal culture was diluted daily with fresh f/2 medium to maintain algae in the exponential growth phase. Experimental media were prepared by assessing cell concentration in batch culture with a Neubauer haemocytometer and then diluted with 0.2-µm filtered seawater.

In the first experimental series, algal species were tested separately and then in a mixture representative of a natural species assemblage in the Gulf of St. Lawrence. Daily initial concentrations used for monoalgal experi-

ments were 1×10^3 cells ml^{-1} for *P. micans*, 1×10^4 cells ml^{-1} for *T. nordenskioldii*, 5×10^4 cells ml^{-1} for *S. costatum*, *C. debilis* and *Navicula* sp., and 1×10^5 cells ml^{-1} for the much smaller flagellates *P. lutheri* and *I. galbana*. The corresponding carbon concentrations were 0.9, 2.0, 1.1, 1.3, 1.0, 0.9 and 0.9 µg C ml^{-1}, respectively, according to the cell volume and carbon content relationships of Strathmann (1967). Based on previous *Calanus* feeding studies (Frost 1972), we estimated that these concentrations were in excess of daily food requirements for female *C. finmarchicus*. Since natural diatom blooms are usually composed of several species, females were also presented with a food mixture consisting of *T. nordenskioldii*, *S. costatum* and *C. debilis*. We estimate that this mixture are representative of natural field conditions as Urban & al. (1992) show that *Thalassiosira* spp., *Chaetoceros* spp. and *S. costatum* are the main components in the faecal pellets of *C. finmarchicus* during the spring in coastal Newfoundland waters. Daily initial concentration in the mixed diet was 3.3×10^3 cells ml^{-1} (0.67 µg C ml^{-1}) of *T. nordenskioldii*, 1.7×10^4 cells ml^{-1} (0.37 µg C ml^{-1}) of *S. costatum* and 1.7×10^4 cells ml^{-1} (0.44 µg C ml^{-1}) of *C. debilis*, representing a proportion of 9 %, 45.5 % and 45.5 %, respectively, by number and 45 %, 25 % and 30 % of total carbon concentration.

In the second experimental series, *C. finmarchicus* females were exposed to the diatom *T. nordenskioldii* at five concentrations. Daily initial concentrations ranged from 0.1×10^3 to 10.0×10^3 cells ml^{-1} (0.02 to 2.0 µg C ml^{-1}). These concentrations were considered to cover the various phases of the functional feeding response of *Calanus* for this diatom, based on results of Frost (1972), and to include cell densities during diatom blooms, which generally range from 10^2 to 10^4 cells ml^{-1} in the St. Lawrence Estuary (Levasseur & al. 1984, 1994). On two days (day 17 and 23), a subsample from each replicate, taken from the experimental medium (well-mixed) immediately after transfer of females to new containers, was preserved in acid Lugol's in order to determine final concentration of algae, for the purpose of calculating feeding rate. Two control containers without copepods were run simultaneously. In these experiments, copepod ingestion rates were calculated by the equations in Frost (1972), and cellular abundances were converted to estimates of carbon (Strathmann 1967). This procedure was also performed in the first experimental series (on day 15 and 22) when females were exposed to the mixed food diet.

RESULTS

Fig. 1 shows the reproductive response of *Calanus finmarchicus* to the monospecific diets in the first experimental series. In the three (of seven) treatments supporting high egg production (Fig. 1A, E, G), the daily rate was at first low, then increased progressively after 3-6 d until a maximum was attained in 8-12 d. The initial lag represents the time required for oocytes to undergo primary and secondary vitellogenesis after preconditioning in filtered seawater (Runge 1984). The average maximum daily egg production rate (i.e. after the initial lag period) in these treatments was 40-45 eggs female^{-1} d^{-1}, which is very similar to earlier laboratory results conducted at 5.5 °C (Runge & Plourde 1996).

In order to take into account the time variations in egg production rate and also in egg viability, initial statistical comparisons between treatments are based on total egg production over the 23-d investigation period. Fig. 2 shows that fecundity was significantly different among food treatments (one-way Anova, $p < 0.01$). Total egg production was similar for copepods fed with the dinoflagellate *P. micans* and the diatoms *S. costatum* and *T. nordenskioldii*. Egg production was significantly lower (F-test, $p < 0.05$) for females fed with the diatoms *C. debilis* and *Navicula* sp. and the flagellates *I. galbana* and *P. lutheri*. Qualitatively, the fecal pellet production followed the same trends (Fig. 2). Fecal pellets were always very abundant when females were exposed to *P. micans*, *T. nordenskioldii* and *S. costatum*, and much lower when exposed to *C. debilis*, *Navicula* sp., *I. galbana*, and *P. lutheri*.

The food treatments also influenced egg viability (one-way Anova, $p < 0.01$). With all non-diatom diets (*P. micans*, *I. galbana*, and *P. lutheri*), hatching success remained relatively high and stable throughout the 23-d period of investigation (Fig. 1). On average, more than 78 % of the total number of eggs produced resulted in healthy nauplii (Fig. 2). However, two of four diatom diets (*T. nordenskioldii*, *Navicula* sp.) induced significantly higher egg mortality (F-test, $p < 0.05$), averaging 56 % (*T. nordenskioldii*) and 40 % (*Navicula* sp.) of the total egg production (Fig. 2). With the other two diatom diets, hatching success was 79 % (*S. costatum*) and 86 % (*C. debilis*). Fig. 3 shows that there was no direct relationship between egg viability and fecundity; both "poor" (*C. debilis*, *Navicula sp*., *I. galbana*, *P. lutheri*) and "good" (*P. micans*, *S. costatum*, *T. nordenskioldii*) food items in term of egg production engendered either high (*P. micans*, *S. costatum*, *C. debilis*, *I. galbana*, *P. lutheri*) or low (*Navicula*, *T. nordenskioldii*) egg viability.

The viability of eggs from the two most inadequate food diets (*T. nordenskioldii* and *Navicula*) changed with time over the course of the experiment (Fig. 1). In the *T. nordenskioldii* treatment, more than 80 % of eggs hatched into healthy nauplii during the first 9 d, after which the proportion of either unhatched eggs or unhealthy nauplii increased progressively until a maxi-

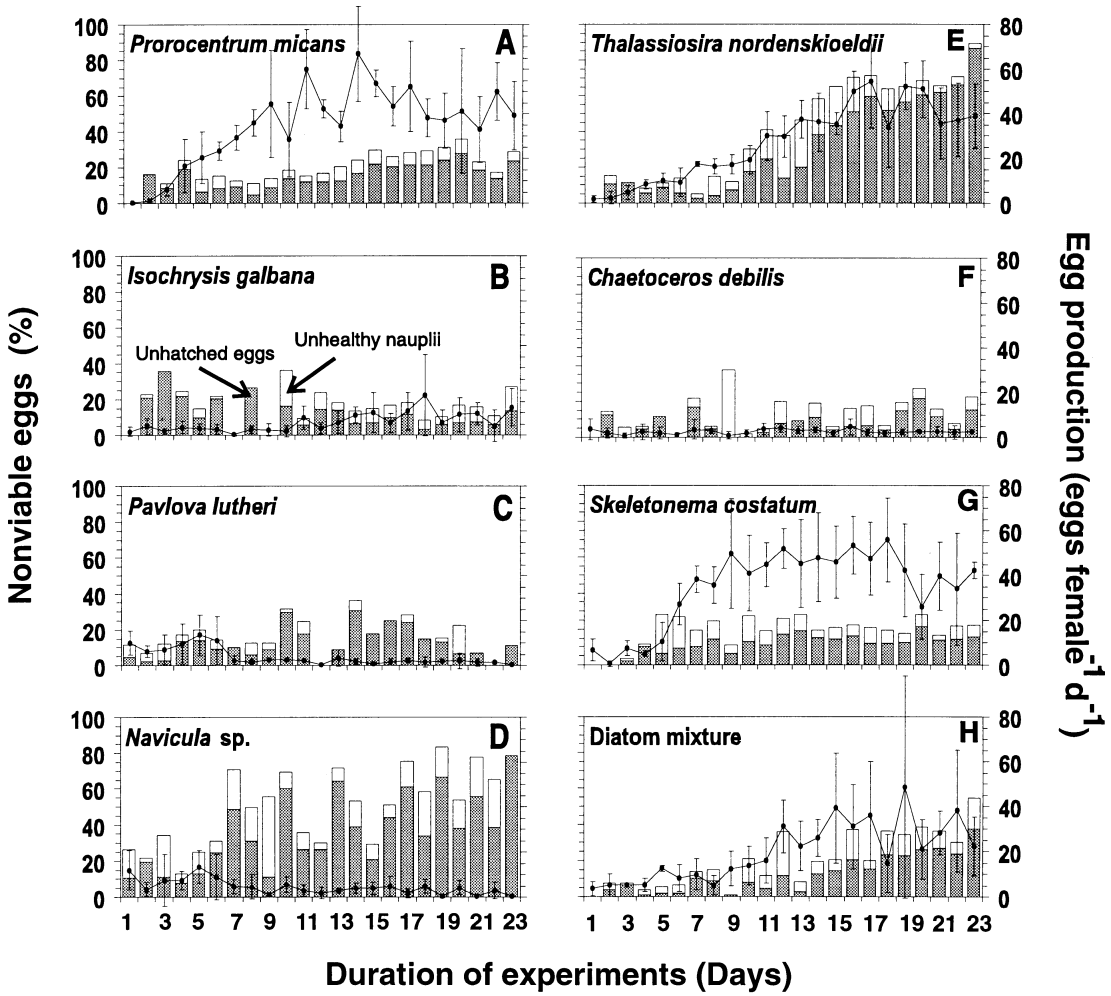

Fig. 1. Mean daily egg production (solid line with standard deviations, 4 replicates) and egg viability (columns) of female *Calanus finmarchicus* fed on 7 unialgal diets (A-G) and a diatom mixture (H) composed of *Thalassiosira nordenskioldii*, *Chaetoceros debilis* and *Skeletonema costatum* in a ratio of 45 % 30 %, and 25 % of total carbon concentration, respectively. Nonviable early life stages were further classified into unhatched eggs (solid columns) and unhealthy nauplii (open columns).

mum was attained in 16 d. In the case of *Navicula*, egg viability was consistently high until day 5; subsequently, the percentage of nonviable eggs increased sharply in 2 d and finally alternated between low (30-40 %) and high (> 60 %) values until the end of incubation. When data were pooled, the proportion of nonviable eggs was significantly less in the *Navicula* treatment compared to the *T. nordenskioldii* treatment (Fig. 2).

Close examination of clutches released by females following ingestion of *T. nordenskioldii* and *Navicula* revealed that eggs underwent strikingly abnormal development (Fig. 4). Abnormal eggs were characterized by a darker color, the presence of globular cytoplasm, and by scattered, irregular, asymmetrical globules corresponding to nuclei. These structural anomalies reflected an abnormal cell division during mitosis. In most cases, abnormal eggs were found dead at the gastrula stage. In some instances late embryos remained encumbered in the egg membrane. Among unhealthy nauplii, most showed strong asymmetrical anatomical anomalies, presenting a crumpled appearance. Appendages were asymmetrical, shortened, and abnormal in segmentation and often fused and the number and length of bristles were atypical. In most cases, deformed nauplii were found dead. Swimming behavior in living but deformed nauplii was convoluted in comparison to nor-

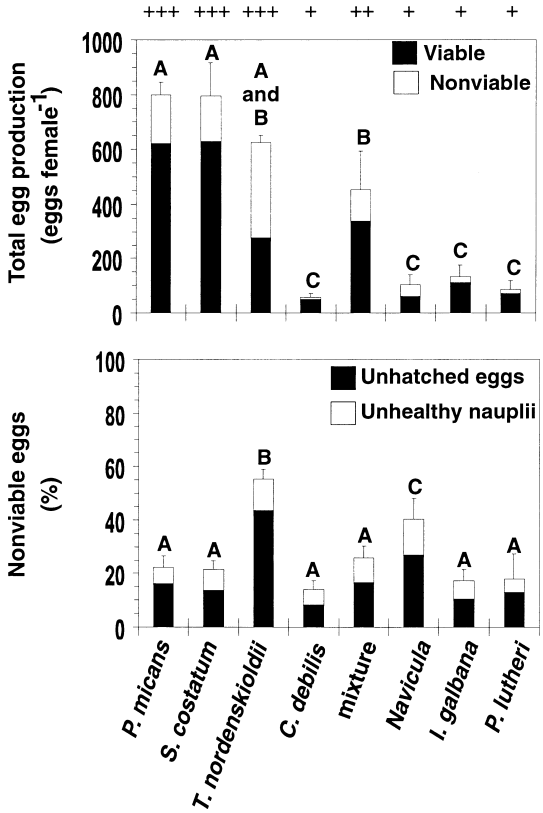

Fig. 2. Total egg production and egg viability of *Calanus finmarchicus* females for each food treatment, integrated over the 23 d experiment. Diets with the same letter are not significantly different (F-test, $P > 0.05$). Plus signs (+) represent qualitative index of fecal pellet production: +++ = fecal pellets very abundant; ++ = abundant; + = rare.

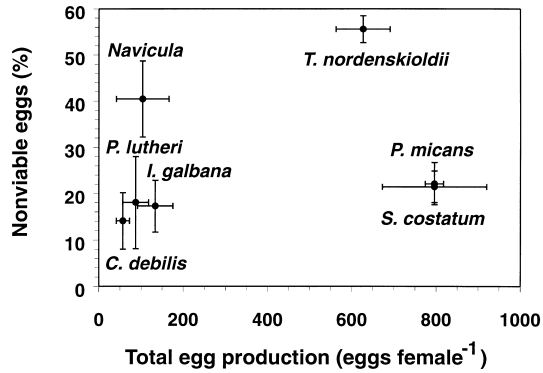

Fig. 3. Correlation between total egg production and egg viability for *Calanus finmarchicus* females fed on various diets. Error bars indicate SD.

mal nauplii. Such anomalies were rarely observed in nauplii from the other algal diets.

Fig. 5 indicates that the negative effect of *T. nordenskioldii* on egg viability of *C. finmarchicus* was significantly dependent on food concentration (one-way Anova, $p < 0.05$). Females produced few eggs over 23 d on a diet of 0.1×10^3 cells ml^{-1} (initial concentration) and a maximum number of eggs at 5×10^3 to 10×10^3 cells ml^{-1} (Fig. 5A). However, Fig. 5B shows that the percentage of nonviable eggs was not constant, but rather increased from 14 % of the total egg production at the low concentration to 68 % at the highest concentration. A consequence of this result is that the production of healthy nauplii increased very little at concentrations greater than 1×10^3 cells ml^{-1} (Fig. 5C). Recruitment rates derived from a diet of *T. nordenskioldii* would thus be substantially less than predicted based on the egg production food concentration relationship in Fig. 5A.

The curves shown in Fig. 5 integrate the reproductive response over the 23-d experimental period. The time course of abnormal egg production is shown in Fig. 6. After day 19 in most of our experiments a drop in the proportion of nonviable eggs is apparent, possibly due to a subtle change in the phytoplankton growth (e.g. Jónasdóttir 1994). Therefore only data for days 1 to 19 were used for further analysis. As in previous experiments, the time course of abnormal egg production (Fig. 6) at cell concentrations $\geq 0.5 \times 10^3$ cells ml^{-1} followed basically the same pattern; the percent of nonviable eggs was at first low, then increased exponentially until a maximum level was attained. The time lag at which 50 % of nonviable eggs varied however with respect to algal concentration. At 10×10^3 and 5×10^3 cells ml^{-1}, the time lag to reach 50 % of nonviable eggs was 7-8 d. At 1×10^3 and 0.5×10^3 cells ml^{-1}, the 50 % level was first observed after 14-18 d. Note that egg viability at the lowest food concentration (0.1×10^3 cells ml^{-1}) is based on very few eggs, resulting in large daily variation. The average threshold levels of nonviable eggs were 80.3 (SD: 2.8), 76.4 (SD: 2.4), 55.7 (SD: 8.9) and 45.4 % (SD: 14.8), at 10×10^3, 5×10^3, 1×10^3 and 0.5×10^3 cells ml^{-1}, respectively.

Ingestion rates of females increased with increasing concentration of *T. nordenskioldii*. Daily ingestion rates ranging from 3.9 µg C female^{-1} day^{-1} at 0.5×10^3 cells ml^{-1} to 28.99 µg C female^{-1} day^{-1} at 10.0×10^3 cells ml^{-1}. Fig. 7 shows that both the time lag to reach 50 % of nonviable eggs and the maximum levels of egg mortality are functions of ingestion rate. The relation between average daily egg production rates produced at each food concentration and average ingestion rates of females was also well described by a linear regression (Fig. 7); hence, no significant difference (F-test, $p > 0.05$) in the

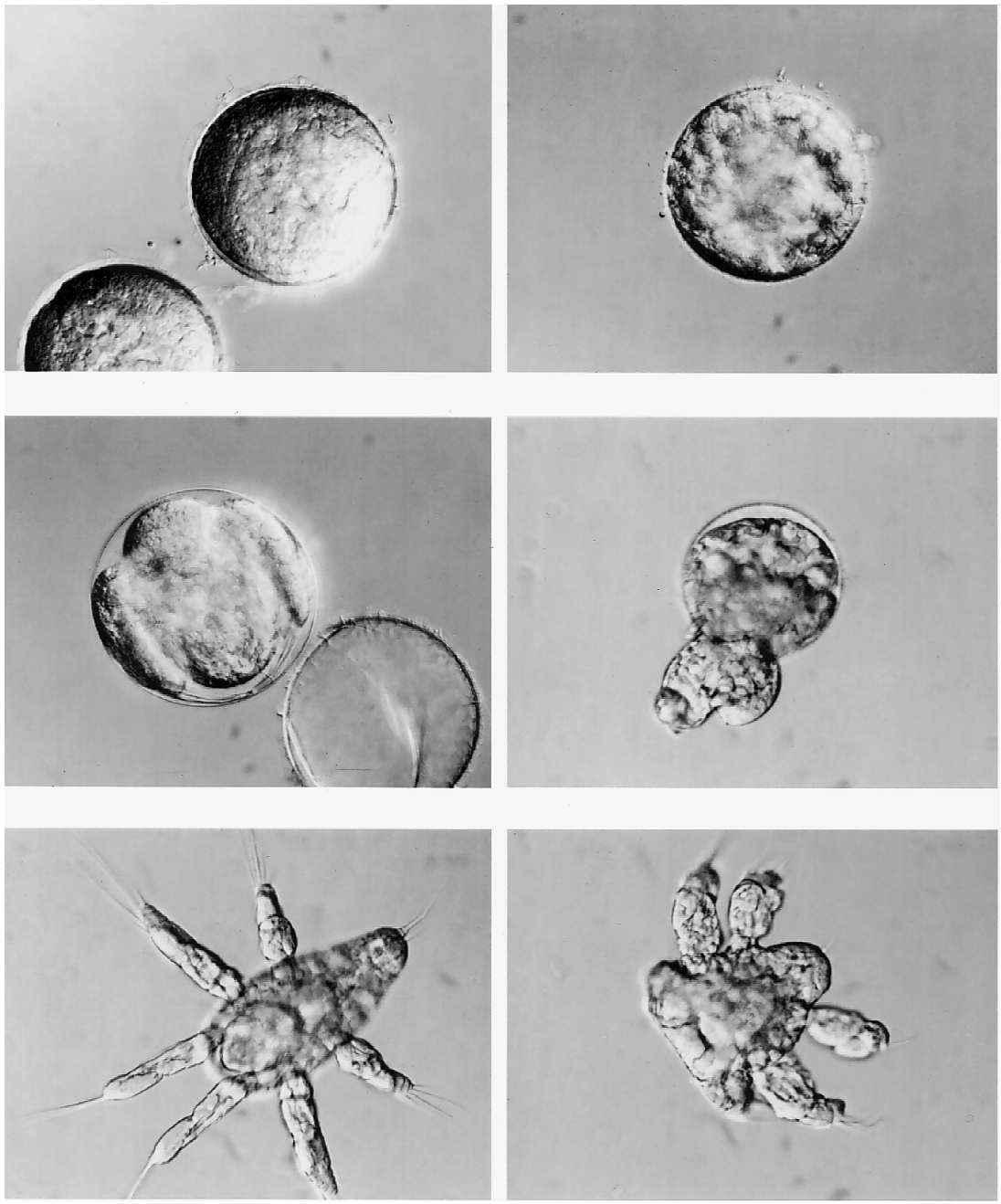

Fig. 4. Light microscope (PL Fluotar 20 ×) images of late embryonic and naupliar stages of *C. finmarchicus*. Left column, normal embryos and nauplii: upper panel = 36h old egg; middle panel = egg just prior to hatching; lower panel = nauplius. Right column, abnormal embryos and nauplii: upper panel = 36h old egg; middle panel = late deformed egg, partially trapped in embryonic membrane; lower panel = deformed nauplius at stage N1.

conversion of food into egg production was found between various food treatments. Assuming a mean value of 0.23 µg C egg^{-1} (Runge & Plourde 1996), the conversion of egg production rates into carbon production indicated a mean gross growth efficiency of 20 %.

Female *C. finmarchicus* presented with the mixed food diet spawned eggs that hatched into healthy nauplii with usually > 80 % success until day 15, after which egg viability decreased sharply in 2 d and finally remained below 70 % until the end of incubation (see Fig. 1H). Due to this lag time, we found no significant difference in egg viability between mixture and good food items when data were pooled over the entire time interval (F-test, $p > 0.05$; Fig. 2). However, if we consider only the experimental period from days 18 to 23 (time at which minimum egg viability is reached), the egg viability was significantly lower (F-test, $p < 0.05$) for copepods fed with the mixed diet than for those fed on the pure cultures of *C. debilis* and *S. costatum* (Table 1). In this experiment, average ingestion rates of *C. finmarchicus* were 52.8×10^3 cells female^{-1} d^{-1} (10.6 µg C female^{-1} d^{-1}; SD: 1.09) for *T. nordenskioldii*, 197.3 $\times 10^3$ cells female^{-1} d^{-1} (4.34 µg C female^{-1}; SD: 0.52) for *S. costatum* and 140.4×10^3 cells female^{-1} d^{-1} (3.65 µg C female^{-1}; SD: 1.08) for *C. debilis*, representing a proportion of 56.9 %, 23.4 % and 19.7 % respectively, of the ingested carbon. Because sedimentation rates for each species were not taken into account during this experiment, our data cannot be used to evaluate feeding preferences of *C. finmarchicus.*

Fig. 7C compares the effect of *T. nordenskioldii* diets during the first and second experimental series as a function of grazing rate of females on *T. nordenskioldii.* The inhibition of egg development during the first experimental series when females fed on *T. nordenskioldii* at 10×10^3 cells ml^{-1} or the mixed diet was approximately

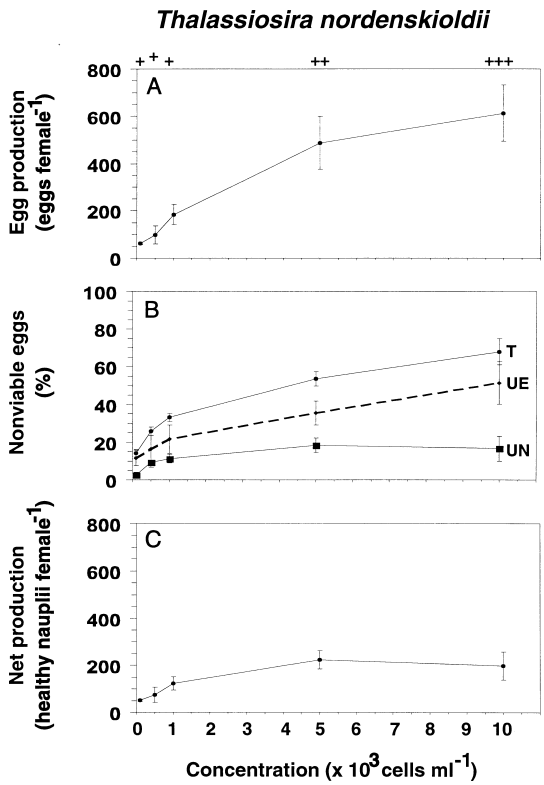

Fig. 5. Total egg production (A), egg viability (B) and output of normal nauplii integrated over a 23 d experiment with *C. finmarchicus* females fed the diatom *Thalassiosira nordenskioldii* at different concentrations. Results are means (± SD) of 4 replicate observations for each food concentration. T = total; UE = unhatched eggs; UN = unhealthy nauplii. Plus signs (+) refer to fecal pellet production: +++ = fecal pellets very abundant; ++ = abundant; + = rare.

Table 1. Variations in egg production and percentage of nonviable eggs of *C. finmarchicus* females exposed to the diatoms *Thalassiosira nordenskioldii*, *Skeletonema costatum* and *Chaetoceros debilis*, or a 45/25/30 mixture of the 3 species at a final carbon concentration of 1-2 µg ml^{-1}. Day 18 to Day 23 inclusively. Diets with the same letter are not significantly different (F-test; $p > 0{,}05$).

Biological response	Type of food				Significance of statistical test
	Thalassiosira nordenskioldii (n = 4)	*Skeletonema costatum* (n = 4)	*Chaetoceros debilis* (n = 4)	Mixture (n = 3)	
Egg production (total eggs female^{-1})	214.78 (SD: 58.28) A	183.96 (SD: 50.0) A	12.21 (SD: 4.5) B	158.09 (SD: 105.75) A	F = 8.8*
Nonviable eggs (%)	71.77 (SD: 2.45) A	20.67 (SD: 2.78) B	20.08 (SD: 1.39) B	41.66 (SD: 13.07) C	F = 33.7*

* Significant at the 95 % level.

15 % less than expected from the second experimental series (for the mixed diet 42 % compared to 57 % from the regression line). In both cases, the percentage of nonviable eggs was outside of the 95 % confidence interval of the *T. nordenskioldii* regression, but not significantly different from the *Thalassiosira* treatment at 10×10^3 cells ml^{-1} and 1×10^3 cells ml^{-1}, respectively.

DISCUSSION

Both fecundity and egg viability of *Calanus finmarchicus* were strongly influenced by food type. When given in excess to females, the dinoflagellate (*P. micans*) and two of the diatom species (*S. costatum*, *T. nordenskioldii*) sustained high egg production, whereas two other diatom (*Navicula* sp., *C. debilis*) and flagellate species (*I. galbana*, *P. lutheri*) did not. Feeding experiments indicate that the lower size limit for particle capture in females of a congeneric species (*C. pacificus*: Frost 1972) is on the order of 5-7 µm; hence low egg production rates with the two flagellate species are likely due to inefficient capture of these small cells. This may also be the case for *Navicula,* which because of its small size and acicular morphology, may be difficult to handle. The chain-forming *Chaetoceros debilis* is adequately sized, but females did not feed well on this species, since few fecal pellets were produced, similar to the small-cell treatments. We hypothesize that the long setae ornamenting *C. debilis* interfere with handling and consequently ingestion rate, as proposed originally by Parsons & al. (1967). These results serve to illustrate that the shape (presence/absence of spines) and size of diatom species have the potential to influence reproductive output. These effects are in addition to the potential effect of the biochemical composition of the algal cells (Pond & al. 1996).

Whatever the reason for differences in egg production among algal diets, at least one diet (*T. nordenskioldii*) yielding high fecundity also rendered nonviable eggs, indicating that characteristics and/or properties of the food controlling egg production rate are different from those affecting egg viability. Feeding on non-diatom food items (*I. galbana, P. lutheri, P. micans*) yielded normal eggs that hatched with > 70 % success. In contrast, two of four diatom diets tested (*T. nordenskioldii* and *Navicula* sp.) induced production of abnormal eggs that either failed to hatch or hatched into unhealthy nauplii. The failure of embryonic development was even apparent, although possibly less severe, when female *C. finmarchicus* were exposed to a diversified diet composed of *T. nordenskioldii* and two innocuous (with respect of egg viability) diatoms. Our cytological inspection indicates that the anomalous embryonic development was due to abnormal cell division during mitosis. Similar reductions of hatching success have recently been reported for many other copepod species feeding upon several unialgal diets of diatoms *ad libidum* (Ban & al. 1997). Morphological deformities in newly-hatched nauplii have also been observed

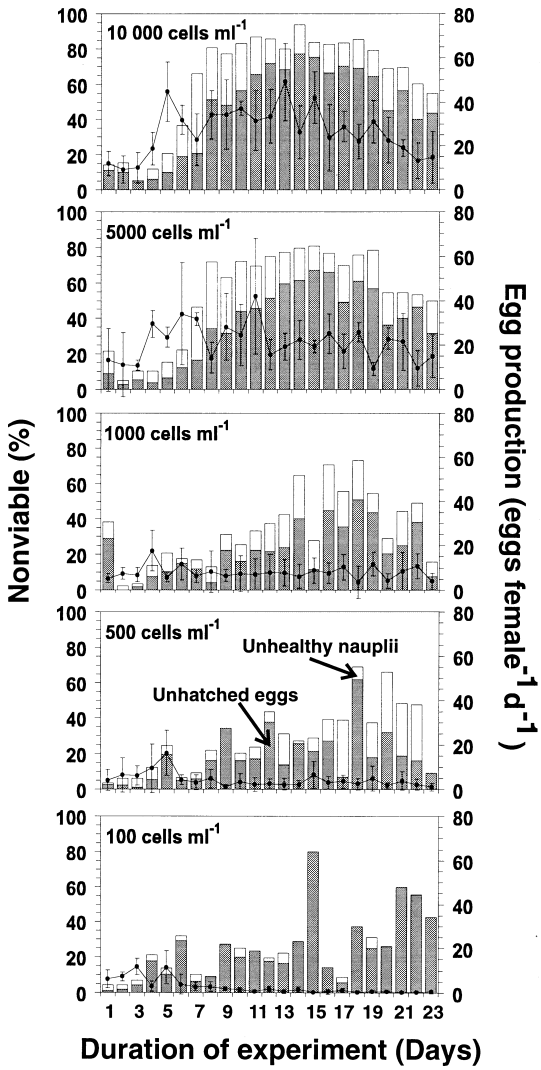

Fig. 6. Mean daily egg production rate (solid line, with standard deviations) and egg viability (columns) of *C. finmarchicus* females fed with the diatom *Thalassiosira nordenskioldii* at different concentrations. Nonviable eggs classified into unhatched eggs (solid columns) and unhealthy nauplii (open columns). Results are means of 4 replicate experiments for each food concentration.

after ingestion of diatoms by female *C. helgolandicus* (Poulet & al. 1995; Laabir & al. 1995) and *C. pacificus* (Uye 1996).

Our study, in addition to supporting the hypothesis that hatching success of copepod eggs is diatom density-dependent (Chaudron & al 1996), reveals that inhibition of egg development is directly proportional to ingestion rate of females on diatoms over a wide range of concentrations. One of the possible causes of anomalous embryonic development is the presence of antimitotic agents within diatom cells (Poulet & al. 1994). By ingesting *T. nordenskioldii* or *Navicula* sp., females would accumulate anti-mitotic agents which would then be transferred to oocytes during vitellogenesis. That these inhibitors must first be accumulated is inferred from the observed initial high viability followed by a progressive diminution in hatching success on successive days. Alternatively, the same effect may be explained by a deficiency in some essential nutritional component within diatom cells (e.g. Jónasdóttir & Kiørboe 1996). Internal body stores of this component could be used by the female initially to supplement the diatom diet and sustain viability, but hatching success would decline as the internal store is depleted. Either alternative could explain results in Figs 1 and 6. However, our results in Fig. 7C, showing a reduced egg viability in a mixture containing high quality food items (with respect of egg viability) making up approximately 40 % of total ingested carbon, imply that the substantial ingestion of high quality food could not completely make up for a biochemical deficit in *T. nordenskioldii*. This may indicate that blocking of copepod embryonic development is chemically mediated. There are a number of examples of various noxious compounds in species of marine benthic algae that serve as chemical defense against herbivores (Hay & Fenical 1988; Hay 1996); we cannot exclude this possibility in phytoplankton. Other potential causes of unsuccessful hatching success, such as the age of food cultures (Jónasdóttir 1994; Jónasdóttir & Kiørboe 1996), age of females (Ianora et al. 1995), infertility caused by the lack of mating (Miralto & al. 1995) and exposure to deoxygenation (Roman & al. 1993) could not have caused the variations in egg viability we observed, as all tests performed during the first experimental series (Fig. 1) were conducted with identical protocols on the same group of females. Differences among diatom species in the capacity to impact *Calanus* egg viability are therefore most likely due to subtle variations in biochemical content of the algae which, when combined with the feeding behaviour for different cell types ultimately determines the quality of the diet for egg production and recruitment.

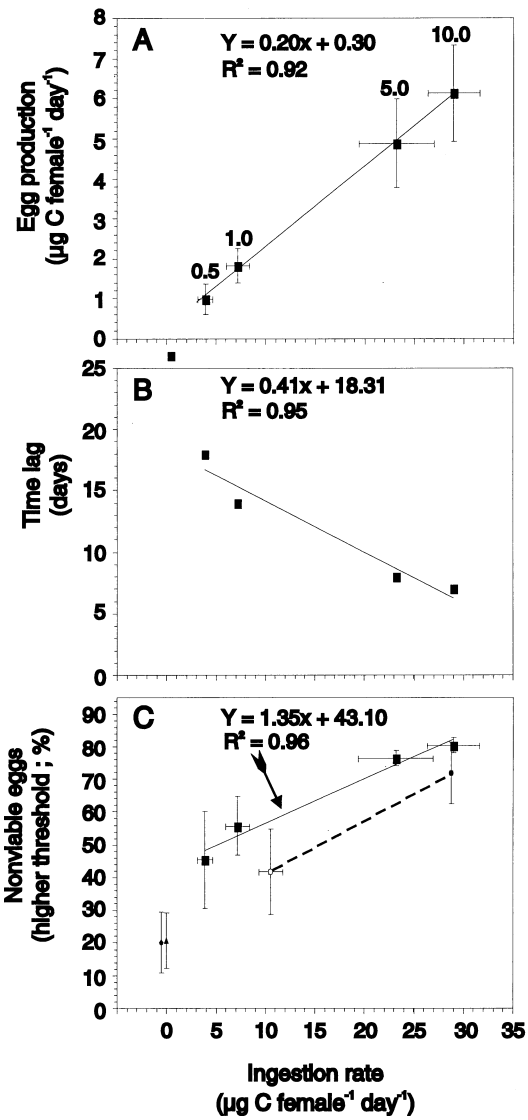

Fig. 7. Relationship between mean ingestion rate of *Calanus finmarchicus* and (A) egg production rate, (B) the time lag to reach 50 % of nonviable eggs and (C) the higher threshold level of egg mortality during the second experimental series (■). Females were exposed to *T. nordenskioldii* at 0.5, 1.0, 5.0 and 10.0×10^3 cells ml^{-1}. Also shown in C, the results of the first experimental series when females fed *ad libidum* the diatoms *Thalassiosira nordenskioldii* (●), *Skeletonema costatum* (◆), *Chaetoceros debilis* (▲) or a 45/25/30 mixture (○) of the 3 species. Estimates of ingestion rate are only for *T. nordenskioldii*. Results are means (±SD) of 8 observations (4 replicates on each of two days).

We conclude from these experiments that extended feeding on certain extremely common diatom species would have a negative impact on *C. finmarchicus* recruitment rates. Based on these results, which generally support results obtained for other diatom and copepod species (e.g. Ban & al. 1997 and references therein), the extent to which reproductive limitation from diatom diets actually occurs in the sea would depend on the duration of feeding on diatoms and the mix of phytoplankton species. The most damaging situations for *Calanus* recruitment would appear to be blooms of diatoms that are difficult to ingest, like *C. debilis*, or that are harmful to hatching success, like *T. nordenskioldii.* In the St. Lawrence Estuary, for example, *Thalassiosira nordenskioldii* regularly dominates the biomass during phytoplankton blooms (Levasseur & al. 1984, 1994). The spawning period of *Calanus* in the Lower Estuary coincides with high *Thalassiosira* concentrations (Plourde & Runge 1993). Based on our experimental evidence from controlled feeding studies, under such conditions *Thalassiosira* could be ingested in quantities high enough to induce anomalies in eggs; the induction time for inhibition at 10^4 cells ml^{-1} is on the order of 1 week, whereas diatom blooms in this region may last 1-2 months.

Whether the diversity in the diet of *C. finmarchicus* is sufficient to relax the potential adverse effects and consistently support a relatively high reproductive output during diatom blooms remains to be determined. While Pond & al. (1996) found that hatching success of *C. helgolandicus* at a station in the English Channel during 1994 was relatively high, Guisande & Harris (1995) and Laabir & al. (1995) observed that egg viability of the same species in the English Channel in other years was more variable, sometimes < 30 %. On Georges Bank, Runge & al. (unpubl. obs.) noted that *Calanus finmarchicus* eggs typically hatched with a success rate of 60-90 %, and on a few occasions only 30-35 % of eggs were viable. Based on these observations and our experimental results, we propose that ingestion of diatoms does at times significantly impact *Calanus* recruitment.

In summary, we have shown that 3 of the 4 diatom species tested induced either low fecundity (*Chaetoceros debilis*) or low egg viability (*Thalassiosira nordenskioldii*) or both (*Navicula* sp.); the exception being *Skeletonema costatum*. The accumulating evidence both in the laboratory and in the field that many diatom species are ultimately insufficient or perhaps harmful sources of nutrition for copepod reproduction opens to question the classical view of a strong linkage between outbursts of diatom-rich phytoplankton and copepod recruitment. Even if egg production in spring is high in temperate and boreal waters, diatom blooms may at times suppress recruitment due to high egg mortality. The frequency with which this actually occurs in the sea, however, is still not well known, as the concurrence of non-harmful diatoms, like *S. costatum,* and other kinds of microplankton such as dinoflagellates and microzooplankton may contribute to significantly lessen the deleterious impacts.

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REFERENCES

Ban S, Burns C, Castel J, Chaudron Y, Christou E, Escribano R, Fonda Umani S, Gasparini S, Ruiz Guerrero F, Hoffmeyer M, Ianora A, Kang H-K, Laabir M, Lacoste A, Miralto A, Poulet S, Ning X, Rodriguez V, Runge J, Shi J, Starr M, Uye S-I, Wang Y. 1997. The paradox of diatom-copepod interactions. *Marine Ecology Progress Series* 157:287-293.

Chaudron Y, Poulet SA, Laabir M, Ianora A, Miralto A. 1996. Is hatching success of copepod eggs diatom density-dependent? *Marine Ecology Progress Series* 114:185-194.

Cushing DH. 1989. A difference in structure between ecosystems in strongly stratified waters and those that are only weakly stratified. *Journal of Plankton Research* 11:1-13.

Frost BW. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus. Limnology and Oceanography* 17:805-815.

Guillard RRL, Ryther JH. 1962. Studies of marine planktonic diatoms. 1 *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gram. *Canadian Journal of Microbiology* 8:229-239.

Guisande C, Harris R. 1995. Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. *Limnology and Oceanography* 40:476-482.

Hay ME. 1996. Marine chemical ecology: what's know and what's next? *Journal of Experimental Marine Biology and Ecology* 200:103-134.

Hay ME, Fenical W. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. *Annu Rev Ecol Syst* 19:111-145.

Ianora A, Poulet SA. 1993. Egg viability in the copepod *Temora stylifera*. *Limnology and Oceanography* 38:1615-1626.

Ianora A, Poulet SA, Miralto A. 1995. A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod *Temora stylifera*. *Marine Biology* 121:533-539.

Ianora A, Poulet SA, Miralto A, Grottoli R. 1996. The diatom *Thalassiosira rotula* affects reproductive success in the copepod *Acartia clausi*. *Marine Biology* 125:279-286.

Jónasdóttir SH. 1994. Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Marine Biology* 121:67-81.

Jónasdóttir SH, Kiørboe T. 1996. Copepod recruitment and food composition: do diatoms affect hatching success? *Marine Biology* 125:743-750.

Kiørboe T, Nielsen TG. 1994. Regulation of zooplankton biomass and production in a temperate coastal ecosystem. 1. Copepods. *Limnology and Oceanography* 39:493-507.

Kleppel GS, Holliday DV, Pieper RE. 1991. Trophic interactions between copepods and microplankton: a question about the role of diatoms. *Limnology and Oceanography* 36:172-178.

Laabir M, Poulet SA, Ianora A, Miralto A, Cueff A. 1995. Reproductive response of *Calanus helgolandicus*. II. *In situ* inhibition of embryonic development. *Marine Ecology Progress Series* 129:97-105.

Legendre L. 1990. The significance of microalgal blooms for fisheries and for the export of particulate organic carbon in oceans. *Journal of Plankton Research* 12:681-699.

Levasseur M, Fortier L, Therriault J-C, Harrison PJ. 1994. Phytoplankton dynamics in a coastal jet frontal region. *Marine Ecology Progress Series* 86:283-295.

Levasseur M, Therriault J-C, Legendre L. 1984. Hierarchical control of phytoplankton succession by physical factors. *Marine Ecology Progress Series* 19:211-222.

Mann KH. 1993. Physical oceanography, food chains, and fish stocks: a review. *ICES Journal of Marine Science* 50:105-119.

Marshall SM, Orr AP. 1955. *The biology of a marine copepod,* Calanus finmarchicus *(Gunnerus)*. London: Oliver & Boyd. 188 p.

Miralto A, Ianora A, Poulet SA. 1995. Food type induces different reproductive responses in the copepod *Centropages typicus*. *Journal of Plankton Research* 17:1521-1534.

Parsons TR, LeBrasseur RJ, Fulton JD. 1967. Some observations on the dependence of zooplankton grazing on the cell size and concentration of phytoplankton blooms. *Journal of the Oceanographic Society of Japan* 23:10-17.

Peterson WT, Kimmerer WJ. 1994. Processes controlling recruitment of the marine calanoid *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. *Limnology and Oceanography* 39:1594-1605.

Plourde S, Runge JA. 1993. Reproduction of the planktonic copepod *Calanus finmarchicus* in the lower St. Lawrence Estuary: relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. *Marine Ecology Progress Series* 102:217-227.

Pond D, Harris R, Head R, Harbour D. 1996. Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. *Marine Ecology Progress Series* 143:45-63.

Poulet SA, Ianora A, Miralto A Meijer L. 1994. Do diatoms arrest embryonic development in copepods? *Marine Ecology Progress Series* 111:79-96.

Poulet SA, Laabir M, Ianora A, Miralto A. 1995. Reproductive response of *Calanus helgolandicus*. I. Abnormal embryonic and naupliar development. *Marine Ecology Progress Series* 129:85-95.

Roman MR, Gauzens AL, Rhinehart WK, White JR. 1993. Effects of low oxygen waters on Chesapeake Bay zooplankton. *Limnology and Oceanography* 38:1603-1614.

Runge JA. 1984. Egg production of the marine, planktonic copepod, *Calanus pacificus* Brodsky: Laboratory observations. *Journal of Experimental Marine Biology and Ecology* 74:53-66.

Runge JA. 1985. Egg production rates of *Calanus finmarchicus* in the sea off Nova Scotia. *Archiv für Hydrobiologie Beith* 21:33-40.

Runge JA, Plourde S. 1996. Fecundity characteristics of *Calanus finmarchicus* in coastal waters of Eastern Canada. *Ophelia* 44:171-187.

Strathmann RR. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnology and Oceanography* 12:411-418.

Urban JL, McKenzie CH, Deibel D. 1992. Seasonal differences in the content of *Oikopleura vanhoeffeni* and *Calanus finmarchicus* feacal pellets - Illustrations of zooplankton food web shifts in coastal Newfoundland waters. *Marine Ecology Progress Series* 84:255-264.

Uye S. 1996. Induction of reproductive failure in the planktonic copepod *Calanus pacificus* by diatoms. *Marine Ecology Progress Series* 133:89-97.

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