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Reproductive success in *Calanus helgolandicus* as a function of diet and egg cannibalism

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ABSTRACT: *Calanus helgolandicus* females were incubated for 6 to 25 d in single and mixed diets of the diatom *Coscinodiscus curvatulus* and the dinoflagellate *Gymnodinium sanguineum* using 2 incubation protocols, and fecundity, hatching success, egg cannibalism and faecal pellet production were measured. Both fecundity and hatching success were significantly reduced by single or mixed diets containing high concentrations of diatoms. The deleterious effect of the diatom was diminished when females were fed diets containing low diatom concentration and also when egg cannibalism represented ca >20% of the total daily egg production. Egg cannibalism was higher with the wheel-incubation method than with the vial-incubation method. The results suggest that diatom inhibition of copepod reproduction can be reduced in various ways, including decreasing the diatom concentration, switching from diatom to dinoflagellate diets, increasing the diversity of food items and also by egg cannibalism (eggs are of high nutrient value).

KEY WORDS: Copepod · *Calanus helgolandicus* · Reproduction · Food

INTRODUCTION

Accumulating laboratory evidence shows that several species of diatoms, at high concentrations ($\geq 10^3$ cells ml^{-1}), are deleterious for copepod reproduction (Ban et al. 1997). Ingestion of diatoms by adult female copepods is followed by low egg production and low hatching success, including abnormal egg and nauplii development (Poulet et al. 1994, 1995, Uye 1996, Lee et al. 1999). The inhibition is reversible when the diatom diet is replaced by a dinoflagellate diet (Laabir et al. 1995b, Uye 1996). The higher the density of diatoms and the longer they are ingested, the lower the hatching success and vice versa (Chaudron et al. 1996). Recently, 3 aldehydes, purified from diatom extracts, have been identified and bioassays have shown that they cause low egg viability (Ianora et al. 1999b, Miralto et al. 1999). In addition to diatom inhibition, other evidence has shown some diatoms species to be insufficiently nutritious to enable successful copepod reproduction (Støttrup & Jensen 1990, Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996). Moreover, some essential fatty acids may be lacking from diatom diets (Jónasdóttir & Kiørboe 1996, Pond et al. 1996). This well-documented evidence of the deleterious effects and nutritional insufficiency of diatoms in laboratory conditions raises questions as to the effect of diatoms on copepods in the field.

In nature, copepod diets are diverse and include various phytoplankton and microzooplankton taxa, bacteria and metazoans (see Mauchline 1998 for review) and probably influence copepod reproduction and consequently recruitment of populations. Despite dietary diversity, it is still unclear what influence food selectivity has on reproduction and how the deleterious effect of diatoms can be mediated by mixed diets. Lee et al. (1999) examined the link between feeding selectivity and reproductive responses in the copepod *Pseudocalanus newmani* and demonstrated the positive influence of mixed diets on its fecundity and hatching success. Mixed diets can provide essential compounds such as vitamins, amino acids and fatty acids that may be lacking in single diets (Harrison 1990).

Calanus spp. are known to eat their own eggs

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(Conover 1960, Turner 1984, Laabir et al. 1995a) and thus in nature, a fraction of the eggs they spawn are lost to cannibalism (Kiørboe et al. 1988, Liang et al. 1994, Peterson & Kimmerer 1994). Laabir et al. (1995a) reported that *C. helgolandicus* ingested their eggs and that cannibalism increased both with increasing female density in the incubators and with increasing level of starvation, and decreased again after addition of food. Therefore, eggs produced by females constitute an additional type of food, that may modify the nutritional value of single or mixed algal diets. This effect of egg cannibalism has never been evaluated during fecundity/hatching measurements.

The aim of this study was thus to evaluate variations in the reproductive response of *Calanus helgolandicus*, to 'modifications' of normal diets (diatom and dinoflagellate in single and mixed diets) by the copepod eggs produced during the incubations. The experiments were performed at different food concentrations and used 2 methods of incubation to determine the effect of various proportions of diatoms among other food components on the selective feeding of copepods.

MATERIALS AND METHODS

Copepod sampling. *Calanus helgolandicus* females were collected from 21 July to 10 October 1998 by towing 500 µm mesh-net obliquely from ~20 m to the surface offshore from Roscoff, France (Western English Channel). They were kept in an insulated box until arrival in the laboratory (within 1 to 2 h after collection). Adult females were sorted and placed in incubators filled with ambient seawater at 13.4 ± 0.4°C. After 24 h acclimation, fecundity and egg viability were estimated; these served to represent the initial reproductive condition of females on the various sampling dates and were used as control values for each experiment.

Phytoplankton culture. Two similar-sized phytoplankton species, the diatom *Coscinodiscus curvatulus* and the dinoflagellate *Gymnodinium sanguineum*, were used in the feeding and fecundity experiments (Table 1). They were cultured in K medium (Keller et al. 1987) with the addition of silicate to the diatoms, at 17°C under a 14 h light:10 h dark cycle at light intensity of ~117 µE m^{-2} s^{-1} and were fed to the copepods in their exponential phase of growth (Uye 1996, Ianora et al. 1999a) (although Jónasdóttir [1994] made distinctions in food quality between the early, mid- and late-exponential phases of growth, this was not done in the present study).

Feeding experiments. Five types of food treatments comprising a diatom-dinoflagellate combination (dia, din) were fed to the females in the following ratios: 1:0 (Sdia), 4:1 (HdiaLdin), 1:1 (Sratio), 1:4 (LdiaHdin), 0:1 (Sdin) (Table 2); total cell volume was constant at 2.5×10^7 µm^3 ml^{-1}. Six 310 ml jars each containing 3 females, and 2 control jars without females were enriched with 1 of the 5 food treatments and placed on a gently rotating wheel (5 to 6 rpm) at 13.4 ± 0.4°C under dim light and a natural light cycle (hereafter 'wheel' incubation method). The females were acclimated to each type of diet for 24 h. Feeding experiments were run for 6 d with the mixed diets (HdiaLdin, Sratio and LdiaHdin) and 7 d with the single diets (Sdia and Sdin). Phytoplankton samples (3 ml) were taken from each experimental and control jar at the beginning and end of each day in each food treatment, fixed with Lugol's solution and then counted on a Sedgewick-Rafter

Table 2. Cell concentration of 5 diet treatments (ratio diatoms:dinoflagellates, based on cell volume) using diatom *Coscinodiscus curvatulus* and dinoflagellate *Gymnodinium sanguineum* in mixed and single diets fed to *Calanus helgolandicus*

Diet treatment	Acronym	Cell concentration (cells ml^{-1})	
		C. curvatulus	*G. sanguineum*
1:0	Sdia	1.6×10^3	0
4:1	HdiaLdin	1.3×10^3	2.1×10^2
1:1	Sratio	8×10^2	5.3×10^2
1:4	LdiaHdin	3.2×10^2	8.5×10^2
0:1	Sdin	0	1.1×10^3

Table 1. Characteristics of phytoplankton species used as diets in feeding and fecundity experiments with *Calanus helgolandicus*. Cell volumes converted into cell-carbon contents according to equation of Strathmann (1967). –: no data

Species	Clone	Size (µm)			Volume (µm^3)	Carbon (pg C)	Location
		Length	Width	Thickness			
Coscinodiscus curvatulus	RCC77	19.1	32.2	–	15554	569	North Atlantic (off Roscoff, France)
Gymnodinium sanguineum	RCC89	50.1	34.1	26.4	23453	2113	North Atlantic (off Florida, USA)

chamber. Female ingestion rates were calculated according to Frost's (1972) equation. In addition to ingestion rate, egg cannibalism, fecundity and egg viability were monitored daily. Egg cannibalism was expressed as the percentage of crumpled and empty egg membranes that remained on the bottom of the jar or were counted in the faecal pellets relative to the total number of eggs produced daily.

Fecundity measurements. Fecundity and egg viability were measured parallel to female ingestion rate. The food treatments were the same as in the feeding experiments (see Table 2). Thirty females were acclimated to the experimental conditions for 24 h, and those that laid eggs ($22 \leq n \geq 30$ females) were each transferred to an individual vial (300 ml vol) containing 100 ml of one of the diets (Table 2) and were maintained at 13.4 ± 0.4°C under dim light and a natural light cycle. Algae were kept in suspension manually 5 to 6 times a day. The females were transferred daily to new containers with fresh food. The experiments were run for 6 d with the mixed diets (HdiaLdin, Sratio and LdiaHdin) and 9 d with the single diets (Sdia and Sdin). Fecundity, egg viability and egg cannibalism were recorded each day, and the number of viable eggs (NVE) was calculated as a product of fecundity and hatching rate. Some females spawned unfertilised eggs (e.g. fragile eggs lacking membrane) during the incubation periods, and these were excluded from the fecundity estimations. Egg viability was determined by incubating each batch of $n \geq 10$ eggs in a chamber containing 2 ml filtered seawater (0.22 µm) for a period of <72 h. Female survival was ~100 % in all dietary treatments.

Diet-shift experiments. To examine the possible deleterious effect of the diatom *Coscinodiscus curvatulus* without any egg-cannibalism artefact and to determine how reproductive success may change as a function of shift in diet, a high concentration (1×10^4 cells ml^{-1}) of the diatom was first provided to the females over a period of 15 d; the diet was then replaced by the dinoflagellate *Gymnodinium sanguineum* (4×10^2 cells ml^{-1}) for 10 further days (Days 16 to 25). Each of the 30 females was individually maintained in a vial (300 ml vol) under the same conditions as the fecundity measurements with the mixed and single diets (hereafter 'vial' incubation method). Fecundity, egg viability, faecal pellet production and egg cannibalism were monitored daily.

Data analysis. Mean and standard deviation of percentage egg viability and egg cannibalism were calculated from arcsine-transformed values. The percentage data were arcsine-transformed (Zar 1984) prior to statistical analyses to normalise the variance, and parametric tests were then applied (e.g. Student's *t*-test or 1-way ANOVA test, followed by a Tukey's post hoc comparison). The ingestion rates, fecundity, faecal pellet production and NVE data were analyzed by non-parametric tests (e.g. Mann-Whitney *U*-test or Kruskal-Wallis ANOVA test, followed by a Mann-Whitney *U*-test post hoc comparison). All statistical analyses used the STATISTICA programme for Windows (StatSoft Inc.).

RESULTS

Feeding selectivity

Non-selective feeding of *Calanus helgolandicus* females was observed for 5 diet treatments (Table 2) for 6 or 7 d, confirming that these females ingested both diets at different rates, depending on food concentration (Fig. 1). In the mixed diet treatments (HdiaLdin, Sratio and LdiaHdin; Fig. 1A,B,C), mean ingestion rates were always significantly higher for diets with high cell concentrations than for those with low cell concentrations (Table 3). With the Sratio diet, ingestion of the diatom *Coscinodiscus curvatulus* was higher than of the dinoflagellate *Gymnodinium sanguineum*, since the relative cell concentration of the diatom was higher than that of the dinoflagellate (cell-volume difference). With HdiaLdin, ingestion of diatoms fluctuated, while ingestion of dinoflagellates was fairly stable. With both the Sratio and LdiaHdin diets, ingestion rates dropped temporarily on Day 5 (Fig. 1B,C); mean ingestion rates of the diatom and dinoflagellate were the same in both treatments (Mann-Whitney *U*-test, $p > 0.05$). For the single-diet treatments (Sdia and Sdin; Fig. 1D), the mean ingestion rate was significantly higher with Sdia than with Sdin (Mann-Whitney *U*-test, $p < 0.01$). A comparison of the mean ingestion rates for 6 d between mixed- and single-diet treatments revealed these rates to be proportional to the concentration of the diatom or dinoflagellate concentrations in the respective diets: ingestion of the diatom in the HdiaLdin and Sdia diets was significantly higher than in the Sratio diet, and was lowest in the LdiaHdin diet (Kruskal-Wallis ANOVA test, H [df = 3, n = 141] = 78.92, $p < 0.0001$). Ingestion of the dinoflagellate from the Sdin diet was significantly higher than from the LdiaHdin and Sratio diets, and lowest in the HdiaLdin diet (Kruskal-Wallis ANOVA test, H [df = 3, n = 142] = 37.64, $p < 0.0001$).

Fecundity and egg viability in mixed- and single-diet experiments

The effects of mixed and single diets on fecundity in the vial experiments were compared. Fecundity of *Calanus helgolandicus* females varied with diet

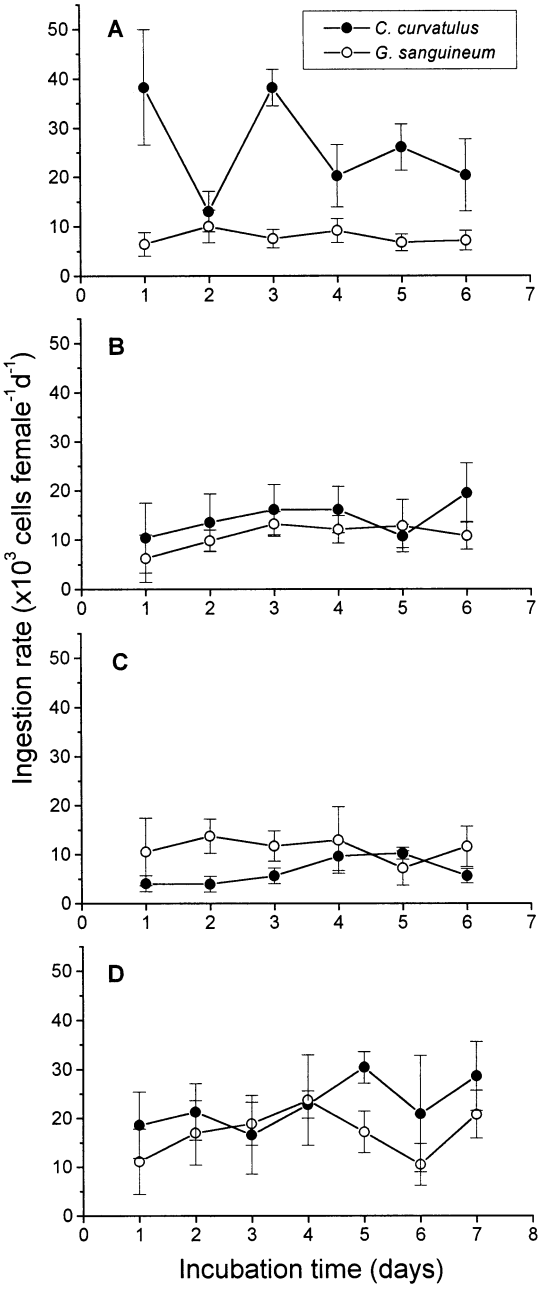

Fig. 1. *Calanus helgolandicus*. Comparison between ingestion rates of females fed mixed (6 d; A,B,C) and single (7 d; D) diets. Diatom *Coscinodiscus curvatulus* and dinoflagellate *Gymnodinium sanguineum* were mixed in ratios 4:1 (A, HdiaLdin), 1:1 (B, Sratio), 1:4 (C, LdiaHdin), and 1:0 (D, Sdia) or 0:1 (D, Sdin), based on cell concentrations (see Table 2), equivalent to a constant cell volume equal to 2.5×10^7 µm^3 ml^{-1}. Data are means ± SD for n = 6 (number of jars)

(Fig. 2). Initial mean *in situ* fecundity was 9.2 ± 4.0 eggs female^{-1} d^{-1} (n = 25) with mixed-diet treatments, and there was no significant difference between sampling dates (21 July, 3 and 17 August; Kruskal-Wallis ANOVA test, H [df = 2, n = 25] = 3.35, p > 0.05), indicating a similar reproductive condition among all females sampled. Initial fecundity in the mixed-diet treatments (HdiaLdin, Sratio and LdiaHdin) increased significantly after the 1st day (Day 2; Fig. 2) (Kruskal-Wallis ANOVA test, H [df = 3, n = 106] = 40.81, p < 0.0001); it then continued to increase gradually and significantly with both the HdiaLdin and the LdiaHdin diets (Table 4). In the single-diet treatments (Sdia and Sdin), initial *in situ* fecundity was close to zero, and mean fecundity increased for 9 d as in the Sdia and Sdin treatments (Table 4). A comparison of the means for 6 d (Days 2 to 7; Fig. 2) between diet treatments revealed that mixed diets resulted in a better fecundity than single diets (Kruskal-Wallis ANOVA test, H [df = 4, n = 796] = 130.45, p < 0.0001).

Mean initial *in situ* egg viability was 83.2 ± 2.6% (n = 70) in mixed-diet treatments, and did not differ significantly between sampling dates (Kruskal-Wallis ANOVA test, F = 0.49, df$_1$ = 2, df$_2$ = 69, p > 0.05). During the incubation periods, egg viability fluctuated slightly with the HdiaLdin and LdiaHdin diets, gradually decreased with the Sratio diet (Fig. 2), and remained significantly high with the LdiaHdin diet (Table 4). In single-diet treatments, egg viability decreased until the 3rd or 4th day, and then gradually increased (Fig. 2), and was significantly higher with the Sdin diet (Table 4). A comparison of egg viability between mixed- and single-diet treatments over 6 d showed that mean egg viability with both the Sdin and LdiaHdin diets was higher than with the Sdia diet (ANOVA test, F = 63.64, df$_1$ = 4, df$_2$ = 661, p < 0.0001). These results indicate that egg viability with either mixed or single diets was proportional to the concentration of the dinoflagellate *Gymnodinium sanguineum* in the diet; the higher the dinoflagellate concentration, the better the copepod egg viability.

The number of viable eggs (NVE) varied as a function of time and dietary treatment. The overall trends for NVE were similar to those for fecundity variation (Fig. 2), showing better rates with LdiaHdin for the mixed diets and with Sdin for the single diets (Table 4). A comparison of the mean NVE over 6 d showed that the recruitment rates for copepods fed mixed diets were higher than for those fed single diets (Kruskal-Wallis ANOVA, H [df = 4, n = 796] = 174.87, p < 0.001), indicating improvement in recruitment of Stage N1 nauplii by mixed diets.

Fecundity and egg viability related to cannibalism

For females incubated individually in vials and fed either mixed or single diets, egg cannibalism was negligible (below ~3% of total daily fecundity), i.e. it represented a negligible artefact in our fecundity and

Table 3. *Calanus helgolandicus*. Ingestion rate (cells female^{-1} d^{-1}) of females fed the mixed and single diets. Mann-Whitney *U*-test revealed significant differences in ingestion rate between *Coscinodiscus curvatulus* and *Gymnodinium sanguineum* for mixed-diet treatments. Data are means of results shown in Fig. 1. –: no data

Diet treatment	*C. curvatulus* Mean ± SD	(n)	*G. sanguineum* Mean ± SD	(n)	Significance
Mixed diets					
HdiaLdin	26342 ± 11387	(35)	7778 ± 2555	(36)	$p < 0.0001$
Sratio	14473 ± 6008	(35)	10959 ± 4016	(35)	$p < 0.01$
LdiaHdin	6487 ± 3048	(36)	11391 ± 5005	(35)	$p < 0.0001$
Single diets					
Sdia	22774 ± 7985	(41)	–	(–)	
Sdin	–	(–)	17026 ± 7168	(42)	

Table 4. *Calanus helgolandicus*. Fecundity, egg viability and number of viable eggs (NVE) of females fed mixed (6 d) and single (9 d) diets with *Coscinodiscus curvatulus* and *Gymnodinium sanguineum*. [a,b,c]Significant differences between diets. Data are means of results in Fig. 2

Diet treatment	Fecundity (eggs female^{-1} d^{-1}) Mean ± SD	(n)	Egg viability (%) Mean ± SD	(n)	NVE (viable eggs female^{-1} d^{-1}) Mean ± SD	(n)
Mixed diets						
HdiaLdin	38.4 ± 15.5	(167)	75.5 ± 1.7	(142)	29.0 ± 12.6	(167)
Sratio	34.0 ± 13.3	(128)	76.6 ± 2.3	(139)	25.9 ± 10.0	(128)
LdiaHdin	41.9 ± 15.7	(175)	84.6 ± 0.9	(141)	35.2 ± 12.9	(175)
Significance	LdiaHdin[a] HdiaLdin[a] > Sratio[b] Kruskal-Wallis ANOVA test H (df = 2, n = 470) = 22.39 $p < 0.0001$		LdiaHdin[a] > Sratio[b] HdiaLdin[b] ANOVA test $F = 33.485$; $df_1 = 2$, $df_2 = 419$ $p < 0.0001$		LdiaHdin[a] > HdiaLdin[b] > Sratio[c] Kruskal-Wallis ANOVA test H (df = 2, n = 470) = 46.58 $p < 0.0001$	
Single diets						
Sdia	30.1 ± 12.7	(242)	70.6 ± 2.0	(203)	21.4 ± 9.9	(242)
Sdin	29.6 ± 18.2	(252)	86.0 ± 1.7	(184)	25.1 ± 15.4	(252)
Significance	Mann-Whitney *U*-test $p > 0.05$ ns		Student's *t*-test $p < 0.0001$		Mann-Whitney *U*-test $p < 0.01$	

egg-viability measurements. In contrast, high egg cannibalism occurred in both mixed and single diets during the classic feeding experiments, using the rotating wheel. In mixed diets, daily egg cannibalism (Fig. 3) constituted ~30 to 55% of total daily fecundity; in single diets cannibalism increased to >25% after the 2nd day (Day 3; Fig. 3). Fig. 4 compares mean fecundity, egg viability and egg cannibalism between the 'wheel' and the 'vial' methods in all diet combinations. There were no significant differences in fecundity between the 2 methods, except for the Sratio and Sdin diets, which for fecundity was higher with the vial method (Fig. 4). Egg viability was significantly higher with the wheel method than with the vial method in all diet treatments except Sdin (Fig. 4). Mean egg cannibalism with the wheel method ranged from 21.4 to 49.2%, and was always significantly lower with the vial method (0.1 to 1.6%) (Fig. 4).

A comparison of mean fecundity, egg viability and egg cannibalism (pooled data for 6 d in mixed and 7 d in single diets; Fig. 5), regardless of diet, revealed egg viability (95.3 ± 4.4%, n = 494) and egg cannibalism (39.6 ± 3.8%, n = 192) in the wheel incubations to be significantly higher than in the vial incubations (egg viability: 78.0 ± 2.4%, n = 714; egg cannibalism: 0.4 ± 1.2%, n = 852). However, mean fecundity did not differ significantly between the 2 incubation methods. These results indicated that egg cannibalism improved egg viability, while fecundity was unaffected.

Changes in fecundity and egg viability as a function of diet

High concentrations of the diatom *Coscinodiscus curvatulus* (1×10^4 cells ml^{-1}) inhibited both fecundity and egg viability (Fig. 6). Mean fecundity of females fed with the diatom for 15 d (1.8 ± 2.7 eggs female^{-1} d^{-1}, n = 30) did not differ significantly from the initial *in situ* fecundity (1.0 ± 1.3 eggs female^{-1} d^{-1}, n = 8) (Mann-Whitney *U*-test, $p > 0.05$). Fecundity increased significantly (12.5 ± 11.5 eggs female^{-1} d^{-1}, n = 74) when the

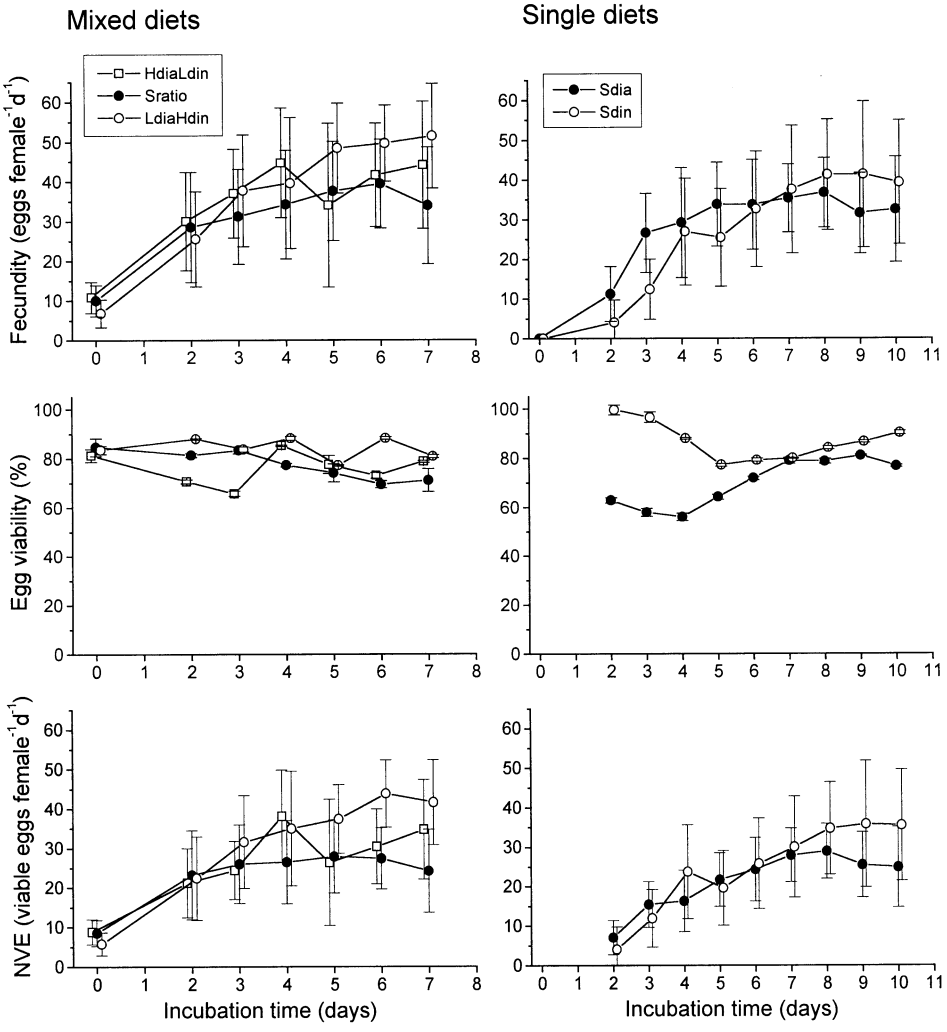

Fig. 2. *Calanus helgolandicus*. Fecundity, egg viability and number of viable eggs (NVE) of females fed on mixed (6 d) and single (9 d) diets of *Coscinodiscus curvatulus* and *Gymnodinium sanguineum*. Concentrations and proportions of diatom and dinoflagellate as in Fig. 1. Day 0 = *in situ* rates prior to start of experiments. Data are means ± SD (n = 6, number of vials for fecundity and NVE; n = 6, number of chambers for egg viability)

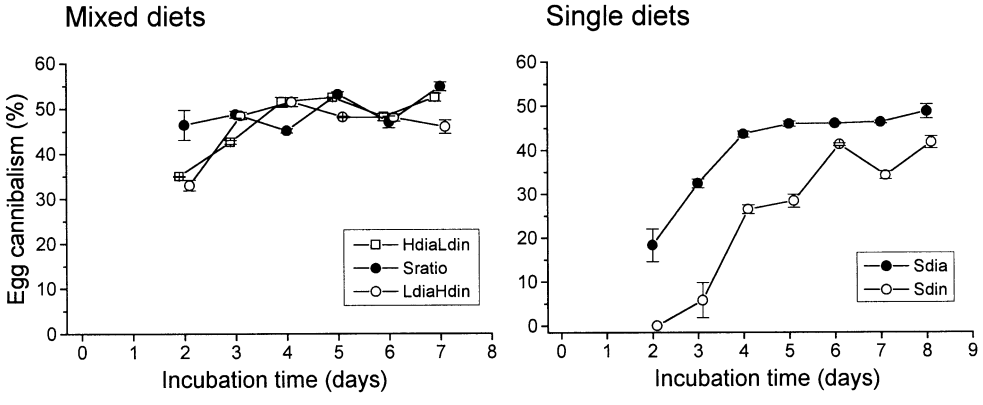

Fig. 3. *Calanus helgolandicus*. Egg cannibalism of females fed mixed (6 d) and single (7 d) diets of *Coscinodiscus curvatulus* and *Gymnodinium sanguineum* in feeding experiments using rotating wheel. Concentrations and proportions of diatom and dinoflagellate in diets as in Fig. 1, incubation time as in Fig. 2. Data are means ± SD (n = 6, number of jars)

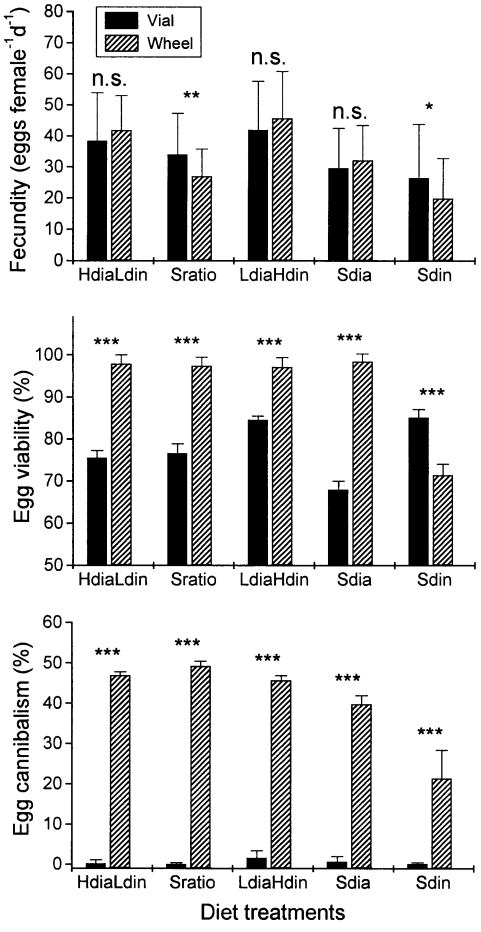

Fig. 4. *Calanus helgolandicus.* Comparison between mean fecundity, egg viability and egg cannibalism of females fed mixed (6 d) and single (7 d) diets using 2 incubation methods: vial and rotating wheel. Concentrations and proportions of diatom and dinoflagellate in diets as in Fig. 1. Data are means ± SD (n = 6, number of vials for fecundity; n = 6, number of jars for egg cannibalism; n = 30, number of chambers for egg viability). Significant differences between the 2 methods were calculated by Mann-Whitney *U*-test for fecundity and Student's *t*-test for egg viability and egg cannibalism (ns = $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$)

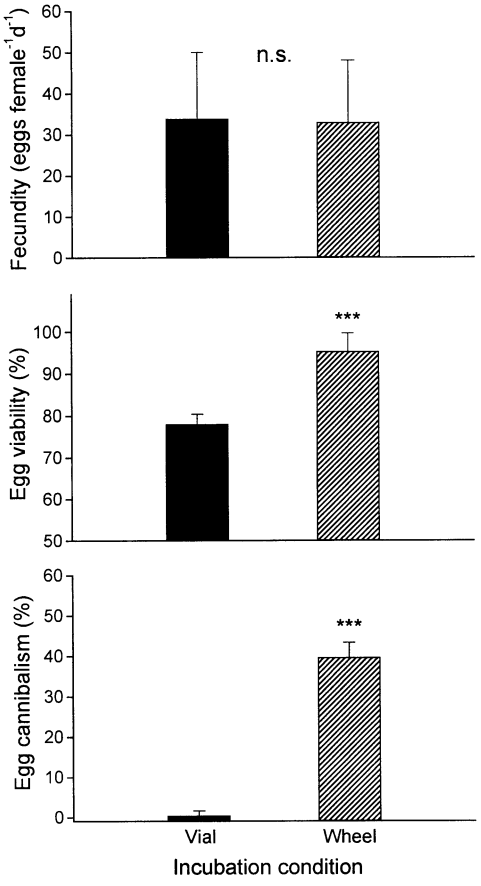

Fig. 5. *Calanus helgolandicus*. Effect of incubation conditions on mean fecundity, egg viability and egg cannibalism calculated from pooled data for mixed (HdiaLdin, Sratio and LdiaHdin, 6 d) and single (Sdia and Sdin, 7 d) diets using 2 different incubation methods: vial and rotating wheel. (ns = $p > 0.05$, $^{***}p < 0.0001$)

diatom was replaced by the dinoflagellate *Gymnodinium sanguineum* at a lower cell concentration (4×10^2 cells ml^{-1}) for 10 d (Mann-Whitney *U*-test, $p < 0.0001$), while egg viability quickly decreased to near-zero for the first 15 d (9.5 ± 6.5%: n = 19); this was significantly lower than the initial *in situ* egg viability (45.5 ± 1.0%, n = 9) (Student's *t*-test, $p < 0.0001$), indicating strong inhibition of hatching success by the diatom. Egg viability, however, increased significantly (95.2 ± 3.3%, n = 56) when the diatom was replaced by the dinoflagellate (Student's *t*-test, $p < 0.0001$). With the diatom diet, faecal pellet production decreased and fluctuated slightly around a mean of 13.2 ± 15.1 pellets female^{-1} d^{-1} (n = 309) for the 15 d experiment. Pellet production increased significantly with the dinoflagellate diet, with a mean of 27.1 ± 20.7 pellets female^{-1} d^{-1} (n = 71) for the 10 d experiment (Mann-Whitney *U*-test, $p < 0.0001$). Egg cannibalism was almost zero over the 25 d period.

DISCUSSION

Our feeding experiments showed that *Calanus helgolandicus* females were non-selective feeders, ingesting either diatoms or dinoflagellates at the same rates in proportion to their concentrations in the diets (Fig. 1, Table 3). Previous examinations of faecal pellets had already revealed that neither *C. helgolandicus* nor *C. finmarchicus* feed selectively on diatoms or dinoflagellates (Urban et al. 1992, Laabir et al. 1995b). Lee et al. (1999) also found that *Pseudocalanus newmani* con-

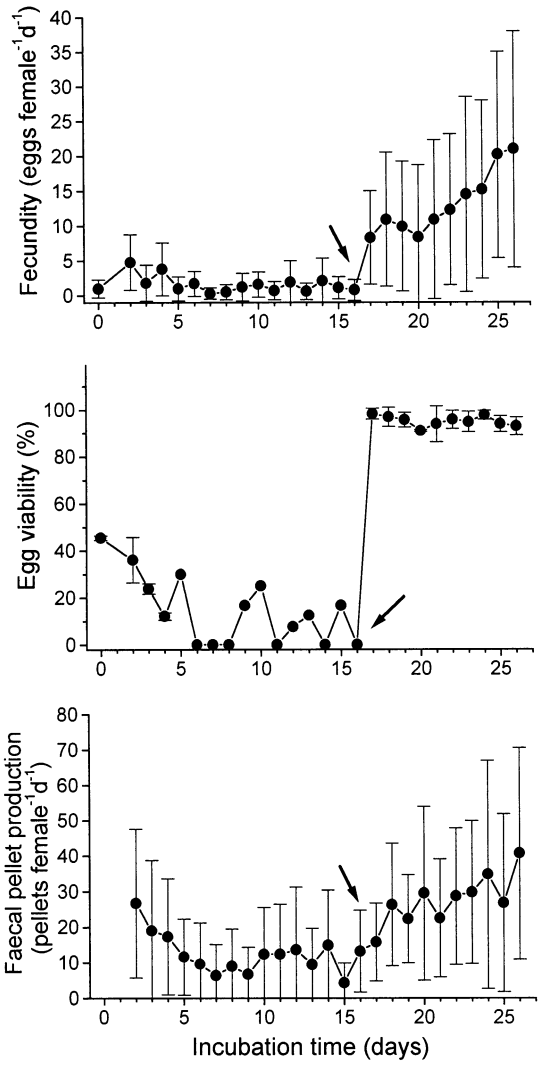

Fig. 6. *Calanus helgolandicus*. Responses of females to dietary shift. Initial food conditions were maintained for 15 d (single diet of diatom *Coscinodiscus curvatulus*: 1×10^4 cells ml^{-1}), and then (arrows) replaced by dinoflagellate *Gymnodinium sanguineum* (4×10^2 cells ml^{-1}). Day 0 = initial *in situ* rates. Data are means ± SD (n = 6, number of vials for fecundity; n = 6, number of vials for faecal pellet production; n = 30, number of chambers for egg viability)

sumes *Chaetoceros gracilis* and *Pavlova* sp. non-selectively in mixed-diet treatments. However, Meyer-Harms et al. (1999) reported that female *C. finmarchicus* positively selected dinoflagellates before and after a spring bloom, despite their minor importance in the natural phytoplankton population, whereas positive selection for diatoms occurred during the bloom. Our results (Figs. 3, 4 & 5, Table 5) also showed that *C. helgolandicus* cannibalise their own eggs at high phytoplankton concentrations. Species of *Calanus* are capable of carnivorous feeding (Landry 1981). Turner (1984) concluded that *Calanus* are mainly omnivorous, being primarily and broadly herbivorous, and shift to carnivory chiefly during periods of phytoplankton scarcity. Omnivory and egg cannibalism provide high nutritional diversity to *C. helgolandicus*, and this appears to lessen the deleterious effect of single-diatom diets on fecundity and egg viability in this genus (Figs. 2, 4 & 6, Table 5).

At what density can diatoms inhibit reproduction in copepods? In laboratory experiments testing several copepod species, diatoms within the 10^3 to 10^5 cells ml^{-1} range and even lower (10^2 cells ml^{-1}) affected fecundity and/or egg viability. *Thalassiosira nordenskioldii* was reported to reduce egg viability in *Calanus finmarchicus* by Ban et al. (1997), while Chaudron et al. (1996) found egg viability to be dependent on diatom in the laboratory experiments, and showed that the higher the density of diatoms and the longer they were ingested, the lower the copepod hatching success, and the lower the diatom concentration, the longer the time-lag inducing blockage of egg development. Under field conditions, Miralto et al. (1999) reported egg viability of *Acartia clausi* in the North Adriatic Sea to be drastically reduced during a diatom bloom (10^2 to 10^3 cells ml^{-1}) dominated by *Skeletonema costatum* and *Pseudonitzschia delicatissima*. In our fecundity experiments using mixed and single diets at a concentration of 10^3 diatoms ml^{-1} (Sdia and HdiaLdin; Table 5, Fig. 2), there was no strong inhibition of egg viability and fecundity, despite egg cannibalism. Inhibition rates were of the same order of magnitude as those recorded in the field by Laabir et al. (1995b), and were lower than that recorded offshore of Plymouth, England by Pond et al. (1996). Single-diatom diets of 10^4 cells ml^{-1} (Hdia; Table 5, Fig. 6) strongly inhibited both egg viability and fecundity, supporting the results of Chaudron et al. (1996).

Our fecundity experiments showed that with 10^4 diatoms ml^{-1} addition of the dinoflagellate *Gymnodinium sanguineum* and high egg cannibalism decreased the inhibition of egg viability and fecundity (HdiaLdin; Table 5), implying that the dinoflagellate and egg cannibalism moderated the deleterious effect of diatoms. Our experiments also showed that at diatom concentrations of $<10^4$ cells ml^{-1}, egg viability increased by 10.1% after the addition of a non-diatom dietary item such as *G. sanguineum* (Sdia and HdiaLdin; Table 5), suggesting that a dinoflagellate diet can improve egg viability. Our results further demonstrated that egg viability significantly increased at time of high egg cannibalism, regardless of dietary treatments, whereas fecundity was not affected (Fig. 5). These new results have revealed an unexpected effect of egg cannibalism on hatching success in copepods. In future, egg cannibalism should be taken into account

Table 5. *Calanus helgolandicus*. Summary of results of the 6 diet treatments, comparing mean fecundity and egg viability in relation to concentrations of diatom *Coscinodiscus curvatulus* and dinoflagellate *Gymnodinium sanguineum* in diets, and to egg cannibalism. Results are shown for 2 incubation methods: vial and wheel

Diet treatment	Hdia	HdiaLdin	Sdia	Sdia	HdiaLdin	HdiaLdin
Cell density in diet (cells ml^{-1})						
Coscinodiscus curvatulus	1×10^4	1×10^4	1.6×10^3	1.6×10^3	1.3×10^3	1.3×10^3
Gymnodinium sanguineum	0	1×10^2	0	0	2.1×10^2	2.1×10^2
Egg cannibalism (%)						
Mean ± SD	~0	68.0 ± 7.1	0.7 ± 1.3	39.8 ± 2.2	0.2 ± 0.9	46.9 ± 0.9
(n)	(162)	(6)	(188)	(42)	(167)	(36)
Significance (Student's *t*-test)	$p < 0.0001$		$p < 0.0001$		$p < 0.0001$	
Incubation						
Condition	Vial	Wheel	Vial	Wheel	Vial	Wheel
Duration (d)	6	6	7	7	6	6
Fecundity (eggs female^{-1} d^{-1})						
Mean ± SD	2.3 ± 3.2	19.1 ± 13.1	29.6 ± 12.9	32.1 ± 11.3	38.4 ± 15.5	41.8 ± 11.2
(n)	(163)	(30)	(188)	(42)	167	36
Significance (Mann-Whitney *U*-test)	$p < 0.0001$		$p > 0.05$ ns		$p > 0.05$ n.s.	
Egg viability (%)						
Mean ± SD	14.8 ± 7.1	86.8 ± 7.8	67.9 ± 2.1	98.4 ± 1.9	75.5 ± 1.7	97.9 ± 2.1
(n)	(10)	(50)	(156)	(105)	142	116
Significance (Student's *t*-test)	$p < 0.0001$		$p < 0.0001$		$p < 0.0001$	
Diatom inhibition						
Fecundity	Strong	Weak	No	No	No	No
Egg viability	Strong	No	Weak	No	Weak	No

in experimental studies concerning the egg viability of copepods. In contrast to the egg cannibalism recorded in laboratory studies, egg cannibalism by copepods is not well known in the field (Kiørboe et al. 1988, Liang et al. 1994, Peterson & Kimmerer 1994). It is likely that some of the eggs sink through the water column, are dispersed by water mixing and currents, or consumed by other predators (Cabal et al. 1997, Tang et al. 1998), thus escaping predation by adults of their own species. The possible coupling of egg predation, cannibalism and egg viability may have several demographic implications that have yet to be addressed.

In what way could egg cannibalism enhance egg viability in *Calanus helgolandicus*? First, eggs constitute a nutritional supplement, and second ingestion of non-diatom foods moderates the effect of the deleterious diatom compounds. Eggs of *C. helgolandicus* are rich in nutrients, with triacylglycerol as the main lipid class (Lee et al. 1972, Gatten et al. 1980), important amounts of the saturated fatty acid 16:0 and the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) (Pond et al. 1996), 4.8 to 13.1% carbohydrate (% body weight) (Guisande & Harris 1995), and 53 ± 5 ng free amino acids per egg (Laabir et al. 1999). They could therefore appear to constitute a good source of food, capable of enhancing egg viability. In addition, egg cannibalism (as well as dinoflagellates) may reduce or delay the accumulation of diatom inhibitors during oogenesis, assuming that these inhibitors can be partially metabolised by digestive enzymes, as indicated by Chaudron et al. (1996).

The present results clarify our conception of the mechanism of diatoms inhibition of copepod reproduction. Egg mortality is proportional to both diatom concentration and copepod ingestion rate. There is clearly a critical threshold concentration of diatoms (10^2 to 10^3 cells ml^{-1} in the present study), which may be masked by ambient food diversity (Table 5). Below this threshold, egg mortality cannot be explained by the diatom-inhibition hypothesis of Ban et al. (1997), but is more probably related to the nutritional value of available food (Jónasdóttir & Kiørboe 1996, Pond et al. 1996); above this threshold, egg mortality can be explained by diatom inhibition. We hypothesize that this critical threshold concentration is species-specific in both copepods and diatoms. The degree of inhibition appears to be diatom-density-dependent (Chaudron et al. 1996, present study). The reproductive response can be moderated when females consume a high variety of food, including dinoflagellates and copepod eggs.

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