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Influence of diatoms on copepod reproduction. I. Field and laboratory observations related to *Calanus helgolandicus* egg production

S. A. Poulet[1,*], T. Wichard[2], J. B. Ledoux[1], B. Lebreton[1], J. Marchetti[1], C. Dancie[1], D. Bonnet[3], A. Cueff[1], P. Morin[1], G. Pohnert[2,4]

[1]Station Biologique de Roscoff, CNRS, INSU, UPMC Paris VI, Unité Mer et Santé, Roscoff 29682, France
[2]Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany
[3]Plymouth Marine Laboratory (PML), The Hoe, Prospect Place, Plymouth PL1 3DH, UK

[4]*Present address:* Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Bâtiment BCH, 1015 Lausanne, Switzerland

ABSTRACT: Egg production rates (EPR) by *Calanus helgolandicus* females were investigated with specimens sampled weekly, from April to November 2003 and from March to October 2004, at a station located in the English Channel off Roscoff. Comparison of results between 1994, 2003 and 2004 showed that *C. helgolandicus* was a late spawner in 1994 and became an early spawner in 2003 and 2004. In all cases high variations in EPR were observed, which could not be correlated to phytoplankton biomass, expressed as diatom, chlorophyll *a*, particulate carbon and nitrogen concentrations in 2003 and 2004. Neither were they correlated to food quality, expressed as C/N ratio. To explain this mismatch between EPR and food concentration, a series of mixed phytoplankton species dominated by diatoms (≥11 µm filtrate representing natural diatom assemblages: NDA) and 7 single diatom species, all occurring during blooms in the field, were assayed as diets with *C. helgolandicus* females. Ingestion of diatoms by females was estimated by faecal pellet production rates and complementary scanning electron microscopy examinations of diatom remains in pellets. Depending on diatom species in diets, EPR was either increased or depressed 2 to 3 d after food uptake by females had started. The EPR decrease was reversible, when diatom diets were replaced by the dinoflagellate *Prorocentrum minimum*. This effect was also observed when females were transferred to natural phytoplankton populations from the English coast of the Channel close to Plymouth, where food composition in the field differed compared to that off Roscoff. EPR ceased completely when the concentration of NDA diets was artificially increased, but recovered after a shift to a dinoflagellate diet. These results indicate that phytoplankton dominated by diatoms can impair *C. helgolandicus* egg production in the field. This effect was not related to the production of polyunsaturated aldehydes by diatoms. Limitations due to unidentified essential compounds not provided by the metabolism of diatoms, or unknown diatom-derived toxins, were probably involved.

KEY WORDS: Copepod · Diatom · Egg production · English Channel

INTRODUCTION

Reproduction success of copepods is influenced by many factors, including temperature, food concentration and quality, presence of chemically mediating factors, as well as the gonad maturity of adult females (see review by Mauchline 1998, Niehoff 2003, Hassett 2004, Irigoien 2004). Egg production and hatching rates, as well as naupliar development, are other important factors contributing to the overall population recruitment success. Several recent studies have described the negative effects of diatom-rich diets on

*Email: poulet@sb-roscoff.fr

the hatching success and larval development of copepods, mediated by deleterious polyunsaturated aldehydes (Miralto et al. 1999, Pohnert et al. 2002, Poulet et al. 2003, Adolph et al. 2004, Ianora et al. 2004), but egg production can also vary significantly depending on diet. Patterns of variability in egg production conform to changes in dinoflagellate and microzooplankton biomass (Kleppel et al. 1991), as well as to phytoplankton blooms mostly dominated by diatoms (Runge & Plourde 1996, Meyer- Harms et al. 1999). The varying influence of different algae on these stages of ontogenesis has been documented by several laboratory studies (Ban et al. 1997, Pohnert et al. 2002). Since copepod diets are temporally strongly variable under field conditions, changes in both the egg production and hatching rates can be expected. This point is particularly relevant during the breeding season of *Calanus helgolandicus* in the English Channel, which occurs from early spring to late fall (Pond et al. 1996, Laabir et al. 1998, Irigoien et al. 2000a). In this area, the reproductive season coincides with the succession of diatom blooms, as well as seasonal shifting in species and cell abundances (Grall 1972a,b, Holligan & Harbour 1977, Martin-Jézéquel 1983, Irigoien et al. 2000a,c). Besides the abundant diatom genera *Chaetoceros*, *Rhizosolenia* and *Thalassiosira*, which have been documented in French coastal waters (Beliaeff et al. 2001), *Guinardia* comes to the fore during the summer in coastal waters off Roscoff (Grall 1972b, present work), whereas other genera like *Leptocylindricus* are missing. The complex roles of diatom diets on copepod reproduction have been discussed intensively among the research community concerned with oceanographic plankton. This is above all due to the fact that most evidence showing that certain diatoms can decrease the reproduction capacity of copepods is based on laboratory experiments (see e.g. Poulet et al. 1994, 1995, Ban et al. 1997, Ianora et al. 2004). It has been claimed that these data do not reflect natural diet characteristics, since high diatom concentrations and single-species diets would not be relevant to field conditions, even under bloom situations (Irigoien et al. 2002, Paffenhöfer et al. 2005). Indeed, field work showed that mean per capita egg production increases during spring/summer phytoplankton blooms, but single values over the year oscillate (Niehoff et al. 1999, Irigoien et al. 2000a,b,c).

This observation was also reported from an earlier series of field estimates performed on the British and French sides of the Western English Channel, off Plymouth and Roscoff (Pond et al. 1996, Laabir et al. 1998), and recently in the Eastern English Channel (Devreker et al. 2005).

In bioassays conducted under laboratory conditions, Ban et al. (1997) found, with combinations of different copepod and diatom species, that certain single-species diatom diets can arrest egg production and/or inhibit hatching. Up to now, it has not been possible to elucidate the reason(s) for the EPR (egg production rate) decrease. It might be caused by low female gonad maturation (Niehoff et al. 2002), by toxic diatom-derived aldehydes (Miralto et al. 1999, Pohnert et al. 2002, Poulet et al. 2003, Romano et al. 2003, Adolph et al. 2004, Ianora et al. 2004), or by the low nutritional value of diatoms (Kleppel et al. 1991, Jónasdóttir et al. 1998, Klein Breteler et al. 1999, Hassett 2004, Jones & Flynn 2005). Neither of these concepts can be seen as exclusively valid, since it is difficult to separate the involved factors in laboratory and field assays. But, other factors also have to be taken into account if copepod population success is to be considered. Ohman et al. (2004), among others, have argued that the decrease of a *Calanus* spp. population in the field is exclusively caused by predation and egg cannibalism. Demographic oscillations might thus be caused by intense predation on eggs and juveniles (Ohman et al. 2004). Mortality of nauplii, caused by unsaturated aldehydes like 2,4-decadienal, might also be a limiting factor (Poulet et al. 2003, Ianora et al. 2004), as well as the egg production deficit, which is described in the present paper.

From 1999 to 2002, we observed that the egg production and hatching rates of *Calanus helgolandicus* were surprisingly abnormal from spring to fall at Roscoff (S. A. Poulet unpubl. data), compared to a previous survey (Laabir et al. 1998). Earlier evidence existed on the negative impact of diatom-rich diets on *C. helgolandicus* reproduction (Laabir et al. 1995a, Poulet et al. 1995), but no follow-up studies had been done to address this aspect systematically for the Roscoff area. Thus, we decided to examine in detail the effects of diatoms on the EPR of *C. helgolandicus*. A field survey was carried out during 2003 and 2004 to compare data with the EPR values observed in 1994 (Laabir et al. 1998). We used protocols (Laabir et al. 1995b, 1998), originally developed for the monitoring of EPR in laboratory investigations when feeding natural phytoplankton or single diatoms, to identify the specific influence of different diatom species on *C. helgolandicus* egg production.

This contribution describes the negative influence of diatom-rich phytoplankton on the egg production of *Calanus helgolandicus* in coastal waters off Roscoff, and is the first part of a series of experiments performed to develop an improved understanding of the very variable reproductive success of calanoids. Also based on the data from this survey (2003 and 2004), the effects of polyunsaturated aldehydes and of essential fatty acids on reproductive success will be described in a separate paper by T. Wichard et al. (unpubl. data)

and the morphology and histology of gonads related to EPR decreases among *C. helgolandicus* females will be reported by S. A. Poulet et al. (unpubl. data).

MATERIALS AND METHODS

Pseudo-field experiments. Field estimates from 1994 of the *Calanus helgolandicus* female EPR prevailing at Roscoff were taken from Laabir et al. (1998). The same methods were used in the 2003 and 2004 experiments described here. *C. helgolandicus* specimens were collected several times a week off Roscoff (48°45′N and 3°58′W, in the Western English Channel, France), during spring/summer surveys, by towing a 500 µm mesh plankton net obliquely from 20 to 0 m. Samples were transported within 1 to 2 h to the laboratory, where adult, sexually mature females (12 to 30 in total) were sorted for each experiment and were incubated individually for 24 h in dishes containing 100 ml of 0.22 µm filtered seawater, in order to estimate initial EPR, corresponding to field conditions (Laabir et al. 1995a).

Diatom isolation and cultivation. All tested diatoms were successfully isolated from Roscoff plankton samples from March to August in 2004 and cultured in filtered seawater enriched with K-medium at 14°C with a 14:10 h light:dark cycle. Isolation, purification and culture of these key diatom species were achieved according to standard methods (Guillard & Ryther 1962, Keller et al. 1987). The diatoms were identified after Drebes (1974) and Hasle et al. (1996). Three of the tested isolates (TR, GD and RS) are responsible for annual local spring and summer blooms that have been observed in Roscoff waters for decades (Grall 1972a,b, Martin-Jézéquel 1983).

Experiments with single diatom species. To test the effect of single-species diets on EPR at various times during the breeding season, single females were transferred after 24 h to dishes with 80 ml filtered seawater, enriched with 20 ml of diatom culture. The 5 different diatom diets used on different dates in 2004 (Fig. 1C: O) were TR (*Thalassiosira rotula*), RS (*Rhizosolenia setigera*), OR (*Odontella regia*), GD (*Guinardia delicatula:* ex. *Rhizosolenia delicatula*) and GS (*Guinardia striata*), set at final cell densities in the incubators corresponding to 2.5×10^4, 8.25×10^3, 0.6×10^3, 6.58×10^4 and 4×10^4 cells ml^{-1}, respectively. These cell densities were based on the concentration of algae in each culture reaching the exponential growth phase before harvesting. The algal culture was renewed daily during the incubation period, which did not exceed 8 d. EPR values were estimated following the techniques described by Laabir et al. (1995a). In 2003, temperatures during incubations were set at 14 ± 2°C before June and subsequently raised to 20 ± 3°C. In 2004, temperature was set at 14 ± 1°C during all incubations. In 1994, during 2 different periods (Fig. 1A: O), *Calanus helgolandicus* females were assayed with 2 diatom species: TW (*Thalassiosira weissflogii*, Strain RCC 76) and SC (*Skeletonema costatum*, Strain RCC 70) (Laabir et al. 1998).

Experiments with diatom-enriched assemblages. Samples of mixed species in natural diatom assemblages (NDA), collected in 2003 and in 2004 at the same station as the copepod females, were used to test their effect on EPR. Subsurface (1 to 2 m depth) seawater samples were gently filtered by gravity through a filtering column formed of 2 Sartorius filtering funnels, the top one supporting a 350 µm mesh, and the one below, a 11 µm mesh Nitex sieve (Millipore, 45 mm diameter). The top one was used to remove zooplankton and large particles, while the second was used to collect diatoms. Samples corresponding to 200 ml seawater were collected on the 11 µm mesh and re-suspended in incubators containing 100 ml filtered seawater (Millipore, 0.22 µm). Thus, the final concentration of NDA diets in each incubator was approximately 2 times higher than the initial concentration in nature (Table 1). Untreated seawater samples were preserved with Lugol's solution to allow identification of the diatom species in the NDA diets, the ratio between microphytoplankton (<11 µm)/diatom numbers in seawater samples (parallel to NDA 2–NDA 4) and to estimate the cell numbers in the incubators (Table 1). Complementary tests achieved with other NDA diets, set at concentrations corresponding to 2, 10 and 50 times the diatom density in the field, were carried out on several occasions. Besides EPR values in controls with *Prorocentrum minimum* (PM), EPR values with *Calanus helgolandicus* fed on naturally occurring phytoplankton in the field and with NDA diets were compared once during the summer 2003 (see Fig. 4). Cell densities in the enriched NDA samples were comparable to those tested with single-species diets (range: 10^2 to 10^4 cells ml^{-1}). Because copepods can be food limited, this approach allowed us to temporarily increase food biomass and, thus, to incubate females above the food-limitation threshold. New seawater stocks were collected twice a week off Roscoff, at the same station as females, and were used to renew NDA diets every day during the entire incubation period. Particles in these seawater samples, kept in 20 l transparent bottles in the same room as the feeding females, were re-suspended by hand twice a day. In order to obtain a representative spectrum of diatom species occurring successively during the spring/summer blooms, samples for NDA 1, 2, 3 & 4 diets were collected from April to August 2003, and samples for NDA 5, 6 & 7

Table 1. Upper table: Relative proportion and abundance of diatoms in seawater samples collected at a fixed station in the Roscoff coastal zone and used as natural diatom species assemblage (NDA) diets assayed with *Calanus helgolandicus* females in 7 different periods during their breeding season in 2003-2004 (x: species [GD, GS, RS, TR] belonging to 3 genera that were isolated during spring/summer blooms and later used to feed copepods with each single diatom species in diets [same as in Fig. 3]). Lower table: Concentrations of chlorophyll *a*, particulate carbon (POC) and nitrogen (PON) and C/N ratios measured at the same station and same date of sampling as *Calanus* females. Ratio between numbers of microorganisms (<11 µm) and diatoms in preserved seawater samples corresponding to NDA 2, 3 & 4 (see Fig. 4)

Diet:	NDA 1	NDA 2	NDA 3	NDA 4	NDA 5	NDA 6	NDA 7
Date of test:	23/04/ 2003 (%)	07/07/ 2003 (%)	17/07/ 2003 (%)	18/08/ 2003 (%)	23/03/ 2004 (%)	29/03/ 2004 (%)	25/05/ 2004 (%)
Diatom							
Chaetoceros	9.9	13.33	0	50.28	0	7.8	0
Coccinodiscus	0.7	0	0	0	4.5	0	0
Cylindrotheca	0.1	0	0	0	0	0	0
Ditylum	0.3	0	0	0	15.7	7.3	0
Guinardia (x)	31.7	54.3	90.5	20.9	0	0	10.8
Lauderia	8.6	0	0	0	0	0	0
Navicula	0.5	0.32	1.25	0.92	4.5	0.5	0
Nitzschia	0	0	0	0	0		27.3
Pleurosigma	0.1	0	0	0	0	0	0
Pseudo-nitzschia	0.4	0	0	0	11.2	0	0
Rhizosolenia (x)	1.6	16.0	3.8	0.4	12.4	9.1	49.8
Skeletonema	0	8	4.42	20.93	0	0	0
Thalassionema	0.3	8	0	6.52	3.9	69.9	0
Thalassiosira (x)	46.0	0	0	0	36	0.9	12
Total (cells ml^{-1} seawater)	61.28	140.46	73.96	24.84	10.3	12.67	74.96
Diatom abundance in the incubators (cells ml^{-1})	122	281	147.92	49.7	20.6	25.34	149.92
Concentration (µg l^{-1})							
Chlorophyll *a*	1.66	2.05	1.36	1.18	0.8	0.71	1.18
Carbon (POC)	112.8	134.6	113.12	149.2	108.7	100.9	134.9
Nitrogen (PON)	17.9	20.7	17.45	22.7	12.8	13	21.7
C/N ratio	6.3	6.5	6.48	6.57	8.49	7.76	6.21
Ratio of cell numbers							
(in fraction <11 µm)/ diatoms		0.07	0.2	1.18			

were collected from March to May 2004 (see dates of experiment start, Fig. 1, Table 1). According to Grall (1972a), diatoms occurring in Roscoff coastal waters are large (≥8 µm). In agreement with this finding, microscopic observations of NDA diets retained on the 11 µm mesh confirmed that they were dominated by diatoms. We assumed that these NDA diets resembled natural assemblages of diatoms, which the *C. helgolandicus* females should have encountered in the field before capture.

During both 2003 and 2004, the dinoflagellate *Prorocentrum minimum* was used as a control diet (Poulet et al. 1994), at concentrations corresponding to 10^3 to 10^4 cells ml^{-1} in the incubators. The growth conditions for this alga have been described earlier (Poulet et al. 1994). This non-diatom diet was often used to test the reproductive capacity of *Calanus helgolandicus* during the season, thus verifying if low EPR values were linked to sterile females. PM was also used to re-initiate egg production when it had collapsed in the field, or following laboratory treatments with single diatom species or with NDA diets.

For the NDA 1 to NDA 7 diets, the dominant diatom species and dates of the bioassays are given in Table 1. Each bioassay with NDA 1 to NDA 7 was conducted once with a different cohort of carefully selected females (Fig. 1B,C), with undamaged antenna, swimming legs and furca, as well as with a well-maturated genital segment. Bioassays with OR, TR, RS, GD and GS were also performed with carefully selected cohorts of fertile *Calanus helgolandicus* females in 2004 (Fig. 1C).

'Copepod-transfer' experiments. To elucidate the role of naturally occurring diatoms on the female egg production of *Calanus helgolandicus*, 2 parallel incubations were performed at 2 close dates in 2003. While egg production was monitored in Plymouth from 28 August, additional females collected off Plymouth were shipped to Roscoff and incubated there for 3 d in local, untreated seawater, with diatom assemblages resembling one of the NDA 3 or 5 diets (Test T1, Tables 1 & 2). Similarly, on 1 September, females used for the weekly egg production field survey in Roscoff were shipped to Plymouth and incubated there for 5 d with a local non-enriched field diet (Test T2, Table 2). In both assays, following their arrival at Roscoff or Plymouth, females were acclimated 24 h in filtered seawater (Millipore, 0.22 µm) before addition of local phytoplankton food.

Faecal pellet analysis. For each type of diet, ingestion of algal cells by individual copepods was estimated indirectly, through counts of the daily faecal pellet production in each incubator. Moreover, with PM, TR, RS, GD, NDA 1 and NDA 3, faecal pellets were collected daily, during the assays for SEM observations, to verify if the diatoms, identified by their frustule remains, were ingested. The methods used for faecal pellet preparation, SEM examination and documentation have previously been described by Laabir et al. (1995b).

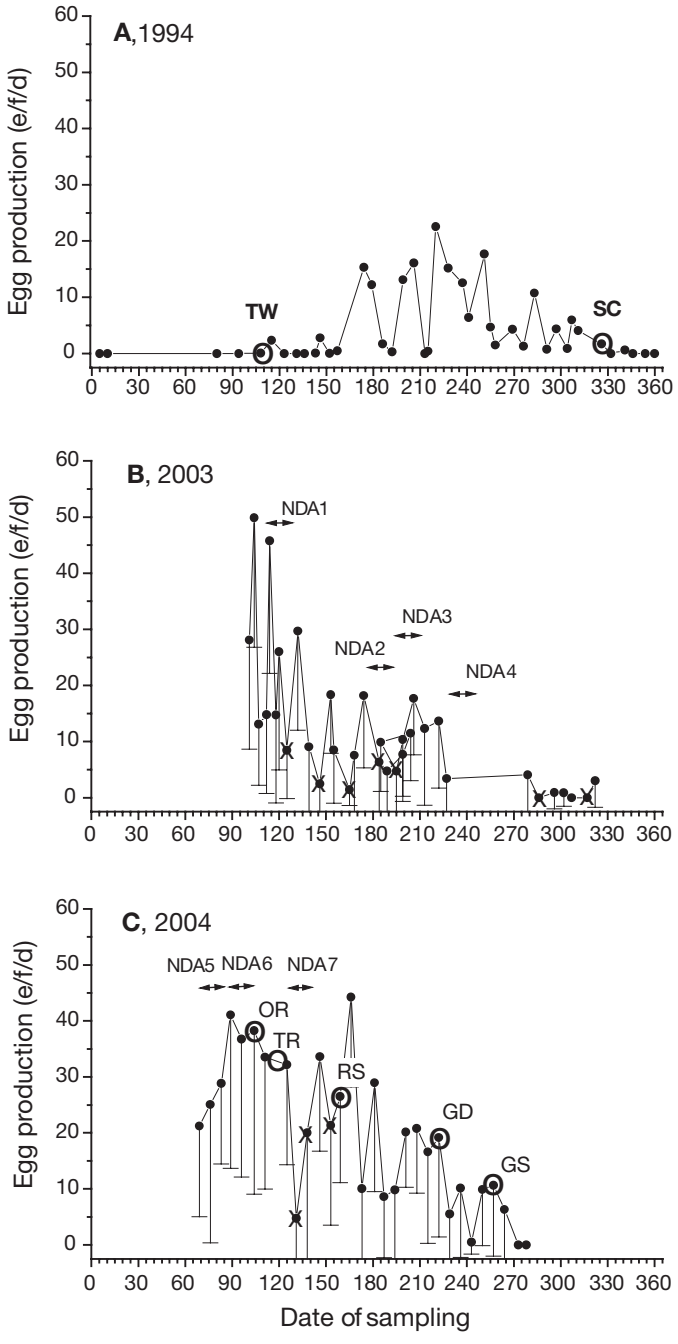


Fig. 1. *Calanus helgolandicus*. Seasonal variation of egg production rates (EPR) in Roscoff coastal waters in (A) 1994, (B) 2003 and (C) 2004. Test periods with natural diatom species assemblages (NDAs, see Fig. 3) are indicated with horizontal arrows. Copepod cohorts fed with NDA 1 to NDA 7 were isolated within this time span. Dates on which EPR induction experiments were performed with single-diatom diets (1994: *Thalassiosira weissflogii*, Strain RCC 76 [TW], *Skeletonema costatum*, Strain RCC 70) [SC]; 2004: *Odontella regia* [OR], *Thalassiosira rotula* [TR], *Rhizosolenia setigera* [RS], *Guinardia delicatula:* ex. *Rhizosolenia delicatula* [GD], *Guinardia striata* [GS]) are indicated with open circles, while dinoflagellates as controls (PM in 2003 and 2004) are indicated with X. Values for 2003 and 2004 are means – SD (bars)

Particulate organic matter and phytoplankton biomass. Every week during 2003 and 2004, untreated subsurface seawater samples were collected at a fixed station off Roscoff at the same time as the *Calanus helgolandicus* females and NDA samples used for the feeding tests. One fraction (250 ml) was preserved with Lugol's solution to determine and evaluate the species and the concentrations of diatoms. Chlorophyll *a* (chl *a*) concentrations were determined by filtering 3 replicate samples (1 l) of seawater from 2 and 60 m depth, collected every 2 wk at the same site as the zooplankton collections, onto GF/F filters and freezing (–30°C) samples for subsequent analyses using a Turner Design fluorometer, according to Yentsch & Menzel's method (1963) and using Lorenzen's (1966) equation. Three further subsamples (1 l) were filtered onto preashed GF/F filters to determine particulate carbon and nitrogen concentrations. Filters were stored at –20°C and then oven dried at 50°C prior to analysis with a Thermo Finnigan CE flash 112 elemental CHN analyser. Only data corresponding to subsurface samples were used in Fig. 2.

RESULTS

The seasonal EPR values of *Calanus helgolandicus* females were extremely variable at Roscoff (Fig. 1). In 1994, peaks of maximum EPR, around 20 to 30 eggs female^{-1} d^{-1}, occurred between the end of May and early August. The rest of the year, lower values were observed. In early spring, autumn and winter, egg production was <10 eggs female^{-1} d^{-1} (Laabir et al. 1998; Fig. 1A). In 2003 and 2004, EPR was evaluated only from March/April to October/November. In contrast to the previous survey, the EPR field estimates were rather different in 2003 and 2004 (Fig. 1B,C). High egg production, with maxima ranging between 30 and 50 eggs female^{-1} d^{-1}, was observed from March to June. The EPR was considerably lower from the end of summer to early fall, with values between 0 and 20 eggs female^{-1} d^{-1} and several drops to 0–5 eggs female^{-1} d^{-1} from August to November. Each year, major oscillations were observed in spring and summer. Mean EPR values (eggs female^{-1} d^{-1} ± SD) were computed for each year from May to November and compared. EPRs were 4.6 ± 4.4 in 1994, 11.9 ± 12 in 2003 and 18.56 ± 13 in 2004. These means were significantly different (*t*-test between years: $t \leq 7.46$, $t > t_\alpha = 1.99$, $\alpha = 0.05$, $82 < df < 76$). In 1994, egg production was linearly correlated to phytoplankton biomass determined in the field in terms of chl *a* and particulate organic carbon and nitrogen concentrations (Laabir et al. 1998). In 2003 and 2004, EPR was not significantly correlated to these environmental food factors, to

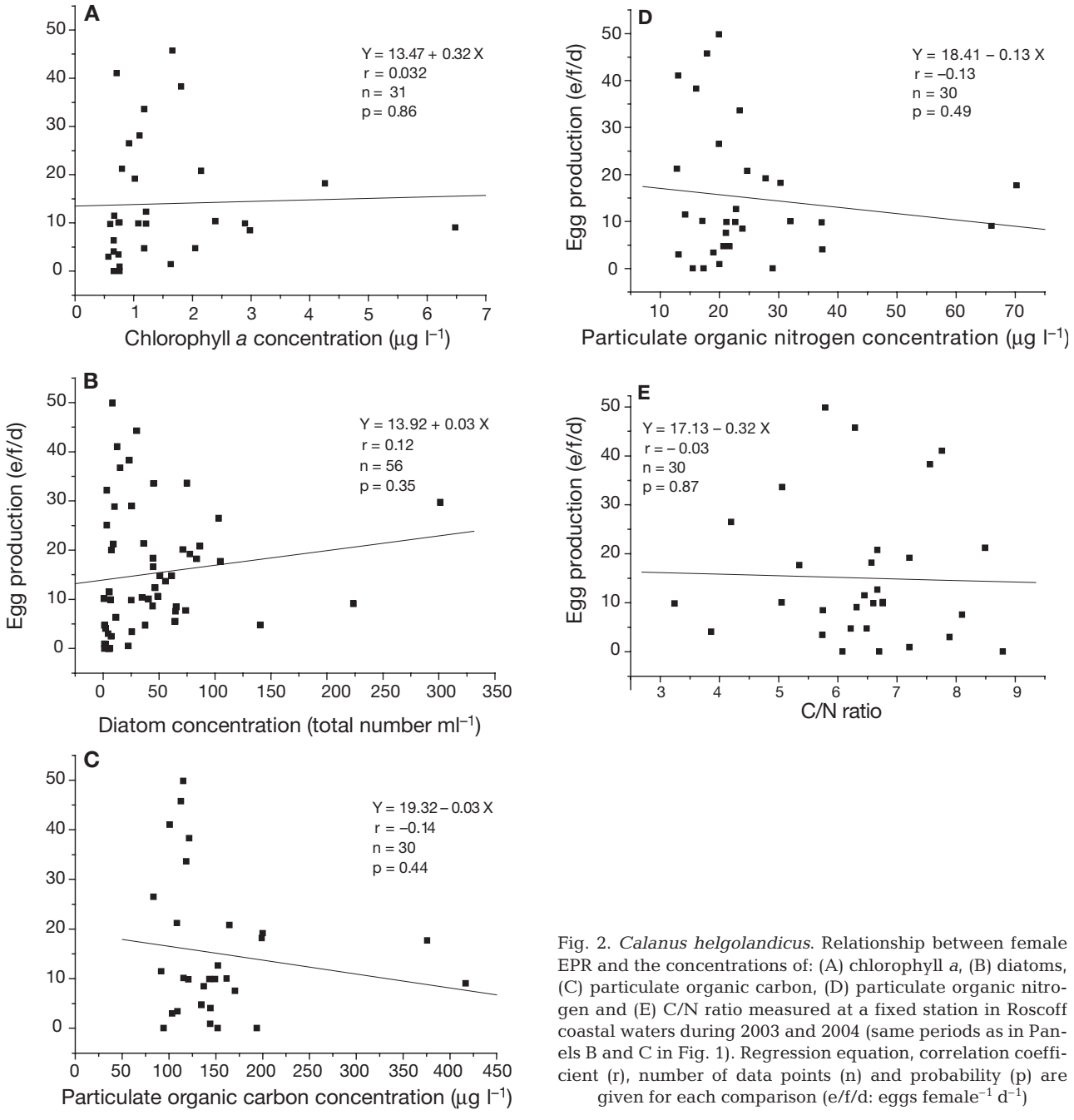


Fig. 2. *Calanus helgolandicus*. Relationship between female EPR and the concentrations of: (A) chlorophyll *a*, (B) diatoms, (C) particulate organic carbon, (D) particulate organic nitrogen and (E) C/N ratio measured at a fixed station in Roscoff coastal waters during 2003 and 2004 (same periods as in Panels B and C in Fig. 1). Regression equation, correlation coefficient (r), number of data points (n) and probability (p) are given for each comparison (e/f/d: eggs female^{-1} d^{-1})

diatom concentrations, or to the C/N ratio, an index of food quality (Fig. 2).

To test the hypothesis that natural assemblages of diatoms can reduce egg production, batches of *Calanus helgolandicus* females belonging to the same cohorts as those used to estimate EPR in the field were exposed to assemblages of mixed diatom species (NDA 1, 2, 3 & 4 in 2003 and NDA 5, 6 & 7 in 2004, see Fig. 1B,C) or to different diets of single diatom species in cultures (TW & SC: see Fig. 1A; TR, RS, OR, GD & GS: see Fig. 1C). A non-diatom diet (PM) was applied as control food. The laboratory tests were performed randomly at times when low or high EPR values had been observed in the field (Fig. 1). Depending on the type of diet, the EPR varied greatly during the incubations. With the natural assemblages of mixed diatom species (NDA 1 to NDA 7; Table 1), the daily mean EPR decreased with time, from 12 to 45 eggs female^{-1} d^{-1} to almost zero, after only 2 or 3 d of incubation (Fig. 3, upper panels). Statistical comparisons of the EPR values between Day 1 and the

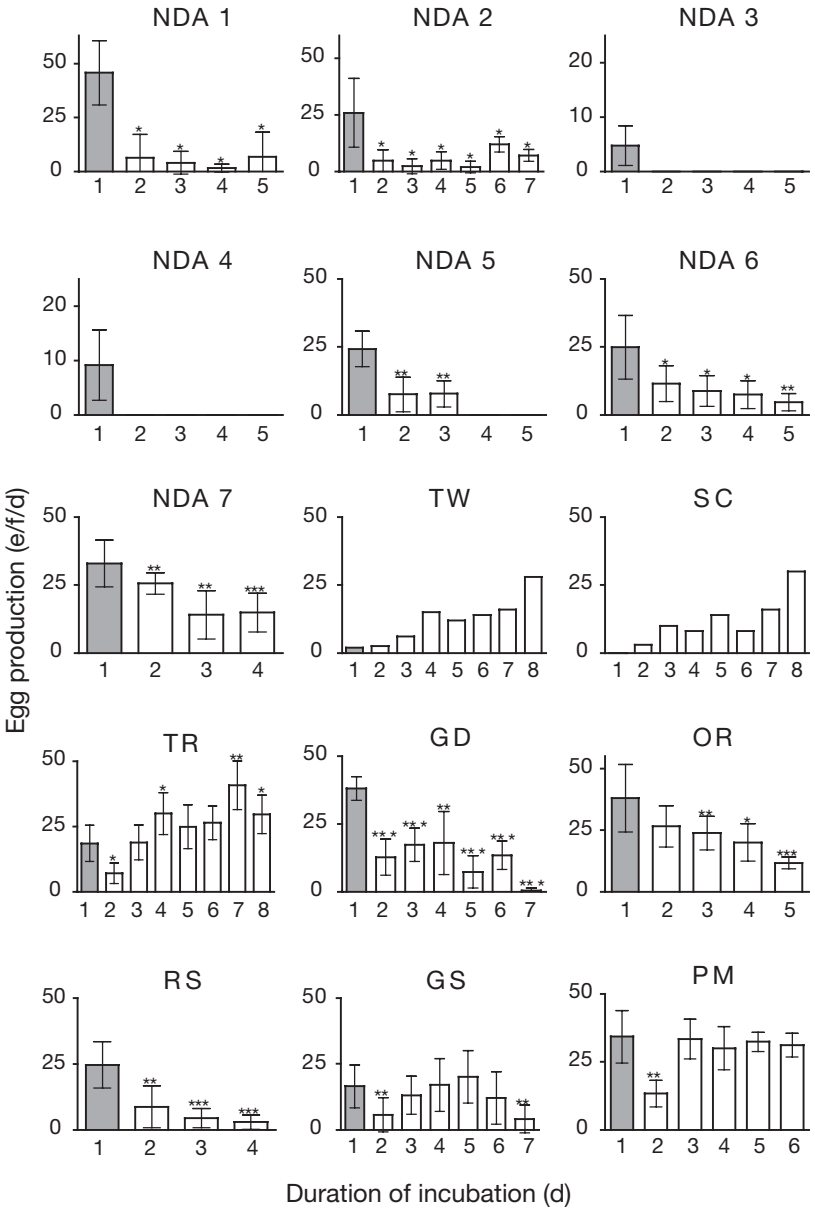


Fig. 3. *Calanus helgolandicus*. Egg production influenced by naturally occurring diatoms. Upper panels: mixed species in NDA 1 to NDA 7 (see composition and abundance of diatoms and corresponding chlorophyll *a*, particulate organic carbon and nitrogen concentrations and C/N ratios in Table 1). Middle and lower panels: isolated species, which were used for feeding experiments. Lower right-hand panel: the non-diatom diet (PM) used as control food. Values on Day 1 (NDA 1 to NDA 7, TW and PM) correspond to initial mean EPR of females estimated in filtered seawater, reflecting EPR in the field (Fig. 1A–C). Values are means ±SD (bars) measured daily in batches of 12 to 20 females per assay. TW and SC were assayed in 1994. NDA 1 to NDA 4 diets were assayed in 2003. NDA 5 to NDA 7 diets were tested in 2004, as well as TR, GD, OR, RS and GS. Error bars based on mean ± 95% CI. Differences of EPR between the initial values on Day 1 (grey bars) and those on the following days with experimental diets were tested with the nonparametric Wilcoxon signed-rank test (significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Note: this test could not be achieved with the daily mean values for TW and SC borrowed from Laabir et al. (1998), because single daily EPR values for each female in each batch were not available

following days during incubation with NDA 1 to NDA 7 diets indicated that mixed diatoms in NDAs had a significantly unfavourable effect on copepod egg production (Fig. 3, upper panels; non-parametric Wilcoxon rank-signed test, $p < 0.05$). Results in Fig. 4 compare, for the same period (August to September 2003), EPR values between *Calanus helgolandicus* females in nature (field diet) and in bioassays using enriched diatom assemblages (NDA diets) and dinoflagellate cells in controls (PM diet). They showed that EPR values in nature were always above those found with NDA diets, and generally below the values obtained with PM diets. When the biomass of NDA 4 was artificially increased to 2, 10, or 50 times its concentration in the field, the EPR was totally depressed from Day 2 to 5 (Fig. 5). These results support the idea that food quantity was not the limiting factor. With diets of the isolates RS, GD, GS (major summer bloom-forming diatoms) and OR, the EPR values decreased significantly in comparison to initial values on Day 1 as well (Fig. 3; non-parametric Wilcoxon rank-signed test, $p < 0.05$ to 0.001). The diatoms TR, TW and SC induced an EPR increase with time in comparison to the initial values measured on Day 1 (Fig. 3, middle panels). The favourable effect of PM on *C. helgolandicus* reproduction was observed several times over the year in 2003 and 2004 (Fig. 1B,C, cross-labelled data points; and Fig. 3, lower right-hand panel). The same effect has been recognised in other studies (Poulet et al. 1994, Laabir et al. 1995b, Uye 1996, Pohnert et al. 2002). These bioassays suggest that several diatom species exerted different influences on EPR, when offered to *C. helgolandicus* at concentrations close or above bloom conditions. In May 2003, at times when the EPR was around 10 eggs female^{-1} d^{-1} in the field (Fig. 1, e.g. Day 1 in Fig. 6), batches of 20 females, incubated individually under the same conditions, were fed alternately with NDA (1.6×10^2 cells ml^{-1}) and PM (10^4 cells ml^{-1}) diets, during a 12 d incubation period. Egg production was quickly depressed due to the adverse effect of

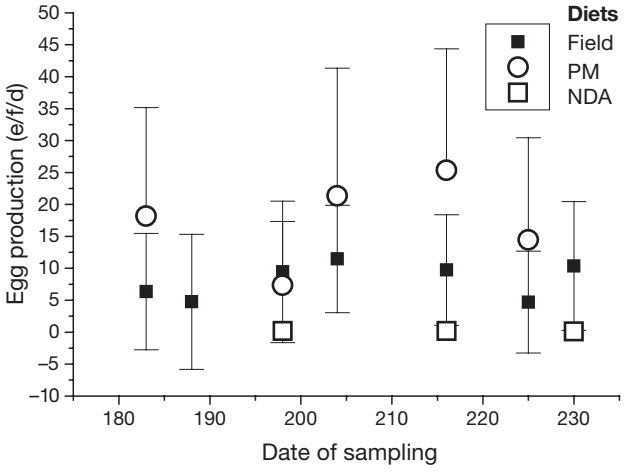


Fig. 4. *Calanus helgolandicus*. Comparison of EPR responses of females to field, PM and NDA diets in July to August 2003. See details in Table 1 for the type of diets, concentration of diatoms and ratio between numbers of microorganisms (<11 µm fraction) and diatoms in seawater-preserved samples corresponding to NDAs 2, 3 & 4

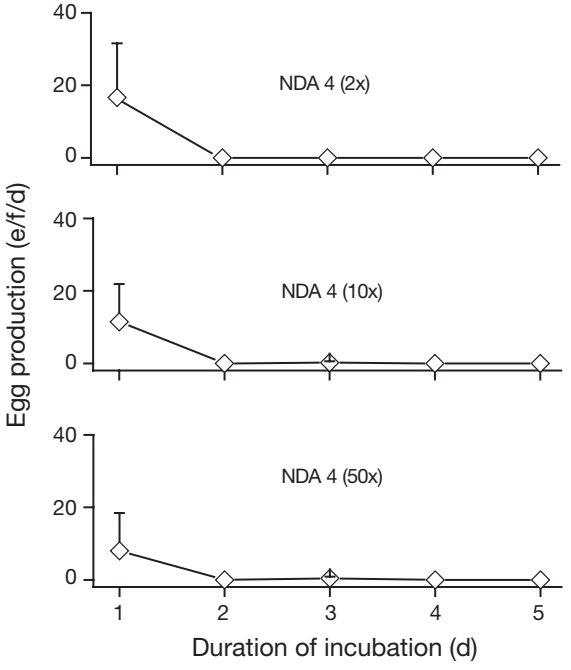


Fig. 5. *Calanus helgolandicus*. EPR responses of females fed the same type of naturally occurring diatoms described for NDA 4 and enriched to 2, 10 and 50 times the field concentration (see Table 1). Values on Day 1 correspond to initial mean EPRs of females in 3 cohorts (10 to 12 females per cohort), belonging to the same population estimated in filtered seawater and reflecting EPR in the field (Fig. 1B). Values are means + SD (bars)

an NDA diet very similar to NDA 1 (Table 1). After the shift of diets, the EPR changed dramatically (Fig. 6), i.e. positive effects with PM and negative effects with NDA were observed repeatedly. The arrest of egg production by NDA was reversible, following a 2 to 3 d period of continuous feeding with the PM diet.

In order to estimate the feeding of *Calanus helgolandicus* on each diet (TW, SC, TR, OR, GD, PM and NDAs), faecal pellet production by females was measured each day for every bioassay. The amount of faecal pellets produced on the respective diets indicates that algae in diets were well ingested by females (Fig. 7). SEM examination of these faecal pellets also showed that the diatoms and non-diatoms (PM) were eaten by the copepods. Remains of TR, RS and GD, mixed in the food with other diatoms, were specifically recognised in the faecal pellets produced under the different NDA diets (see arrows: Fig. 7).

For demonstration of the importance of different diatoms on the reproductive success of *Calanus helgolandicus*, copepod females were exchanged between the biological stations in Roscoff and Plymouth. Two naturally occurring diets in non-enriched seawater samples (Roscoff: T1, Plymouth: T2) reflected the local phytoplankton, particulate matter biomass prevailing in each coastal zone at the time of sampling (Table 2). The main differences between T1 and T2 were a higher chl *a*, POC and PON biomass, a higher density of diatoms and an absence of *Guinardia* spp. in the Plymouth diet (Table 2), which is typical for Plymouth waters (Irigoien et al. 2000a,b). When 'British females', shipped to Roscoff, were exposed to the T1 diet, the EPR decreased sharply from 10 ± 1 to 0 eggs

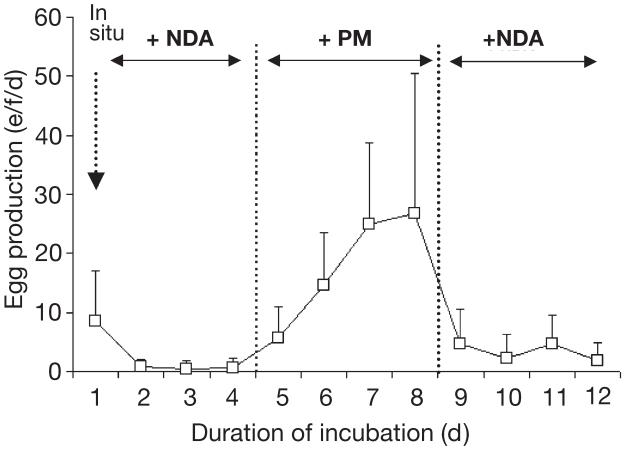
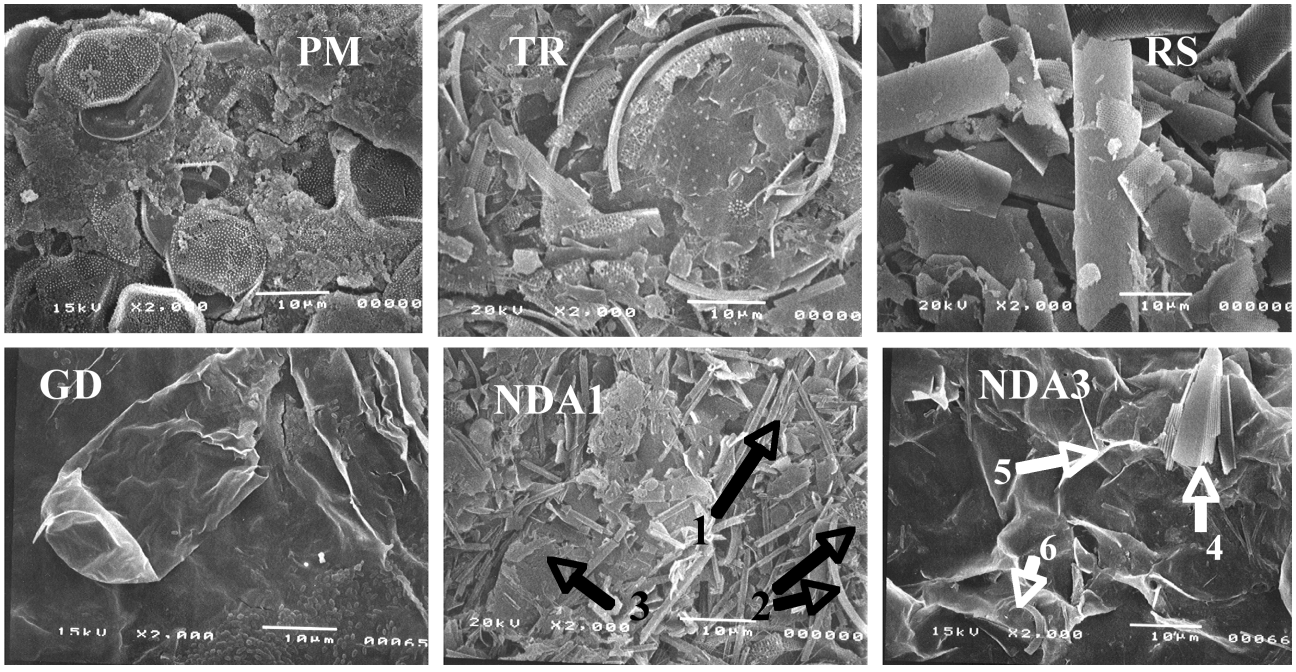


Fig. 6. *Calanus helgolandicus*. Mean daily EPRs with alternating NDA and PM diets. Mean value on Day 1 (5 May 2003) represents estimated rate in filtered seawater, reflecting EPR in the field. Two new NDAs were provided to copepods from Day 1 to 3 and from Day 8 to 12, respectively, alternating with PM provided from Day 4 to 7. The types and concentrations of diatoms in the 2 NDA diets were checked under the microscope and were close to the status of NDA 1 sampled in April (see Table 1). Values are means + SD (bars)



TYPE OF DIET								
PM	TW	SC	**TR**	OR	**RS**	**GD**	**NDA1**	**NDA3**
51–94	>20	>20	56–90	25–43	10–87	27–77	2–30	23–105

Faecal pellet production (p/f/d)

Fig. 7. *Calanus helgolandicus*. Daily production of faecal pellets and SEM pictures of algal food remains in copepod faeces. Different single-species diets were tested, as well as natural diatom mixed-species assemblages (NDA 1 & 3 diets: see composition in Table 1). Diatom remains identified in pellets are shown by arrows (1: *Chaetoceros* spp.; 2: *Thalassiosira* spp.; 3: *Guinardia* spp.; 4: *Navicula* spp.; 5: *Guinardia* spp.; 6: *Chaetoceros* spp.). Ranges of daily pellet production per female recorded during the assays (see Fig. 3) are given for 9 different diets. Pellets produced with 6 diets (bold letters) were investigated by scanning electron microscope for species identification (p/f/d: pellets female^{-1} d^{-1})

female^{-1} d^{-1} at the end of the incubation period. In contrast, at Plymouth, the T2 diet led to a slight EPR increase, from 12 ± 13 to 16 ± 7 eggs female^{-1} d^{-1}, along with high hatching rates of 'French females' (Table 3).

DISCUSSION

Over the past 3 decades, the major diatom species causing spring/summer blooms in Roscoff coastal waters have remained the same, including *Guinardia delicatula* (ex *Rhizosolenia delicatula*), *G. striata*, *G. flacida*, *Rhizosolenia setigera*, *Rhizosolenia* spp., *Thalassiosira rotula*, *Chaetoceros* sp. and *Odontella regia* (Grall 1972a, b, Martin-Jézéquel 1983, Gaillard et al. 2002; see Table 1). The increases of chl *a* observed during spring/summer at Roscoff (Waffar et al. 1983, Sournia & Birrien 1995, Laabir et al. 1998, P. Morin unpubl. data) were mainly due to the same diatoms as those that have occurred for decades and matched the species composition observed in the NDA diets in 2003 and 2004 (Table 1).

Observations reported in Fig. 1B,C for 2003 and 2004 reveal that EPR values were highly unstable at Roscoff, resembling data obtained in 1994 by Laabir et al. (1998). However, the seasonal variations showed a major difference between 2003/2004 and 1994. A decade ago *Calanus helgolandicus* was a late spawner (Laabir et al. 1998), while it became an early spawner in 2003 and 2004 (Fig. 1).

This might be explained by different climatic conditions, since 2003 and 2004 were exceptional years in terms of the number of sunny days, mediating higher chlorophyll concentrations during spring/summer (P. Morin unpubl. data). Arrest of egg production in the field has frequently been observed, mainly during fall and winter, corresponding to the lowest chl *a* values (Laabir et al. 1998). Our data now show that this parameter can also be very variable during spring and summer (Fig. 1). The fluctuations of EPR were not correlated to the phyto-

Table 2. Upper table: proportion and abundance of diatom species in seawater samples collected at 2 fixed stations, located in the Plymouth and Roscoff coastal zones, and used as non-enriched diets (see Tests T1 and T2) with *Calanus helgolandicus* females for the trans-Channel parallel bioassays in August to September 2003 (see results in Table 3). Lower table: concentrations of chlorophyll *a*, particulate carbon (POC), nitrogen (PON) and the C/N ratios measured at the same 2 stations

Diatoms	Plymouth 28/08/2003 (T2 diet) (%)	Roscoff 01/09/2003 (T1 diet) (%)
Cerataulina	0.39	0
Chaetoceros	15.59	30.20
Eucampia	0.04	0
Guinardia	0	10.30
Leptocylindricus	27.27	0
Navicula	0	1.52
Nitzschia	48.75	50.42
Rhizosolenia	0.64	0
Roperia	0.004	0
Stauroneis	0.27	0
Thalassionema	1.97	2.89
Thalassiosira	4.77	4.55
Total (cells ml^{-1})	414.15	46.59
Concentration (µg l^{-1})		
Chlorophyll *a*	0.73	1.21
Carbon (POC)	301.7	152.2
Nitrogen (PON)	43.9	22.8
C/N	6.9	6.1

plankton biomass in the field or to the other biological parameters considered in this work as food factors (Fig. 2). This finding is in accordance with *in situ* observations in the southern Gulf of St. Lawrence (Maps et al. 2005) and in the North Sea (Arendt et al. 2005), where EPR values in *Temora longicornis* did not correlate with chl *a* or temperature; or in Florida Bay, where egg production of *Acartia tonsa* varied independently of the concentrations of proteins, carbohydrates, lipids and most fatty acids in the seston (Hazzard & Kleppel 2003). Assuming that only biotic parameters influence the EPR, one should expect optimum EPR values with *Calanus helgolandicus* under high chlorophyll conditions (estimated to be ≥33 eggs $female^{-1}$ d^{-1}: see Mauchline 1998, Ianora et al. 2003). This value is nearly always higher than the EPR observed in the coastal waters off Roscoff (1993, 1994: Laabir et al. 1998; 2003 and 2004: Fig. 1), while a similar discrepancy was detected in the NE Atlantic with *T. longicornis* (Devreker et al. 2005). This phenomenon could be partly due to the age of females, some of which are arrested at the pre- or post-reproductive stages (Niehoff et al. 1999, 2002, Niehoff 2003). However, results with our control tests (PM diet) showed that the sexually mature females were not arrested at pre- or post-reproductive stages, although they only reached 50 to 80% of the optimum specific value of 40 eggs.

Table 3. *Calanus helgolandicus.* Reproduction rates of 2 female cohorts used in bioassays in response to different natural food conditions prevailing at 2 stations located on each side of the English Channel (see type of diatoms and phytoplankton biomass in Table 2). Egg production and hatching rates of females at Plymouth (Test 1, 28 August 2003) and Roscoff (Test 2, 1 September 2003) (e/f/d: eggs $female^{-1}$ d^{-1})

	Test 1		Test 2	
	Plymouth (initial)	Roscoff (final)	Roscoff (initial)	Plymouth (final)
Egg production (e/f/d)	10 ± 1	0	12 ± 13	16 ± 7
Hatching (%)	87	?	0	90

The increase of EPRs observed during the early spring in 2003 and in 2004 coincided with diatom assemblages with different dominant species (Table 1). However, even during these periods, short phases of strongly reduced EPRs were observed, which might be attributed to short time shifts in NDAs (Fig. 1B,C). An EPR decrease was observed during late-spring and summer months, coinciding with specific diatom blooms (Table 1, Fig. 3). We suspected that EPR variation might be due to fluctuations in natural diet composition and designed a series of experiments to elucidate the influence of food on egg production. In our bioassays every natural assemblage of mixed diatoms (NDAs), enriched 2 or more times the field concentrations (Figs. 3, 4 & 5, Table 1), as well as some of the isolated individual species (OR, RS, GD, GS), were detrimental to copepod egg production (Figs. 3 & 5). Other diatoms (SC, TR), as well as the dinoflagellate PM, could restore or even increase the EPR (Table 1, Fig. 3). It is interesting that particularly RS and GD, which could be made responsible for reduced EPRs, belong to the spring- to summer-bloom diatoms. Notably all NDA diets (1 to 7) contained GD and RS — albeit not always as major species — whereas the beneficial diet from Plymouth lacked these species (T2 diet; Table 2). Therefore, it is tempting to conclude that the frequent oscillations in the fecundity of *Calanus helgolandicus* females, observed during spring and summer of 2003 and 2004, were linked to successions of a few specific diatoms appearing, or co-occurring during the blooms.

A food effect on fluctuating EPRs would require that the detrimental effects of food are completely reversible under fluctuating food regimes. This reversibility was observed in a laboratory assay in which copepod egg production decreased as the result of a diet of natural diatom assemblages, while it repeatedly recovered when copepods were fed a dinoflagellate diet (PM, Fig. 6). Apparently, the reversible effect illus-

trated in Fig. 6 was not simply due to variation in the cell concentrations between the NDA 1 (2×) and PM diets, set at 1.22×10^2 and 10^4 cells ml^{-1}, respectively. In fact, when NDA 4 (50×) was artificially increased up to 1.25×10^3 cells ml^{-1}, corresponding to a food concentration 1 order of magnitude below PM and 2 orders of magnitude higher than NDA 4 in nature (Day 1), EPR values were consistently depressed (Table 1, Fig. 5). Carbon concentrations in copepod diets were estimated using the values 274 pg $cell^{-1}$ (PM), 624 pg $cell^{-1}$ (TR) and 226 pg $cell^{-1}$ (GD), based on measurements made by Ianora & Poulet (1993) and Wichard et al. (2005). By comparison, the carbon concentrations in the GD and TR diets were 15.6 µg ml^{-1} (with TR), >15 µg ml^{-1} (with GD), and >2.74 µg ml^{-1} (with PM), further suggesting that food biomass was not the limiting factor (Figs. 3, 5 & 6), because TR and GD had inverse effects on EPR. Moreover, the increase of carbon concentration from 0.03 to 0.14 µg ml^{-1} (estimating carbon content of NDA 4 diets equivalent to the cumulated values of cell carbon in each dominant species occurring in NDA 4, Table 1) by increasing the cell concentration (NDA 4: 10× to 50×; Fig. 5) did not result in higher EPR.

Polyunsaturated aldehydes (PUA), produced by several diatoms, have been discussed to be involved in the chemical defence of diatoms, since they have a negative influence on egg and naupliar development (Miralto et al. 1999, Ianora et al. 2004). A recent survey of the production of PUA allowed us to elucidate whether these chemicals may also be responsible for the observed effects, since the isolates used in this work belonged both to PUA producers and non-producers (Wichard et al. 2005). The PUA producers TR and GD had opposite positive and negative effects, respectively. A positive effect was observed for SC (Laabir et al. 1998), also known to be a PUA producer (Wichard et al. 2005). In contrast, OR, which only produces traces of PUA, and 3 non-producers had either a negative (RS, GS), or a positive (TW) effect on EPR. This complete lack of correlation between PUA production and EPR indicates that these metabolites are not an influencing factor in the egg production process. Other chemical factors missing in diets (Hassett 2004), unbalanced diets (Jónasdóttir et al. 1998, Hazzard & Kleppel 2003, Jones & Flynn 2005), or starvation, as caused by fall to winter conditions in the English Channel, might influence copepod population size (Irigoien 2004). But we still do not know which factors in phytoplankton diets determine favourable or less favourable conditions for *Calanus helgolandicus* egg production.

Until now it remains an open question whether the coastal waters off Roscoff are an exceptionally unfavourable site for *Calanus helgolandicus* reproduction. Other sites in the world, apparently, are better for *Calanus* spp. fecundity, such as Plymouth in the Northern Hemisphere (Pond et al. 1996, Laabir et al. 1998, Irigoien et al. 2000a) or the Benguella upwelling system in the Southern Hemisphere (Richardson & Verheye 1999, Richardson et al. 2001). Other ecosystems might be similar or intermediate between the Roscoff and Plymouth cases, for example the North Adriatic Sea (Miralto et al. 1999, 2003, Ianora et al. 2004), or sites where variable spring to summer fecundity and low hatching rate values (0 to 80%) have also been reported (Irigoien et al. 2000b, 2002). The contrasting reproductive responses (EPR and hatching; Table 3) of *C. helgolandicus* between Plymouth and Roscoff were assumed to be due to different hydrological conditions (stratified versus homogeneous water mass) supporting phytoplankton blooms formed of different local species (Holligan & Harbour 1977, Laabir et al. 1998, Irigoien et al. 2000a; Tables 1 & 2; plankton composition in Plymouth can be found at www.pml.ac.uk/L4/). In this context the results of the trans-channel reproduction tests (Tables 2 & 3) allowed us to draw 3 conclusions: (1) the reproductive responses of *C. helgolandicus*, reflecting their past feeding history in the field, were very different between Plymouth and Roscoff at the sampling times in late 2004, even though they were closer at other periods (Irigoien & Harris 2003); (2) the past feeding history had no long-term influence on reproductive success, because French copepod females were able to spawn again after a diet shift corresponding to a new Plymouth food assemblage; (3) these differences were supposedly triggered by different natural diets prevailing in the coastal zones on each side of the English Channel off Roscoff and Plymouth (Tables 1 & 2). Moreover, the elevated reproductive success of the 'French females' at Plymouth was not due to the higher diatom density in Plymouth, because 2× to 50× higher cell densities in NDA diets fed to copepods off Roscoff did not increase EPR (Figs. 3 & 5).

We have not explored the response of *Calanus helgolandicus* reproduction to microorganisms (<11 µm, e.g. dinoflagellates and ciliates, which were removed from the NDA diets) belonging to the microbial foodweb. Of course, these small-sized planktonic particles also have a key role in the food transfer to copepods. Interestingly, Gailhard & co-workers (2002) recorded the presence of *Prorocentrum* sp. along with *Rhizosolenia* sp. in the summer months from June to August along the French coast from 1992 to 2000. This natural phytoplankton assemblage could overcome the negative effect of some diatom species in nature, and may explain the differences in EPR values on the starting day and the final day of the feeding experiments with the NDA diets. Different effects of phytoplankton on

EPR could be dependant on the dominant diatom species in the food or on modifications of the proportions of diatom and non-diatom cells. Thus, for example, Kleppel et al. (1991) and Kleppel (1993) might not have observed any negative effects of diatoms, because, either these were not detrimental, like TW, SC and TR (Fig. 2), or negative effects were compensated for by dinoflagellates, ciliates, or other microphytoplankton present at that time. Such a situation prevails in Plymouth coastal waters, where non-diatom organisms are abundant and where reproductive responses were always higher than at Roscoff (Laabir et al. 1998, Irigoien et al. 2000a,b; www.pml.ac.uk/L4/; Table 3). Interestingly, Lacoste et al. (2001), Kang & Poulet (2000), Turner et al. (2001) and Jones & Flynn (2005) have performed tests with mixed diets of diatoms/dinoflagellates and with plankton eggs and larvae in comparison to mono-diets of diatoms; they found improved reproductive success of *Calanus helgolandicus*, *Temora stylifera* and *Arcatia tonsa* fed on the mixed diets, or with eggs and larvae. Whereas Turner et al. (2001) claimed a dilution effect of toxic compounds (e.g. unsaturated aldehydes), Kang & Poulet (2000) and Jones & Flynn (2005) noted the improved nutritional status of mixed-organism diets. First approximations of our results for the summer of 2003 seem ambiguous (Fig. 4); they do not clearly show whether the decreases in egg production observed at Roscoff with both the field and NDA diets, in comparison to the PM diet, were due to food deficiency (i.e. related to the removal of organisms in the <11 µm fraction), or to increased toxicity in the NDA diets enriched with diatoms. During July and August (Fig. 4), proportions of non-diatom organisms (<11 µm; corresponding to flagellates, Gymnodineae, Cryptophyceae and coccolithophorids) over diatoms in seawater samples collected at the same time as females and NDA 2, 3 & 4 (see Table 1) were 0.07, 0.2 and 1.18, respectively. These values might suggest that organisms belonging to the non-diatom diets (<11 µm fraction) were not abundant enough to exert a beneficial effect against the deleterious activity of diatoms.

Other factors, such as UV (Lacuna & Uye 2001), pollutants (Micic et al. 2001), or nitrogen availability (Checkley 1980, Jones & Flynn 2005), also known to affect reproduction in invertebrates, were not investigated as potential causes of fecundity anomalies, but can be excluded partially. Copepod females were not exposed to UV light in the laboratory, and possible seawater pollution did not affect the control diets with PM that systematically induced the recovery of egg production, as shown in Fig. 6. Finally EPR was not correlated to particulate nitrogen concentrations.

In summary, we suggest that the *Calanus helgolandicus* egg production is temporarily depressed due to a food factor, related either to *quantitative* food limitation (mainly during fall and winter, when phytoplankton is scarce: Phase 1 in Fig. 7) or to *qualitative* food limitation or composition. This effect could be caused by the deficiency of essential chemicals (when phytoplankton is abundant or sufficient during spring and summer; Fig. 1; Jónasdóttir et al. 1998, Rey-Rassat et al. 2002, Hazzard & Kleppel 2003, Hassett 2004, Arendt et al. 2005), or the presence of hitherto unidentified chemical inhibitors from diatoms. Our work supports the idea that the biomass of other organisms (e.g. autotrophic/heterotrophic flagellates, dinoflagellates, protistan microzooplankton and meroplankton, zooplankton eggs and larvae) could have a highly beneficial effect on copepod growth and egg production only when their proportions are higher than those of diatoms in diets encountered in the field (Roman & Gauzens 1997, Kang & Poulet 2000, Kang et al. 2000, Lacoste et al. 2001, Turner et al. 2001, Arendt et al. 2005). Thus, *C. helgolandicus* egg production in the field is strongly influenced by the succession of different assemblages and densities of phytoplankton, containing either suitable or unsuitable food, mainly related to diatoms. For all these reasons, females can only occasionally reach, or maintain, their optimum EPR (≥33 eggs female^{-1} d^{-1}) during short favourable periods of the breeding season in Roscoff coastal waters.

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