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Copepod egg production during highly productive late spring conditions: importance of freshly ingested food and lipid storage

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ABSTRACT: The reproductive activity of 2 calanoid copepods, *Calanus helgolandicus* and *Calanoides carinatus*, under post-bloom conditions was investigated during a cruise in the central Cantabrian Sea (SW Bay of Biscay) in May 2000. Several mesoscale structures, such as an upwelling of rich waters associated with the topography of one submarine canyon, were observed on the continental shelf. Consequently, a high biomass of large phytoplankton was found in coastal and mid-shelf areas. These unexpected productive conditions after the spring bloom supplied copepods with favourable food resources. Accordingly, females showed mature gonads, high percentages of spawning and high egg production rates (EPRs). A surface saline current was detected flowing parallel to the coast along the slope. A microbial food web prevailed there and, unlike the coast and mid-shelf areas, low chlorophyll concentrations and a high proportion of small phytoplankton were found. The microbial food web did not seem to be suitable to support copepod production; thus, only a few females had mature gonads and fecundity was low. The number of eggs produced increased as herbivorous feeding increased, but feeding on phytoplankton did not cover the carbon requirements at all stations, and a mixed diet was suggested. The relationship between EPR and diatom concentration showed a saturation response for both species, but interestingly the saturation concentration was low and fecundity values were sub-maximal. Females had low lipid levels and low C:N ratios, indicating that egg production could be entirely fuelled by freshly ingested food. The low lipid reserves of females could also help to explain the sub-maximal EPR of *C. helgolandicus*. Nevertheless, low lipid storage was not a constraint for *C. carinatus* because it is well known that this species uses only freshly ingested food to support egg production. The implications of this extra productive time at the end of spring on the annual recruitment of *C. helgolandicus* and *C. carinatus* are discussed.

KEY WORDS: Copepods · Egg production · Lipid storage · Herbivorous feeding · Late spring · *Calanus helgolandicus* · *Calanoides carinatus* · SW Bay of Biscay

INTRODUCTION

The link between the spring phytoplankton bloom and the bulk of copepod productivity is widely recognized (e.g. Runge 1988, Cushing 1989). Because of the lower growth rate of herbivorous copepods compared to phytoplankton, a time lag between the production peaks of the primary producers and their consumers is usually observed in temperate waters. During the late phase of the spring bloom, the phytoplankton community is normally dominated by large cells, and mesozooplankton have already developed a high biomass in response to the abundant food resources supplied by the active phytoplankton growth. According to the trophic pathway classification proposed by Legendre & Rassoulzadegan (1996), this situation corresponds to a herbivorous food web, where mesozooplankton exert strong grazing pressure on large phytoplankton.

A long time-series study in the central Cantabrian Sea (SW Bay of Biscay) shows an uncoupling between

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spring peaks of phytoplankton and mesozooplankton biomass (M. Llope et al. unpubl. data). Phytoplankton typically develops its highest biomass and production level between March and April, coinciding with the transition from mixed to stratified water conditions (Fernández & Bode 1991), whereas the highest mesozooplankton biomass occurs in May under post-bloom conditions. Nevertheless, this seasonal pattern may be modulated by some shelf-related mesoscale processes that occur during the late spring in the central Cantabrian Sea. In May 1996, González-Quirós et al. (2003) observed an additional input of nutrients into the euphotic layer due to a dome-shaped density feature associated with the shelf break front and with the topography of the Avilés submarine canyon. The phytoplankton community responded with high values of biomass and production, which were very similar to those of the previous spring phytoplankton bloom. A comprehensive survey (Sardina cruise) was conducted in May 2000 to describe shelf-related mesoscale structures and their effects on primary production, food web structure and the fish larvae retention process (González-Quirós et at. 2004). The hydrodynamics were characterised by a slope saline current, in which the microbial food web prevailed, and a dome-shaped nutrient feature as well as an upwelling of deep water associated with the topography of the Avilés canyon; both of the last 2 features induced a high productivity of large phytoplankton in mid-shelf and coastal waters. Under these conditions, an increase in the energy flow towards higher trophic levels is expected at the continental shelf. The high biomass of mesozooplankton detected in nearshore waters would support this idea (González-Quirós et al. 2004). These conditions also suggest an additional opportunity for recruitment at the end of spring for herbivorous copepods that reproduce seasonally. Given that they reach their maximum abundance at this time of the year, this would result in an important increase in their annual productivity.

In the present work, we studied the ability of 2 herbivorous calanoid copepods, *Calanus helgolandicus* and *Calanoides carinatus*, to exploit these post-bloom spring conditions. We measured their reproductive activity during a cruise in the central Cantabrian Sea. Our hypothesis is that these 2 species take full advantage of the favorable food conditions promoted by the shelf-related mesoscale structures, so high reproductive activity is expected in the shelf and coastal areas.

Calanoides carinatus inhabits all upwelling areas around the Africa continent (see Verheye et al. 1991 and references therein). Its reproduction is strongly coupled to the high food availability (mainly diatoms) induced by the upwelling events (e.g. Smith 2001). *C. carinatus* is also found in the Bay of Biscay (e.g. John et al. 1998), but its biology remains fairly unknown in this area. A recent study of its reproductive response to an upwelling event off NW Spain suggested a reproductive strategy less dependent on upwelling episodes (Ceballos et al. 2004). Moreover, our previous observations in the central Cantabrian Sea show that *C. carinatus* and *Calanus helgolandicus* attain highest abundances during spring, when their main reproductive output also occurs (Ceballos & Álvarez-Marqués 2006). *In situ* production of *C. helgolandicus* in areas near the Cantabrian Sea also showed a strong link between recruitment events and periods of phytoplankton productivity, for example, the phytoplankton spring bloom in the English Channel (Pond et al. 1996, Laabir et al. 1998), and the summer upwelling off the NW coast of Spain (Ceballos et al. 2004). These species are very representative of the copepod community in the Bay of Biscay (*C. helgolandicus*), or in the Cantabrian Sea (both species), because they rank among the 10 most abundant and frequently occurring species (Álvarez-Marqués 1982, Poulet et al. 1996). Both species attained high abundances during spring and are the most frequent and abundant Calanidae thoughout the year (Álvarez- Marqués 1982, 1984)

In addition to freshly ingested food, *Calanus* spp. can also use internal energy stores to fuel reproduction, although their role is complex. It is normally accepted that Vitellogenesis I occurs in Stage V copepodites and that it is fuelled by lipid reserves, whereas Vitellogenesis II and final maturation of the ova in young females depend on freshly ingested food. However, some populations of *C. finmarchicus* can produce eggs before the spring bloom, apparently using only stored lipids (e.g. Richardson et al. 1999), and Niehoff et al. (2002) reported that *C. hyperboreus* egg production is independent of food supply. As for *C. helgolandicus*, recent laboratory studies (Rey-Rassat et al. 2002b) suggest that egg production over the entire lifespan does not rely solely on immediate carbon intake, but also on a buffer of lipid reserves, which reflect past feeding conditions and the developmental condition of the females. Likewise, *Calanoides carinatus* has large lipid reserves that also play an important role in reproduction, although unlike *C. helgolandicus*, lipids do not seem to be directly involved in its reproductive activity. Armstrong et al. (1991) showed that these lipid reserves are used to enhance the survival of *C. carinatus* females in the southern Benguela Current when food is scarce, such as during the non-upwelling season or between upwelling pulses. This strategy ensures a rapid response of females to subsequent high food supply. Because of this, our secondary goal was to measure the herbivorous feeding rate and lipid storage of females in order to examine the influence of both factors on the *in situ* egg production rates recorded during our cruise.

MATERIALS AND METHODS

Sampling procedures. Data were collected in the central Cantabrian Sea (SW Bay of Biscay) during the Sardina cruise on board the RV 'García del Cid', from 2 to 17 May 2000. A total of 11 stations were sampled during the daytime (09:00 to 13:00 h) along 4 transects perpendicular to the coast (Fig. 1). Vertical profiles of temperature, salinity and fluorescence were taken at each station using a Mark III CTD with a Sea-Tech fluorometer attached to a rosette of 24 Teflon Niskin bottles. Live experimental animals were collected with a triple WP2 net (0.60 m diameter, 200 µm mesh) equipped with 3 l cod-ends. Hauls were made vertically from 200 m to the surface, or from 5 m above the bottom to the surface at the coastal stations. Once on deck, 1 or 2 cod-ends were immediately processed for gut pigment content analysis. Another haul was made and the 3 cod-ends were diluted in a 5 l plastic jar filled with 100 µm-filtered seawater to ensure that the animals remained alive for our experiments. Water used for the incubations was collected with Van Dorn bottles (15 l) from the depth of maximum fluorescence (MFD). Bongo net hauls were performed to collect zooplankton samples for abundance estimations as described in González-Quirós et al. (2004). A bongo net (0.60 m diameter, 200 µm mesh) equipped with calibrated flowmeters was towed obliquely from 200 m to the surface, or from 5 m above the bottom to the surface at coastal stations. Samples were preserved in 4% buffered formaldehyde.

Food availability. Chlorophyll *a* (chl *a*) concentration and microplankton composition were used as indicators of potential copepod food availability. Chl *a* concentration was determined as described in González-Quirós et al. (2004). Briefly, water samples of 100 ml were collected from depths with 100 and 1% of incident light, and from the MFD; depending on the station depth, 1 or 2 additional samples were taken at increasing intervals. Samples were filtered sequentially through 5 and 0.2 µm polycarbonate filters because it has been suggested that 5 µm is the size above which copepods graze efficiently (e.g. Cushing 1989). Pigments were extracted in 5 ml of 95% acetone at 4°C overnight and the chl *a* concentration was measured fluorometrically before and after acidification (1 N HCl) using a Turner Design Model 10 fluorometer. Total chl *a* was calculated as the sum of the 2 size fractions (0.2 to 5 µm and >5 µm). Chl *a* concentration was integrated in the water column. For most stations the integration depth was 100 m, but for the coastal stations (Stns A1, A2, B1 and C1) the integration depth was 23, 65, 40 and 40 m, respectively. The maximum value of chl *a* (chl a_{max}) was also used to describe the food environment of the copepods.

For microplankton composition and abundance, 125 ml water samples were taken at 3 depths within the euphotic zone (100 and 1% of light incidence and the MFD). Samples were preserved with Lugol's solution (3% final concentration) and concentrated by gravimetric sedimentation in Utermöhl chambers (25 ml). When possible, cells >5 µm were identified to the species level using an inverted microscope. Microplankton cell abundance was transformed into carbon units. Cells were measured using a digital analysis system. The carbon content was estimated from cell volume by approximation to the nearest geometric shape (Hillebrand et al. 1999) and by applying the carbon conversion factors proposed by Putt & Stoecker (1989) for ciliates, and by Menden-Deuer & Lessard (2000) for the remaining groups. The abundances were integrated in the euphotic zone (cells m^{-2}, mg C m^{-2}). The euphotic depth varied from 40 to 60 m, but it was shallower at the coastal stations (Stns A1 and A2), where it was 23 and 28 m, respectively. The mean abundance for the euphotic zone was also calculated (cells ml^{-1}).

Biomass, prosome length, gonad development and oil sac volume of females. Measurements of body carbon (C) and nitrogen (N) content of females were made on 3 replicates of 4 individuals at each station. Live

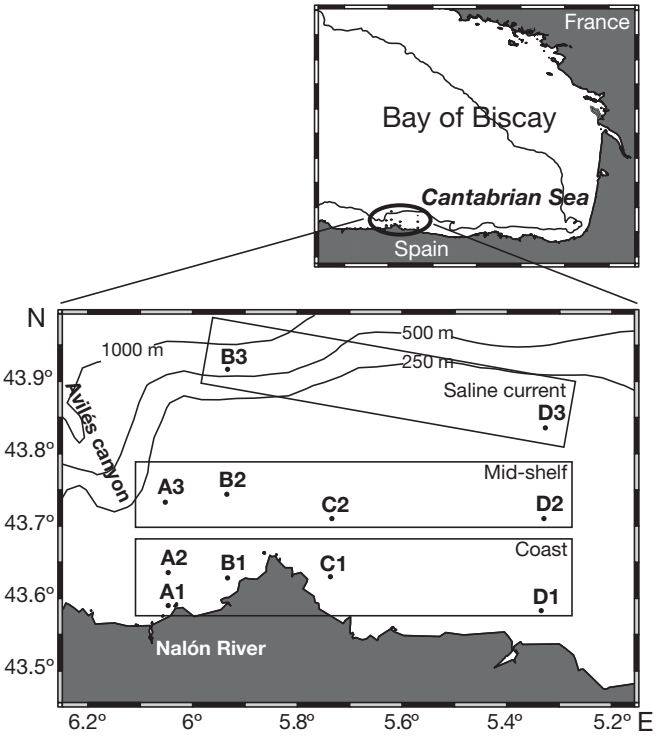

Fig. 1. Map showing study area. Stations were grouped into coastal (<100 m depth), mid-shelf (100 to 200 m depth) and slope saline current stations

females were picked under a dissecting microscope, rinsed in distilled water to remove adhering material, and transferred to pre-combusted GF/A filters. Excess water was removed by filtering with low vacuum pressure, and then the filters were frozen (–20°C). In the laboratory, the filters were dried at 60°C for 48 h before estimating C and N content with a Perkin Elmer 2400 Elemental Analyzer. The prosome length of 40 preserved females for each station was measured in lateral view using a video image analysing system. This system was also used to measure the oil sac volume (estimated as an ellipse volume; Plourde & Runge 1993) of preserved females from each station. The volume was converted into wax ester (WE) content following Rey-Rassat et al. (2002b). In order to normalize the effect of female size, WE content was divided by prosome volume, which was estimated following Mauchline (1998).

Gonad development of females was also used to describe the reproductive status of the *Calanus helgolandicus* and *Calanoides carinatus* populations. The females used to measure the prosome length were stained and classified into 6 gonad development stages following Ceballos et al. (2004). The reproductive index (RI) was calculated as the proportion of females with mature gonads and therefore ready to spawn in 24 h incubations (e.g. Runge & Roff 2000).

Egg production experiments. Between 10 and 15 healthy and fertilised females (i.e. with full spermatheca) of *Calanus helgolandicus* and *Calanoides carinatus* were gently sorted, under a dissecting microscope, and placed individually in Nalgene, bottles containing 200 ml of 100 µm-filtered seawater from the MFD. Experiments were run by maintaining the bottles in a deck incubator equipped with a temperature control system (±0.1°C) at the temperature of the MFD (12.7 to 13.6°C). This was done under dim light with a natural photoperiod brought about by covering the container during the dark hours to avoid the influence of the ship's lights. The bottles were gently shaken 2 to 3 times per day to avoid cells settling. At the end of incubation (24 h), the viability of the females was checked and the eggs were filtered onto a 33 µm mesh and preserved at –20°C in vials, until being counted in the laboratory. Egg production rates (EPR) were calculated as the number of egg spawned per female per day. Only live females were considered in this calculation, although mortality was insignificant. The percentage of females that spawned during the incubation and the mean clutch size (CS) including only the bottles where females produced eggs were also calculated; it was assumed that females produced only 1 clutch during the incubation. Egg cannibalism was accounted for by including crumpled egg membranes in egg production counts (e.g. Ianora et al. 1995, Runge & Roff 2000), but cannibalism was negligible.

The population EPR (eggs m^{-3} d^{-1}) was calculated as the product of the EPR and female abundance, and could be considered as a gross estimation of the potential recruitment of *Calanus helgolandicus* and *Calanoides carinatus*. The population EPR was also converted into carbon units (mg C m^{-3} d^{-1}) by multiplying by the egg carbon content, which was estimated using a carbon/volume relationship of 0.14×10^{-6} µg C $µm^{-3}$ assuming that eggs are spherical (Kiørboe et al. 1985). At each station a number of eggs were measured with an ocular micrometer attached to a microscope at ×10 magnification. To determine *C. carinatus* egg diameter, the perivitelline space was not considered. No significant differences in egg size were found between stations for either species ($F_{9,352} = 1.90$, $p > 0.05$ for *C. helgolandicus* and $F_{9,390} = 1.54$, $p > 0.05$ for *C. carinatus*); the mean values of egg diameter were 165.82 µm (0.33 µg C) and 135.65 µm (0.18 µg C) for *C. helgolandicus* and *C. carinatus*, respectively.

Herbivorous feeding. Daily ingestion rates (I) of phytoplankton by *Calanus helgolandicus* and *Calanoides carinatus* females were estimated from the gut fluorescence method (Mackas & Bohrer 1976) using:

$$I = GF \times K$$

where GF is the gut fluorescence and K is the gut clearance rate, which was calculated following the equation shown in Båmstedt et al. (2000):

$$K = 0.0124 \times e^{(0.07675 \times T)} \ (\text{min}^{-1})$$

where T is *in situ* temperature (mean column temperature here). K values ranged between 0.033 and 0.037 min^{-1}.

The content of 1 cod-end of the WP2 net was immediately filtered through an Albet® filter paper (47 mm diameter) on a low vacuum; the disc was wrapped in aluminium foil and frozen at –20°C. The entire process lasted <3 min. Samples were analysed 1 mo after they were frozen (Morales et al. 1993). For chlorophyll pigment extraction, 3 to 4 replicates of 10 to 15 females were quickly picked from frozen samples using a dissecting microscope under dim light, and placed in 90% acetone for 1 night in darkness at 4°C. Extracts were analysed with a Turner Design Model 10 fluorometer before and after acidification (1 N HCl). Ingestion rates could be interpreted as conservative since our sampling was conducted during the daytime and both species usually have highest feeding activity during dark hours (*Calanus helgolandicus*: Harris & Malej 1986; *Calanoides carinatus*: Timonin et al. 1992).

Ingested chl *a* was converted into carbon units using a C:chl *a* ratio of 156.13, which is the slope of the linear regression between a chl *a* concentration of >5 µm and the carbon concentration of photosynthetic cells (C =

156.13 chl *a* + 8.80, R^2 = 0.75, $F_{1,33}$ = 99.19 p < 0.001, N = 34). The linear regression equation was calculated using all the sampled depths for microplankton composition (i.e. MFD, 100 and 1% of incident light) at all stations.

Statistical analysis. To test for differences between means 1-way ANOVAs and *t*-tests were applied. Kolmogorov-Smirnov and Bartlett tests were first performed to check for normality and homogeneity of variance. The differences in female carbon content between stations were examined using the Kruskal-Wallis test due to lack of data normality. Lipid data were log-transformed to achieve normality. When ANOVA results were significant ($p < 0.05$), post-hoc comparisons were performed with the Tukey Honestly Significant Difference (HSD) test. The relationships between variables were explored using linear regression analyses and the Ivlev function: $Y = Y_{max}(1 - e^{(-\alpha X)})$.

This function represents a type II numerical/functional response (Dam et al. 1994), where Y_{max} is the corresponding maximum value of the rate (e.g. EPR or phytoplankton ingestion rate) and X and α are the food concentration and the rate at which the biological rate attains the maximum value, respectively.

RESULTS

Hydrology

The summer thermal stratification was starting to develop at the time of sampling. Well-mixed water was restricted to the upper 10 to 20 m; a weak thermocline was observed and a subsurface fluorescence maximum was detected (Fig. 2). Surface temperatures ranged from 13.2 to 15.4°C, with highest temperatures along Transect A (Table 1). Surface salinity showed a coastal–oceanic gradient, with lowest salinities at the coastal stations of Transect A due to the fresh water runoff from the Nalón River (Fig. 1, Table 1).

Several mesoscale structures were encountered during the Sardina cruise (for detailed descriptions of these structures and of the biological effects of the hydrodynamics see González-Quirós et al. 2004). Firstly, a saline current (>35.64) was detected flowing along the slope. Two stations, Stns B3 and D3, were located inside the saline current (Fig. 1). Offshore and over the slope a surface meandering circulation was found. The inferred quasigeostrophic (QG) velocities

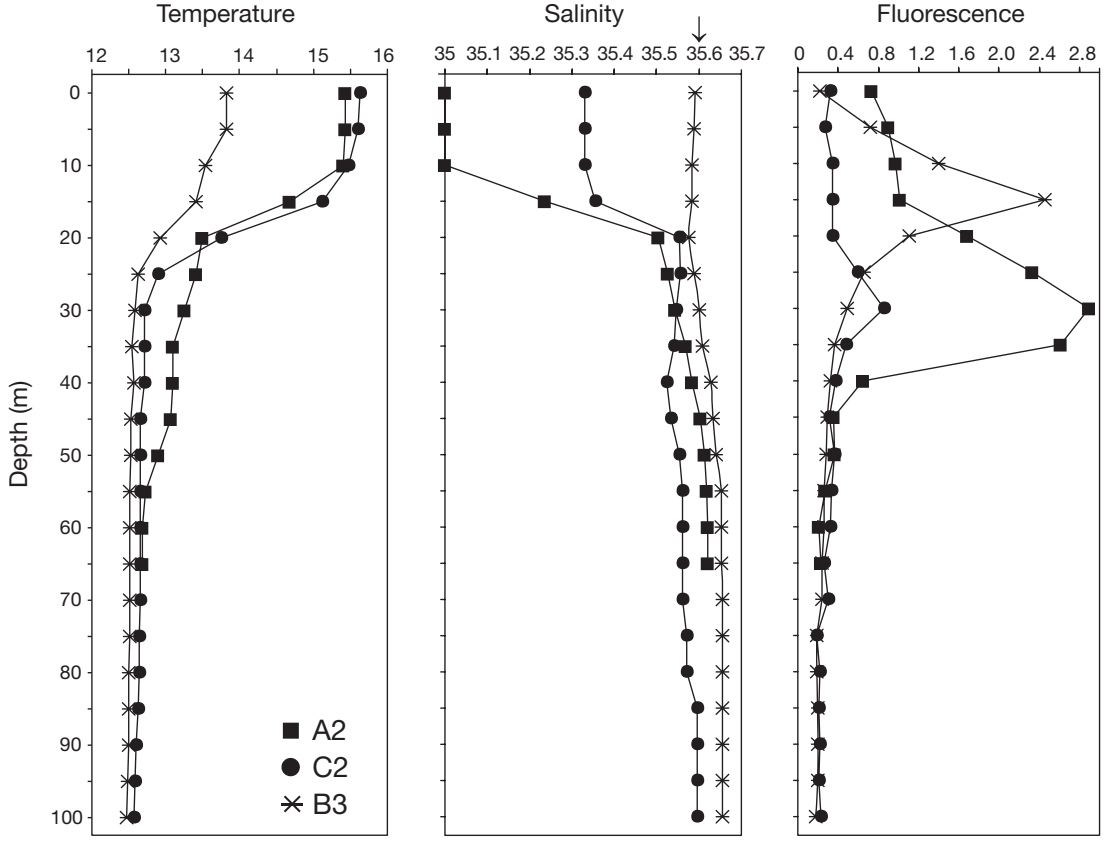

Fig. 2. Vertical profiles of temperature (°C), salinity (arrow: salinity of the slope salinity current) and fluorescence (arbitrary units) at 3 selected stations that represent coastal (Stn A2), mid-shelf (Stn C2) and slope (Stn B3) conditions

Table 1. Environmental variables in the study area. Surface and mean water column temperature (°C), surface salinity and total and size-fractioned chlorophyll *a* (chl *a*). Chl *a* is represented as the integrated (mg m^{-2}), maximum and mean values in the water column (µg l^{-1}). C: coast, MS: mid-shelf, SC: slope current, Max.: maximum value. See 'Materials and methods' for integration depth data

Transect	Zone	Stn	Temperature		Salinity	Chl *a*					
			Surface	Water column		Integrated 0.2–5 µm	Integrated >5 µm	Max. >5 µm	Max. total	Mean >5 µm	Mean total
A	C	A1	15.1	13.6	34.2	5.0	16.3	1.6	2.1	0.7	0.9
	C	A2	15.4	13.3	35.0	5.8	63.2	5.3	5.4	0.9	1.0
	MS	A3	15.1	13.8	35.5	10.9	13.1	0.3	0.6	0.1	0.3
B	C	B1	13.5	12.8	35.2	4.0	9.0	0.6	0.7	0.2	0.3
	MS	B2	14.2	12.9	35.4	16.8	29.9	1.5	1.8	0.4	0.6
	SC	B3	13.8	13.0	35.5	34.6	5.2	0.1	1.2	0.1	0.5
C	C	C1	13.2	12.7	35.3	4.3	37.8	1.9	2.0	0.8	0.9
	MS	C2	15.6	13.3	35.3	5.5	19.0	0.8	0.8	0.2	0.3
D	C	D1	14.5	12.7	35.5	10.3	20.4	1.1	1.3	0.4	0.5
	MS	D2	13.8	12.9	35.2	9.4	19.4	1.0	1.3	0.3	0.4
	SC	D3	13.6	13.6	35.6	14.6	5.1	0.2	0.4	0.1	0.2

showed downwelling of water at Stn B3 and upwelling at Stn D3. Secondly, a dome-shaped feature in the cross-shelf vertical distribution of nutrients (higher concentrations in the euphotic layer) was observed, which may be related to internal waves. Thirdly, an upwelling of deep water associated with the topography of the Avilés Canyon was also detected on the shelf.

Food availability

The phytoplankton community was dominated by the small size class (0.2 to 5 µm) in the saline slope current. This fraction accounted for 87 and 74% of total chl *a* at Stns B3 and D3, respectively. However, phytoplankton composition in the coastal and mid-shelf areas comprised mostly large cells (>5 µm) (55 to 92% of total chl *a* biomass) for all transects (Table 1). Total phytoplankton biomass showed high variability throughout the study area, ranging between 13 and 69 mg chl *a* m^{-2}. Subsurface (15 to 30 m) chl a_{max} was detected at all stations.

Dinoflagellates (*Prorocentrum* spp., *Gyrodinium* spp., *Gymnodinium* spp., *Dinophysis* spp., *Ceratium* spp. or *Protoperidinium* spp.) dominated the microplankton community in both cell abundance and carbon concentration along Transects A and D (Table 2). *Prorocentrum balticum* accounted for 60% of dinoflagellate carbon at Stn A1. This photosynthetic species has been

Table 2. Microplankton abundance. Abundance is represented as the percentage (%) of total microplankton cells, the mean (M) and the integrated (Int) concentration of cells in the euphotic layer. Abundance is also shown as carbon concentration integrated in the euphotic layer (Int C). C: coast, MS: mid-shelf, SC: slope current. Values in M: (cells ml^{-1}); Int: (cells m^{-2}); Int C: (mg C m^{-2}). See 'Materials and methods' for euphotic layer depth

Transect	Zone	Stn	Diatoms				Dinoflagellates				Ciliates				Other groups			
			%	M	Int	Int C	%	M	Int	Int C	%	M	Int	Int C	%	M	Int	Int C
A	C	A1	2	6	159	203.0	96	301	6702	5687.2	1	3	88	183.3	0	0	0	0
	C	A2	1	21	425	46.6	97	1150	30850	8312.1	1	17	476	395.8	0	0	0	0
	MS	A3	18	11	540	63.9	72	50	2212	555.3	4	3	116	89.1	6	4	197	26.0
B	C	B1	71	36	1588	536.3	17	10	385	196.0	9	5	190	206.8	3	1	64	8.4
	MS	B2	87	557	2953	4533.4	12	84	3913	1153.4	1	7	259	348.5	1	7	281	420.7
	SC	B3	<1	1	31	37.1	1	27	1135	768.4	<1	7	300	110.9	99	2379	112700	485.9
C	C	C1	69	153	10242	2403.4	13	56	1898	889.4	1	4	222	563.4	17	52	2576	320.5
	MS	C2	18	72	6836	1377.8	15	62	5604	2443.0	63	242	23420	1962.9	3	13	1250	947.5
D	C	D1	26	41	2166	1444.9	46	99	3904	447.7	11	17	920	336.5	17	27	1432	68.0
	MS	D2	39	115	6079	677.6	57	145	8870	2945.3	3	6	424	725.5	2	5	258	55.2
	SC	D3	3	1	77	2.8	73	39	1692	297.2	17	8	385	153.5	7	2	162	33.4

reported to form red tides in waters with low salinity (Edler at al. 1984), as occurred at Stn A1 (see Table 1). At stations of the slope saline current, dinoflagellates were mainly composed of small species of the family Gymnodiniaceae. Diatoms were the most important group in the coastal and mid-shelf waters of Transect B and at Stn C1 (Table 2), whereas they accounted for between 1 and 39% of the total microplankton in Transects A and D. Maximum diatom abundance was attained at Stn B2, where their concentration was more than 3 times higher than at the other stations. This maximum was due to *Thalassiosira* spp., mainly *T. rotula*, which represented 94% of the diatom cells and 96% of diatom carbon. A low diversity of diatoms was also observed at other stations with high diatom abundance; *Nitzschia pungens* represented 82% of total diatom abundance at Stn C1, *Detonula pumila* and *Rhizosolenia delicatula* represented 65% of diatom cells at Stn D1 and 3 species of *Nitzschia* and *R. delicatula* comprised >95% of diatom abundance at Stn C2. The density of ciliates was generally low but they dominated the microplanktonic community at Stn C2. 'Other groups' were composed of Chrysophyceae, Cryptophyceae, Euglenophyceae and microflagellates. All these groups showed very low diversity (1 or 2 species) and were really scarce, except microflagellates at Stn B3, where they represented 99% of cell abundance but only 38% of microplankton carbon biomass (Table 2).

Population status

The distribution of *Calanus helgolandicus* and *Calanoides carinatus* populations were not related to food availability or physical factors (Fig. 3). For both species, the highest densities of females and total population were detected at Stn D2, although there was no clear pattern of abundance. Copepodites V and females were the dominant stages. Female abundance varied considerably between stations, ranging from 4 to 491 females m^{-3} for *C. helgolandicus* and from 12 to 748 females m^{-3} for *C. carinatus* (Fig. 3). Males were scarce or absent and the sex ratio was always biased towards females.

Egg production rate

The fecundity of both species followed the same coastal–ocean pattern, with the highest EPRs attained in the mid-shelf area, where significant differences between stations were found (*Calanus helgolandicus*: $F_{3,57} = 3.96$, $p < 0.05$; *Calanoides carinatus*: $F_{3,56} = 5.29$, $p < 0.01$). *C. helgolandicus* females produced eggs at rates between 10 ± 3 eggs d^{-1} at Stn A3 and 24 ± 2 eggs d^{-1} at Stn D2. *C. carinatus* fecundity ranged between 13 ± 2 eggs $female^{-1}$ d^{-1} at Stn A3 and 28 ± 3 eggs $female^{-1}$ d^{-1} at Stn D2. In the coastal area there were no differences in fecundity between transects (*C. helgo-*

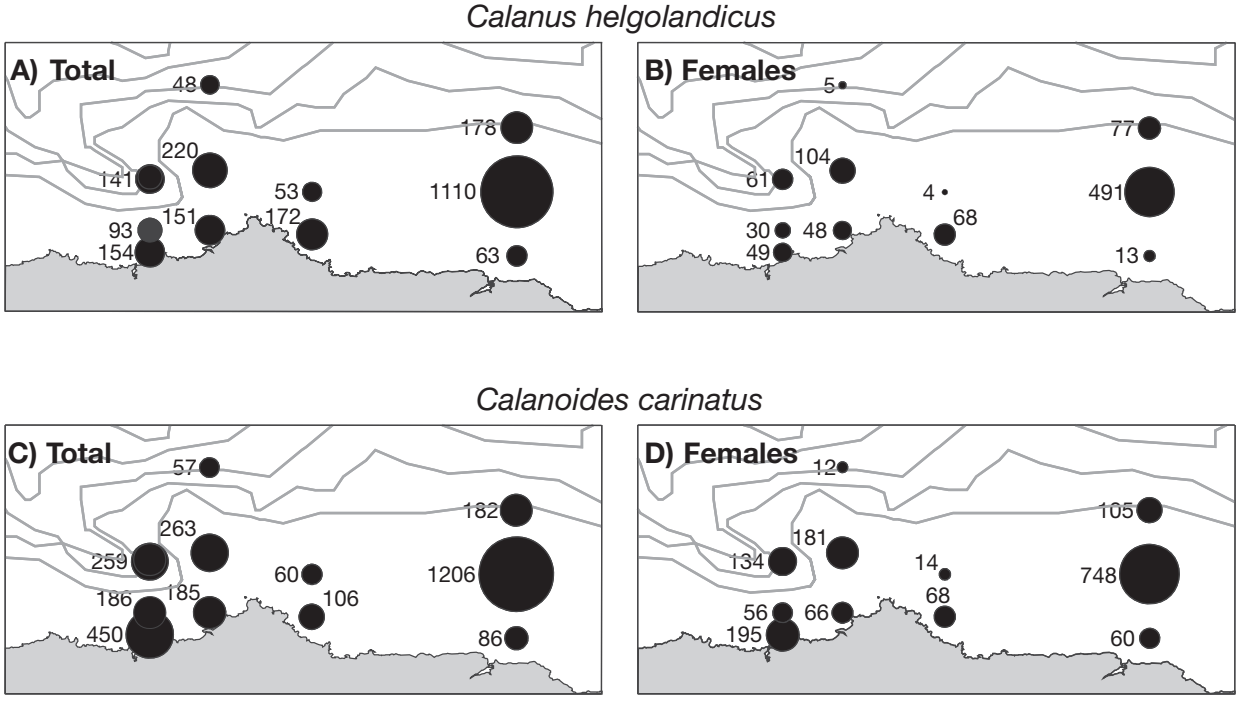

Fig. 3. *Calanus helgolandicus* and *Calanoides carinatus*. (A,C) Total and (B,D) female population abundance (CIV to adults, ind. m^{-3}) during the Sardina cruise

landicus: $F_{4,56}$ = 1.99, p > 0.05; *C. carinatus:* $F_{4,56}$ = 184, p > 0.05) and fecundity had intermediate values, i.e. 14 eggs female^{-1} d^{-1} for *C. helgolandicus* and 16 eggs female^{-1} d^{-1} for *C. carinatus*. Females produced eggs at low rates (mean value of 1 egg d^{-1}) in stations in the offshore saline current, with no difference in *C. helgolandicus* egg production between stations (*t*-test, T_{18} = 1.68, p > 0.05). *C. carinatus* did show a difference in egg production at these stations (T_{15} = 2.75, p < 0.05) with 8 ± 3 eggs female^{-1} d^{-1} at Stn B3 and 0.3 ± 0.2 eggs female^{-1} d^{-1} at Stn D3.

The lowest percentage of spawning and CS were found at the slope saline current (Fig. 4). The average percentage of spawning females in mid-shelf was 80% for *Calanus helgolandicus* and 98% for *Calanoides carinatus*. In nearshore waters, fewer females produced eggs than in mid-shelf zones, the overall means for coast being 70% for *C. helgolandicus* and 80% for *C. carinatus* (Fig. 4). The mean CS was similar for mid-shelf and coastal waters (around 20 eggs female^{-1} d^{-1}); for all shelf and coastal stations CS ranged between 15 and 26 eggs female^{-1} d^{-1} for *C. helgolandicus* and between 16 and 25 eggs female^{-1} d^{-1} for *C. carinatus* (Fig. 4). The maximum individual EPR was found nearshore on Transect D, with 54 eggs d^{-1} for *C. helgolandicus* and 49 eggs d^{-1} for *C. carinatus.*

For both species, the mean population production rates were highest for the mid-shelf station group and lowest for the saline current station group (Table 3); the overall mean population egg production was the same for both species (T_{15} = 0.71, p > 0.05). There were no environmental factors that explained the variability in population production rates (p > 0.05 for regression analysis with temperature and copepod food concentration as the independent variable).

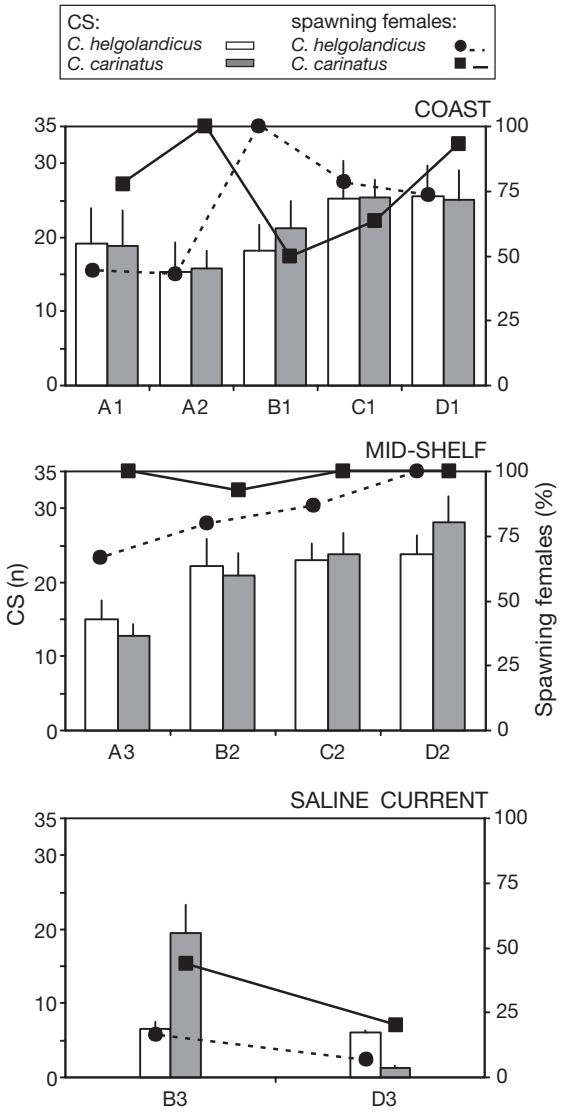

Fig. 4. *Calanus helgolandicus* and *Calanoides carinatus*. Reproductive response of females for coastal, mid-shelf and saline slope current stations. Spawning females is given in % and clutch size (CS) is given in mean number of eggs (±1 SE)

Egg production and food availability

The relationship between EPR of both species showed a saturation response with diatom concentration (Fig. 5A). This relationship explained 89 and 62% of the variability in egg production for *Calanus helgolandicus* and *Calanoides carinatus*. The EPR of *C. carinatus* also showed a functional response with the concentration of chl *a* > 5 µm, although the coefficient of determination was lower (R^2 = 0.53) A saturation response of CS to diatom concentration was also observed (R^2 = 0.80 for both species). We have run similar analyses, using linear regressions and the Ivlev function with the remaining descriptors of food availability (total chl *a*, dinoflagellates, ciliates, etc.), but interestingly no other significant relationships between EPR and food availability were found.

Table 3. *Calanus helgolandicus* and *Calanoides carinatus*. Population production rates. Values are shown as range of coastal, mid-shelf and slope saline current stations

	Production rate	
	Eggs (m^{-3} d^{-1})	Carbon (µg C m^{-3} d^{-1})
C. helgolandicus		
Coast	196–1347	66.9–455.4
Mid-shelf	81–12000	27.6–3900
Slope current	9–31	3.1–10.4
C. carinatus		
Coast	703–2858	28.5–517
Mid-shelf	336–21000	61.9–3900
Slope current	28–99	5.1–18

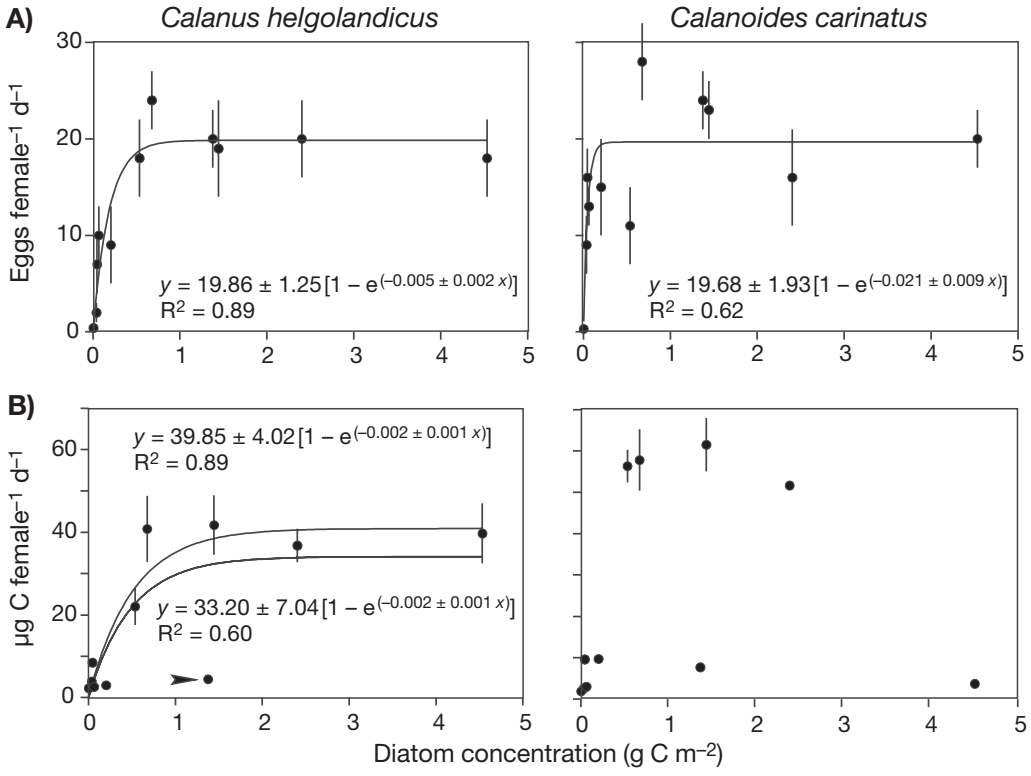

Fig. 5. *Calanus helgolandicus* and *Calanoides carinatus*. Relationship between diatom concentration and (A) egg production rate and (B) ingestion rate. Relationship between ingestion rate of *C. helgolandicus* and diatom concentration is shown excluding Stn C2 (arrowhead) (see 'Results' for more details). Data are mean ± 1 SE

Female gonad maturation stage, mass and lipid reserves

The RI attained high values (80 to 90%) at most stations and was always higher than 50%, except at the slope current station (Stn D3), where the percentages of females in the spawning state were 32 and 37% for *Calanus helgolandicus* and *Calanoides carinatus*, respectively. Moreover, there was a positive relationship between RI and spawning percentage (for *C. helgolandicus*: $R^2 = 0.62$, $p < 0.01$, $N = 11$; for *C. carinatus*: $R^2 = 0.50$, $p < 0.05$, $N = 11$). For both species, all the females analysed for gonad development had full spermatheca, indicating that they were fertilized. Only 6 out of 880 females analysed were senescent. These results suggest an active reproduction season.

The carbon content of *Calanus helgolandicus* females showed no differences between zones (Kruskal-Wallis test, $H_9 = 14.32$, $p > 0.05$) with an overall mean of 88.3 ± 11.5 µg C female^{-1}, whereas the biomass of *Calanoides carinatus* females changed between stations ($H_9 = 16.23$, $p < 0.05$), ranging from 40.6 to 56.9 µg C female^{-1}. However, this difference in biomass had no effect on EPR, since the parameters were not related ($R^2 = 0.29$, $p > 0.05$, $N = 11$). The C:N ratio was similar for species and ranged from 3.6 to 4.5 (3.9 ± 0.1) for *C. helgolandicus* and from 3.8 to 4.4 (3.9 ± 0.1) for *C. carinatus*.

Calanus helgolandicus females had no visible lipid sac at Stns A1 and B1, but they had several tiny drops scattered along the cephalothorax. At the remaining stations, more than 70% of females had oil sacs. For *Calanoides carinatus*, 80 to 93% of the females had oil sacs, except at Stn A2, where females had only oil drops. Lipid storage of *C. carinatus* was higher than that of *C. helgolandicus* only as relative WE content values (Table 4). In addition, the oil sacs of *C. carinatus* females were darker in colour. Lipid storage of both species varied between stations ($F_{8,103} = 11.49$, $p < 0.01$ for *C. helgolandicus*; $F_{9,102} = 7.94$, $p < 0.01$ for *C. carinatus*). The post-hoc Tukey test separated the stations into 2 groups: one for Stns A3 and C2, which had the highest values, and another one for the remaining stations (Table 4).

Herbivorous feeding of females

Gut pigment content (GPC) and ingestion rate of *Calanoides carinatus* females showed a coastal–ocean gradient, reaching the highest values at the coastal sta-

Table 4. *Calanus helgolandicus* and *Calanoides carinatus*. Oil sac volume, lipid content as wax ester (WE) and relative lipid content (WE/prosome volume) of females. Stations are separated into 2 groups according to results of the post-hoc test (see 'Results; Female gonad maturation state, mass and lipid reserves'). Data are minimum and maximum values

	Oil sac vol. (10^6 µm^3)	WE (µg C)	Relative WE
Stns A3 and C2			
C. helgolandicus	13.8–21.3	6.4–9.9	4.3–6.8
C. carinatus	13.7–15.5	6.6–9.1	8.4–9.6
Other stations			
C. helgolandicus	4.4–6.1	2.1–2.8	1.2–1.8
C. carinatus	2.2–4.9	1.0–2.2	1.1–2.3

Table 5. *Calanus helgolandicus* and *Calanoides carinatus*. Gut pigment content (GPC, in ng chl *a* female^{-1} d^{-1}, mean ± 1 SE) and ingestion rate (in µg C female^{-1} d^{-1}) for coastal, mid-shelf and saline slope current stations

	GPC	Ingestion rate	
		Mean	Range
C. helgolandicus			
Coast	2.9 ± 1.0	21.8	2.9–40.7
Mid-shelf	2.8 ± 1.4	21.3	2.5–39.8
Slope current	0.4 ± 0.1	3.0	2.2–3.8
C. carinatus			
Coast	5.0 ± 1.6	37.3	9.4–60.4
Mid-shelf	2.4 ± 1.8	17.7	3.0–56.8
Slope current	0.4 ± 0.1	2.2	1.9–2.6

tions (Table 5). For *Calanus helgolandicus*, the mean values for the coastal and mid-shelf areas were very similar, and the lowest herbivorous feeding activity was also measured in the slope saline current. The ingestion rate expressed as the daily ratio of body weight ranged between 2.5 and 46.5% body carbon d^{-1} (19.8 ± 5.5) for *C. helgolandicus* and between 4.2 and 125.5% body carbon d^{-1} (45.5 ± 15.5) for *C. carinatus*. For both species the highest and lowest GPC and ingestion rates were attained at Stn D1 (coast) and Stn D3 (slope saline current), respectively (Table 5). The relationship between the ingestion rate of *C. helgolandicus* and diatom concentration showed a type II functional response (Fig. 5B). At Stn C2 the ingestion rate was lower than expected, and ciliates were the most abundant microplankton group (Table 2, Fig. 5B). If Stn C2 is omitted, the coefficient of determination increases from 0.60 to 0.89. No other significant relationships were found between *C. helgolandicus* feeding rate and the remaining parameter used to describe the copepods' food environment (i.e. total chl *a*, chl *a* > 5 µm and the abundance of the remaining phytoplankton groups). Interestingly, the ingestion rate of *C. carinatus* was not related to food availability. CS and herbivore feeding rates were positively correlated for both species ($r = 0.67$ and 0.65, $p < 0.05$, for *C. helgolandicus* and *C. carinatus*, respectively).

DISCUSSION

Food availability, herbivorous feeding and egg production

The potential food conditions for females in the slope saline current differed clearly from those observed at coastal and mid-shelf stations (González-Quirós et al. 2004). Phytoplankton was dominated by the small size fraction in the slope current and, thus, the microbial food web prevailed there, which is in agreement with previous findings (Fernández et al. 1993). In contrast, large cells (diatoms and dinoflagellates) were predominant in the coastal and mid-shelf zones, suggesting the importance of the classical food chain (e.g. Cushing 1989). The typical situation for the central Cantabrian Sea under post-bloom conditions, when the water stratification has just started, is that dinoflagellates dominate the microplankton community (Fernández & Bode 1994), as we found at several stations along the shelf, but the nutrient input induced by the mesoscale structure also allowed diatoms to attain high abundance in coastal and mid-shelf waters. Therefore, high abundances of dinoflagellates and diatoms prevailed simultaneously in nearshore waters.

The 2 kinds of food webs found during the cruise induced different reproductive responses in *Calanoides carinatus* and *Calanus helgolandicus.* In the slope current, food availability was scarce due to the low concentration of large phytoplankton. Consequently, the ingestion of phytoplankton was lowest in this area because of the low efficiency of large calanoids feeding on small phytoplankton cells (e.g. Hansen et al. 1994). Given the general relationship between egg production and diatom abundance observed during the cruise, the low fecundity of both species may be linked to the scarcity of diatoms there. This is more evident for *C. helgolandicus*, since the general relationship between ingestion rate and diatom concentration suggests that this species may select for diatoms. At the salinity current stations only a few females spawned because few females had mature oocytes, and the CSs were low. This is in agreement with previous works that showed that reproduction of *Calanus finmarchicus* (Båmstedt et al. 1999), *C. carinatus* and *C. helgolandicus* (Armstrong et al. 1991, Ceballos et al. 2004) is food limited when the phytoplankton assemblage is dominated by small algae in sub-bloom concentrations. Therefore, food concentration and its size distribution appear to be the main constraint on egg production in the saline current.

In the slope current the ingestion of phytoplankton by *Calanoides carinatus* and *Calanus helgolandicus* females was insufficient to meet their carbon demands (Table 6). Moreover, although fecundity and herbivorous feeding were positively related for both species during the cruise, the ingestion of phytoplankton did not explain ca. 35% of the variability in fecundity. Therefore, females needed to use alternative food items to obtain supplementary carbon, and a mixed diet could be argued. The microbial food web could sustain copepod populations through the heterotrophic protozoa (Sherr & Sherr 1994). Ciliates and non-autotrophic dinoflagellates might be a good alternative prey when phytoplankton is dominated by small cells (e.g. Nielsen et al. 1993). Furthermore, *Calanus* spp. generally prey on ciliates and heterotrophic dinoflagellates more efficiently than on phytoplankton because their fast mobility increases encounter rates and thus capture efficiency (Levinsen et al. 2000). Hence, females could gain a nutritional advantage which may help to ensure survival by preying on protozoa, even when they are not highly abundant. However, this heterotrophic assemblage may not have been adequate for egg production because egg production is more nutritionally demanding than female basal metabolism. It should be also considered that our feeding rates are conservative values (see 'Materials and methods'), and that this fact may help to explain herbivore feeding which is not sufficient to support carbon demands.

Table 6. *Calanus helgolandicus* and *Calanoides carinatus*. Daily carbon requirements (in µg C female^{-1} d^{-1}) for egg production rate (EPR) and basal metabolism (respiration, *R*) and percentage of requirements covered by ingestion on phytoplankton (C_{ing}/C_{req}) for coastal, mid-shelf and saline slope current stations. *R* was calculated according to Ikeda et al. (2001). Our *R* ranged between 0.38 and 0.45 µl O_2 female^{-1} h^{-1} for *C. helgolandicus*, and between 0.26 and 0.32 µl O_2 female^{-1} h^{-1} for *C. carinatus*. Carbon requirements for metabolism were estimated as $C = R \times RQ \times (12/22.4)$ using a RQ (respiratory quotient) of 0.97 (Omori & Ikeda 1984). Carbon requirements for egg production were estimated by multiplying EPR per egg carbon content and applying a gross growth efficiency of 30%

Zone	Stn	C requirements		C_{ing}/C_{req}
		R	EPR	
C. helgolandicus				
Coast	A1	5.3	9.5	19.8
	A2	4.7	7.4	68.2
	B1	5.7	19.8	84.3
	C1	6.2	22.2	>100
	D1	5.3	20.6	>100
Mid-shelf	A3	6.0	10.8	15.1
	B2	6.0	19.9	>100
	C2	5.0	22.8	15.6
	D2	5.4	26.4	>100
Slope current	B3	5.6	2.0	50.3
	D3	5.2	0.4	38.7
C. carinatus				
Coast	A1	4.0	26.3	31.4
	A2	3.7	28.7	29.1
	B1	4.0	19.2	>100
	C1	3.9	29.3	>100
	D1	3.9	42.9	>100
Mid-shelf	A3	3.8	22.8	11.2
	B2	3.8	36.0	9.2
	C2	3.6	43.7	15.9
	D2	3.8	51.7	>100
Slope current	B3	3.6	15.2	13.7
	D3	3.2	0.5	50.6

The food resources available in the saline current were apparently not suitable for the population EPR. Applying an annual mean egg hatching success of 82% for *Calanus helgolandicus* and 71% for *Calanoides carinatus*, obtained at 2 stations close to the study area (Ceballos & Álvarez-Marqués 2006), the potential recruitment was only 8 to 25 nauplii d^{-1} m^{-3} for *C. helgolandicus* and 20 to 71 nauplii d^{-1} m^{-3} for *C. carinatus*.

Primary production and phytoplankton biomass at coastal and mid-shelf stations were similar to levels measured in the early spring bloom in these waters (González-Quirós et al. 2004). These conditions seemed to enhance the production of both species, compared with that in the slope saline current. The mean EPR at the coast and mid-shelf was similar to EPRs measured during the productive seasons in the study area (Ceballos & Álvarez-Marqués 2006) and adjacent areas (e.g. Pond et al. 1996). Although female ingestion rates were higher in nearshore waters than at the slope current stations, the carbon requirement for respiration and egg production was almost fulfilled by herbivorous feeding at only 4 or 5 stations in this zone, for *Calanus helgolandicus* and *Calanoides carinatus*, respectively, and a big deficit was found at some other stations (Table 6). As proposed for the saline current, protozoa could also have provided an alternative prey for females, for example at Stn C2, where ciliates were abundant. Interestingly, there was no indicator of potential food that directly explained herbivorous ingestion by *C. carinatus*. However, the relationship between diatoms and egg production described here, as well as previous information on the importance of diatoms for *C. carinatus* ingestion and production (Smith 2001), indicates that females could feed actively on diatoms. *C. carinatus* shows a high selectivity on a species basis, and the highest rates were found in assemblages dominated by *Nitzschia delicatissima* and *Rhizosolenia styliformis* (Smith 1995). In agreement with this, stations where ingestion rates were highest during our cruise were characterised by moderately high or high diatom abundance and high concentrations of *Nitzschia* spp. and *Rhizosolenia* spp. The selective feeding behaviour of copepods is enhanced in situations with low food diver-

sity (Turner & Tester 1989), so *C. carinatus* would probably have selected these diatom species during the cruise. This may also explain why the *C. carinatus* ingestion rate did not show high variability between the remaining stations, even though diatom concentration did (see Fig. 5).

EPR of both species showed a saturation response to diatom concentration. This relationship was also observed for *Calanus helgolandicus* ingestion. The highest carbon ingestion rates by *C. helgolandicus* and *Calanoides carinatus* found in the present study were in the upper range of those reported in the literature (see Tables 19 & 21 in Mauchline 1998). In terms of ingestion on chl *a*, although we found some very high values, they were not close to the maximum values previously reported for *Calanus* spp. or *C. carinatus.* This is due to the fact that most of the authors used the standard ratio C:chl *a* of 50, whereas we used a higher ratio, which was measured during the cruise, and similar to other *in situ* ratios (e.g. Irigoien et al. 2000).

Although EPRs of both species attained high values at several stations, a comparison of the maximum EPR found here with previously reported *in situ* maximum rates (>50 eggs female^{-1} d^{-1}, e.g. in Laabir et al. 1998 for *Calanus helgolandicus* and >60 eggs female^{-1} d^{-1}, e.g. in Richardson et al. 2001 for *Calanoides carinatus*) implies that both species were far from saturation. Moreover, Poulet et al. (1996) pointed out that the food concentration needed to obtain a saturation response in egg production for *C. helgolandicus* ranged between 80 and 250 µg C l^{-1}, and we had only about 13 µg C l^{-1}. Accordingly, the saturation relationship between egg production and diatoms is somewhat surprising, because it seems that something not related to physiological constraints was slowing EPR down at stations with a high diatom abundance. It is possible that a non-suitable food may explain these results. At low food concentrations, the egg production of copepods is limited by the quantity rather than by the quality of the food, whereas at high concentration, food quantity is important only if food is not diverse enough to compensate for poor nutritional value (Guisande et al. 2000, and references therein). Assuming that females fed mainly on diatoms, egg production would have been limited by food quantity at those stations with low diatom concentrations, whereas high diatom concentration would have induced an increase in egg production. Nevertheless, at stations with the highest diatom concentration (where functional response saturates), the diatom diversity was very low and, therefore, egg production may have been limited due to a nearly mono-specific diet. Several studies have screened diatom species and examined the role of essential nutrients (e.g. fatty acids and amino acids) in an attempt to explain why copepod fecundity is sometimes low when animals feed on certain species of diatoms, although the results to date are not consistent (e.g. Lacoste et al. 2001, Pohnert et al. 2002). Different field studies have shown that some diatoms may depress copepod recruitment by producing secondary metabolites that reduce egg production and/or egg hatching success (Miralto et al. 2003, Ianora et al. 2004). We cannot rule out the possibility that some species of diatoms were able to produce these kinds of toxins at the stations where functional response saturated. In addition, silica frustules of the diatoms could hinder its ingestion (Besiktepe & Dam 2002); as a consequence, a diet based mostly on diatoms might lead to a reduction in assimilation efficiency.

Lipid reserves and egg production

The egg production recorded during our cruise seems to be supported exclusively by freshly ingested food and not by lipid reserves. Lipid storage of *Calanus helgolandicus* and *Calanoides carinatus* females was low compared with previous data (Rey-Rassat et al. 2002a,b for *C. helgolandicus* and Arashkevich & Drits 1997 for *C. carinatus*). The C:N ratio of females suggests low lipid reserves and a protein-based metabolism (Tande 1982, and references therein). Female lipid reserves were highest at Stns A3 and C2 and it is likely that females at these stations had better food conditions during development and growth, which enhanced lipid deposition. It is also possible that the advection process had moved copepods from one place to another during the time when lipid deposition occurred, explaining the high lipid content at these 2 stations and the same amount of lipid reserves at the remaining ones. However, this difference in lipid deposition between stations did not result in differences in reproduction.

Calanoides carinatus lipid stores are used during gonad development in the CV stage and, therefore, partially, to produce the first eggs of young females (Arashkevich et al. 1996); however, there is no information to date on lipid support of egg production after that stage. In fact, *C. carinatus* shows a strong relationship between current feeding and egg production, responding to the availability of food in the previous 24 h (Huggett 2001). The low lipid content of females found here should not be a constraint for egg production. Moreover, low lipid storage in *C. carinatus* females was consistent with the use of lipids to fuel gonad development, since females have mature gonads with oocytes in Vitellogenesis II. This may also be valid for *Calanus helgolandicus*, but this species needs both good current conditions and a buffer of lipids to produce at high rates during the entirety of its spawning

life (Rey-Rassat et al. 2002b). The low WE content of *C. helgolandicus* found during the cruise could hence be a limiting factor for fecundity. This may help us to understand why egg production was not maximum and explain the relationship between egg production and ingestion rates with diatom concentration. High food levels (i.e. here high diatom concentrations) could not compensate for the constraints that low lipid reserves of *C. helgolandicus* females placed on fecundity, since lipid reserves could limit the egg production even at stations with sufficient food to attain high ingestion rates. This explanation agrees with Rey-Rassat et al. (2002b), who underlined the importance of evaluating the environmental conditions not only at the time of sampling but also those in the recent past as well as the growth history of the females. Hence, estimates of lipid storage should be made routinely to help understand the *in situ* egg production of species such as *C. helgolandicus*. The egg production of *C. helgolandicus* was measured in the central Cantabrian Sea in May 1996 by González-Quirós et al. (2003). Although they found high phytoplankton biomass, the EPRs showed low values that were uncorrelated to the phytoplankton biomass. Low lipid reserves could help us to explain this result, but unfortunately, lipid stores were not measured in this work. Finally, we cannot exclude the possibility that *C. carinatus* females use lipid storage in the same way *C. helgolandicus* does in the Cantabrian Sea; more work is needed to understand the role of *C. carinatus* lipid reserves outside African upwelling systems.

Productive late spring conditions and population recruitment of *Calanus helgolandicus* and *Calanoides carinatus*

The mesoscale structures found during our study may be a regular phenomenon at the end of the spring season (González-Quirós et al. 2004), and the resulting extra time for production after the spring bloom would provide several advantages for copepod annual recruitment. Firstly, females can produce eggs over a longer period. Secondly, these conditions may enhance naupliar survival. The risk of starvation for young stages born during the spring blooms is high due to the short duration of these blooms. It has been suggested that the best time for naupliar birth is before the spring bloom onset because this ensures a suitable food supply during the whole period of development and growth (e.g. Melle & Skjoldal 1998). Accordingly, conditions that prolong the spring bloom, such as were found here, may reduce nauplii starvation. Thirdly, late copepodite stages may be able to take advantage of the long period of high food conditions by storing lipid reserves that allow them to develop into a successful overwintering generation. In the present study, for example, the late copepodites (CIV and CV) had large, bright orange lipid sacs extending longitudinally and ventrally through the cephalothorax at all stations (data not shown).

There is not much information on overwintering strategies of *Calanoides carinatus* and *Calanus helgolandicus* populations in the Cantabrian Sea. In the northwest African upwelling areas, *C. carinatus* is known to diapause, as Stage V copepodites, in deep waters during the non-upwelling season and to inhabit surface waters only during the upwelling (e.g. Binet & Suisse de Sainte Claire 1975). However, in northern Benguela, where upwelling is also seasonal, *C. carinatus* inhabits continental shelf waters throughout the year; its population consists of active individuals that feed and reproduce in surface waters over the shelf and the slope, and of diapausing Stage V copepodites in the deep oceanic waters. During the non-upwelling season active individuals appear in lower numbers (Timonin et al. 1992, Arashkevich et al. 1996). Given this, it is likely that during winter at least a fraction of the *C. carinatus* population in the central Cantabrian Sea undergo diapause in oceanic waters. The dormancy phase in *Calanus* spp. has been well established for many species (see review in Hirche 1996), although for *C. helgolandicus* there is no clear evidence of it for populations that inhabit coastal waters. However, a diapause phase during Stage CV in deep waters seems to be feasible for offshore populations (e.g. Williams & Conway 1988, Andersen et al. 2001). Williams & Conway (1982, 1984) showed that the overwintering stock in shallow waters off the Celtic Sea shelf consists of adults and Stage V copepodites distributed homogeneously throughout the water column. These animals showed low metabolic rates but fed and probably produced eggs. Hirche (1983) also showed that some *Calanus finmarchicus* populations remain in shallow waters and fiords during winter, the copepods exhibiting reduced metabolism but not yet total diapause. Similarly, Durbin et al. (1997) reported continuous reproduction of *C. finmarchicus* during winter in the Gulf of Maine. This might also occur in the central Cantabrian Sea. A mixed strategy that includes a first resting stock at the end of the spring bloom and an active population at the surface during summer, which in turn produces a second resting stock, might also be possible. In this case, the high lipid stores, accumulated during the extended productive springtime, may allow copepodites to survive during the subsequent and prolonged summer, when food resources are not suitable. In the Cantabrian Sea, light conditions are still favourable at the end of summer, and the breaking of the thermal stratification induces an input of nutri-

ents into the euphotic layer, leading to an autumn phytoplankton bloom (Fernández & Bode 1991). If a large number of *C. helgolandicus* and *C. carinatus* individuals were able to survive until this time in a good nutritional condition, a successful reproductive event associated with the autumn bloom might occur. This could lead to a large winter surface population and/or a large diapause stock.

An interesting result of our study was the lack of a pattern in the spatial distribution of the population production rates of *Calanus helgolandicus* and *Calanoides carinatus*. This was because egg production was correlated with food availability, but abundances were not related to any environmental parameter. The uncoupling of primary producers and herbivorous biomass has been observed before, for example, in Mediterranean copepod populations (Calbet et al. 2002, and references therein). It can be argued that a number of factors such as predation or advection may explain this result. Nevertheless, during our cruise the distribution of the more important fish larval species (e.g. sardine and mackerel), as well as of other copepod predators such as chaetognaths, was uncoupled from *C. carinatus* and *C. helgolandicus* population abundance (data not shown). Processes resulting in the importation/exportation of animals cannot be ruled out, but the presence of the subsurface saline slope current and its associated saline front would restrict the ocean–coast transport of animals; transport parallel to the coast may be feasible however. In fact, a general eastern transport of water along the slope during the cruise was described (González-Quirós et al. 2004).

González-Quirós et al. (2004) proposed that, given the high values of large phytoplankton production and of mesozooplankton biomass found on the continental shelf, the mesoscale features that occur during the late spring might have an important influence on the energy flow towards higher trophic levels (fish) after the phytoplankton spring bloom in coastal areas. In this paper, we show that the reproductive activity of 2 large herbivorous copepods suggests that they may efficiently exploit the high biomass of large phytoplankton cells induced by these mesoscale structures, so our results seem to complement the findings of these authors.

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