Egg production and hatching success in the peri-Antarctic copepod *Calanus simillimus*

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Abstract. *In situ* rates of egg production and hatching success are reported for *Calanus simillimus*, one of the most abundant calanoid species in peri-Antarctic regions, during the Italian 'Italica' cruise in the Straits of Magellan in March–April 1995. Low fecundity (8.6 eggs female^{-1} day^{-1}) and fecal pellet production (0.8 fecal pellets female^{-1} day^{-1}) in this period indicate that the species was feeding very poorly. Sixty-six of the 126 females sampled did not produce eggs and 80 of these did not produce any fecal pellets during the 24 h period of incubation. Striking abnormal naupliar and embryonic development was recorded in 81.8% of the eggs spawned. Aberrant eggs did not undergo normal cleavage, and failed to develop to hatching. Deformed nauplii were asymmetrical and presented strong anatomical anomalies in the total body length and number of swimming appendages. These results are discussed in the light of recent findings on the causes of low hatching success in copepods.

Introduction

Calanus simillimus is widely distributed over northern Antarctic and sub-Antarctic oceanic areas. The species ranges up to 1000 m and exhibits marked and rapid diurnal vertical migrations (Hardy and Gunther, 1935). According to Atkinson (1991), *C.simillimus* overwinters as late copepodite stages, mainly below the 250 m depth, and rises to spawn the following spring. The summer generation develops very rapidly and may give rise to a small second generation in late summer. The lifespan of *C.simillimus*, however, seems almost certainly to last only 1 year.

Not much is known about the reproductive biology of this species. Ward and Shreeve (1995) reported that *C.simillimus* generally spawns in the spring in the central part of its geographic range, with peak recruitment rates in November through January. The same authors found no significant relationship between egg production rates and measured surface chlorophyll (Chl) levels, suggesting the use of stored reserves to fuel reproduction.

The present study reports egg production rates and hatching success for *C.simillimus* in the Straits of Magellan at the beginning of the austral autumn. This is the most common *Calanus* species in the Straits (Mazzocchi *et al.*, 1995), with maximum abundances in the central area where it has been sampled at all depths. We show that, in this period, females continue to spawn, but most of the eggs produced are non-viable or hatch into malformed nauplii.

Method

Zooplankton samples were collected at the beginning of the austral autumn (27 March–1 April 1995) by the R/V 'Italica'. *Calanus simillimus* stage composition and vertical distributions were determined at eight stations (S1–S8) from west of

Cabo Deseado to off Punta Arenas (Figure 1). Samples were taken at discrete depths, from the surface to 700 m during both day and night, with an EZNET BIONESS opening/closing net equipped with 12 230-μm nets arranged on the main frame (mouth opening of 1 m²), and fixed immediately with 4% sea water–formalin. The net was equipped with a KMII (ME Meerestichnik Elektronic GmbH9) multiparametric probe which continuously monitored temperature, salinity and light attenuance. Flow velocity through the nets was monitored with external and internal acoustic Doppler flowmeters (SM 21H-ME Meerestichnik Elektronic GmbH). Chlorophyll *a* concentration was calculated from fluorescence values measured with a Mie-Backscat Fluorometer (Haardt Optik Mikroelektronik) mounted on the net. The EZNET BIONESS was towed at a speed of 1.5–2 m s⁻¹ and the nets were opened and closed at the desired depth by command from the ship. The volume of water filtered for each sample varied from 56 to 1234 m³ with greater volumes filtered at increasing depths.

Calanus simillimus egg production rates were determined at eight stations (S4, S6–S12), four of which were the same as before, in the central and Paso Ancho areas of the Straits where greatest abundances of adult females were recorded.
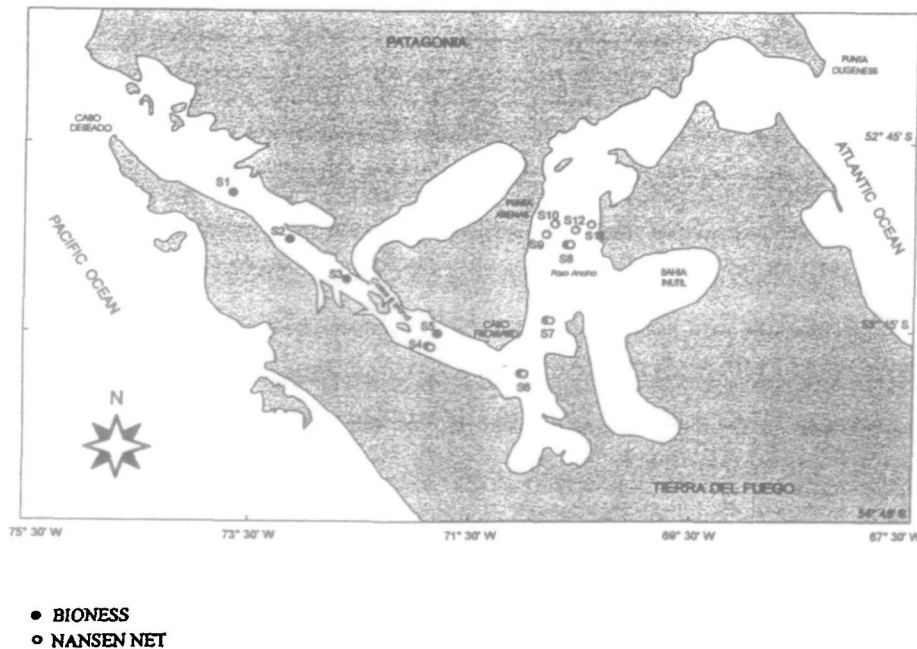

- ● *BIONESS*
- ○ NANSEN NET

Fig. 1. Location of sampling sites in the Straits of Magellan during the R/V 'Italica' cruise in March–April 1995 where black circles indicate zooplankton samples collected with the EZNET BIONESS opening/closing net and gray circles indicate live *C.simillimus* samples collected with a NANSEN net.

Hatching success and egg and naupliar mortality were calculated at the five central stations in the Paso Ancho area (S8–S12).

Adult females were sorted from live plankton samples collected by oblique tows with a Nansen net from 50 m to the surface. Females were placed individually in crystallizing dishes containing 100 ml of unfiltered sea water collected at the same sampling site. They were then incubated in temperature- and light-controlled chambers at 8°C and 12 h light:12 h dark, similar to ambient conditions. After 24 h, females were removed and eggs and fecal pellets counted with an inverted microscope. After an additional 72–96 h, egg viability was determined by counting the number of empty egg membranes and unhatched eggs, which was equal to the number of eggs at the start of the incubation, indicating that membranes were not lost after hatching. A tally was also kept of the total number of abnormal nauplii. Eggs and nauplii were considered abnormal when there was a lack of symmetry in the arrangement of the cellular nuclei of the dividing embryo or when the hatched nauplii were characterized by an asymmetry in the body appendages such as those previously described for *Calanus helgolandicus* (Poulet *et al.*, 1995). Egg and naupliar mortality were calculated by assuming that all of the malformed embryos and nauplii would develop abnormally and die shortly afterwards.

To test whether low spawning activity was due to low ambient food concentrations, five replicate samples of five females were incubated for 5 days in crystallizing dishes with unfiltered water enriched with dense concentrations of phytoplankton cells obtained by horizontally towing a 40 μm phytoplankton net at the surface for 10 min. Each day, females were transferred to new containers with fresh media and the original container bottom was examined for the presence of eggs and fecal pellets.

Results

Although *C.simillimus* was widely distributed, the greatest concentrations occurred in the central area of the Straits (Figure 2A and B). The three most Pacific stations (S1, S2 and S3), where lowest adult *C.simillimus* abundances were recorded, were characterized by relatively low Chl *a* values and stratified water masses, suggesting the intrusion of Pacific waters up and until Carlos III Island (Figure 2C and D). The species was much more abundant at the three intermediate stations (S4, S5 and S6), characterized by more mixed waters and somewhat higher Chl *a* values at all depths. High abundances also occurred in the Paso Ancho area (S7, S8) where the highest Chl *a* values were measured, especially in the upper 70 m. The stage composition of the population at the time of sampling (beginning of the austral autumn) was characterized by a strong dominance of the copepodite V (CV) stage (91.9%). The rest of the population consisted of females (6.8%) and males (1.1%); all other stages were lacking.

Calanus simillimus adults occurred in high numbers at all depths sampled, except for the Pacific area (S1, S2, S3) where they were absent from surface waters (Figure 2C). CVs were more abundant below 100 m with standing stocks that were an order of magnitude higher than at the surface; adults were more
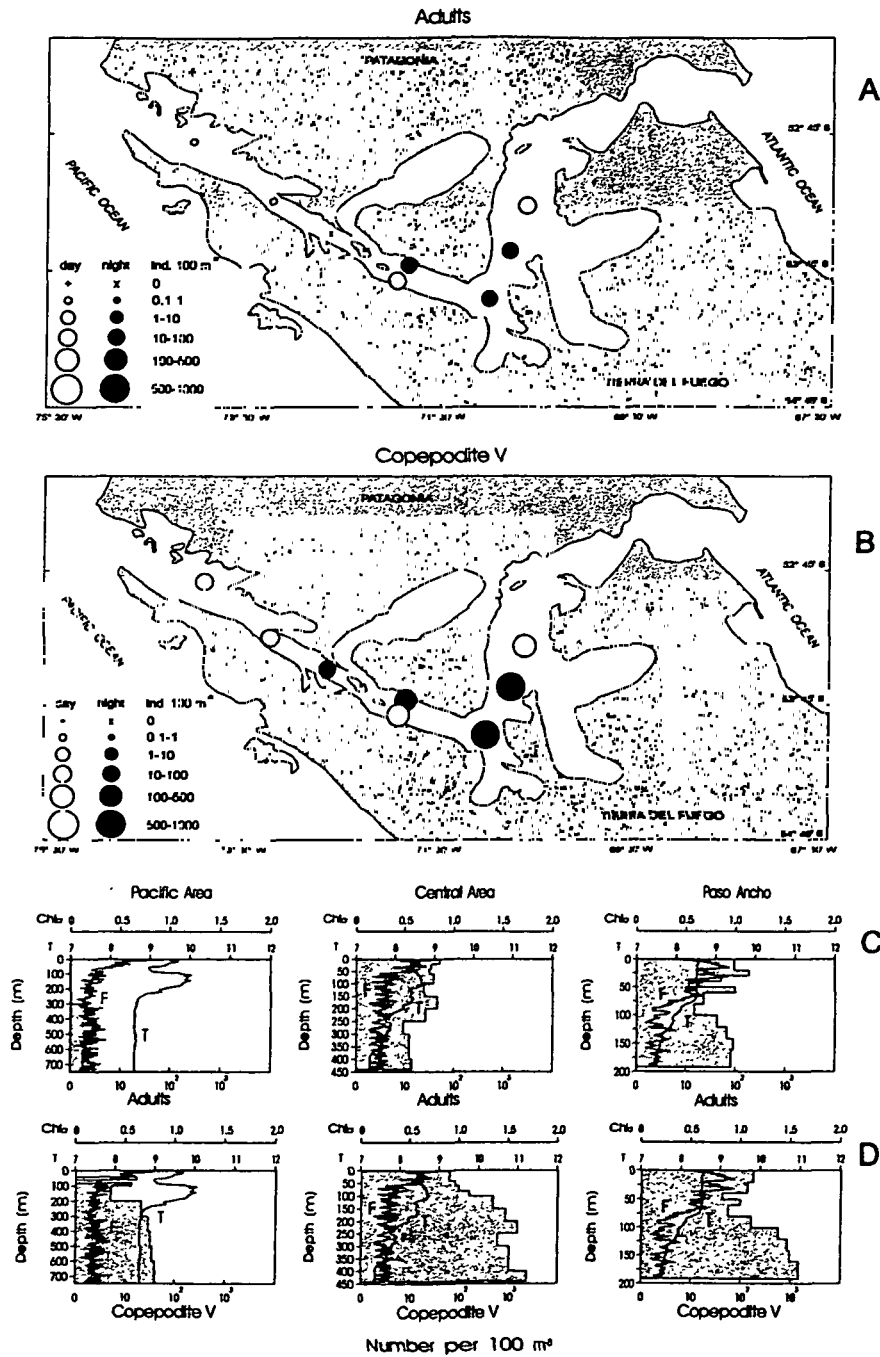
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Fig. 2. *Calanus simillimus*. Spatial distribution of adults (A) and copepodite V (B) stages obtained by grouping all data at the different depths. Vertical distribution of adults (C) and copepodite V (D) stages obtained by grouping data for Pacific (S1, S2, S3), Central (S4, S5, S6) and Paso Ancho (S7, S8) areas. Vertical profiles are also given for temperature (T) and fluorescence (F) where *in vivo* fluorescence values ranging from 0 to 10 volts correspond to 0–50 mg m^{-3} Chl *a* with <10% variability.

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evenly distributed throughout the water column. The two stations where both day and night samples were taken (S4, S8) indicate that the population does not undergo diurnal migrations in this period.

Egg and fecal pellet (fp) production, and egg hatching success, were determined for 126 adult females. Mean fecundity (8.6 eggs female^{-1} day^{-1}) and fecal pellet production (0.8 fp female^{-1} day^{-1}) were very low throughout the area, indicating that animals were feeding at very low rates at the time of collection (Figure 3A and B). Sixty-six of the 126 females (52.4%) sampled did not produce any eggs and 80 (63.5%) did not produce fecal pellets during 24 h (Figure 3C and D). Although they were not feeding, females did not appear to be in diapause since they swam very actively and showed no sign of retarded movements. However, when females were provided excess food obtained from net phytoplankton hauls, there was no resumption of egg and fecal pellet production after 5 days, indicating that females were not responding to the presence of food (Table I). Only in one case (trial 2) did females produce 29 fecal pellets (5.8 fp female^{-1} day^{-1}) on the fifth day.

Development times to hatching were <72 h. Nonetheless, unhatched eggs were again observed 96 h after spawning to avoid underestimation of hatching success.

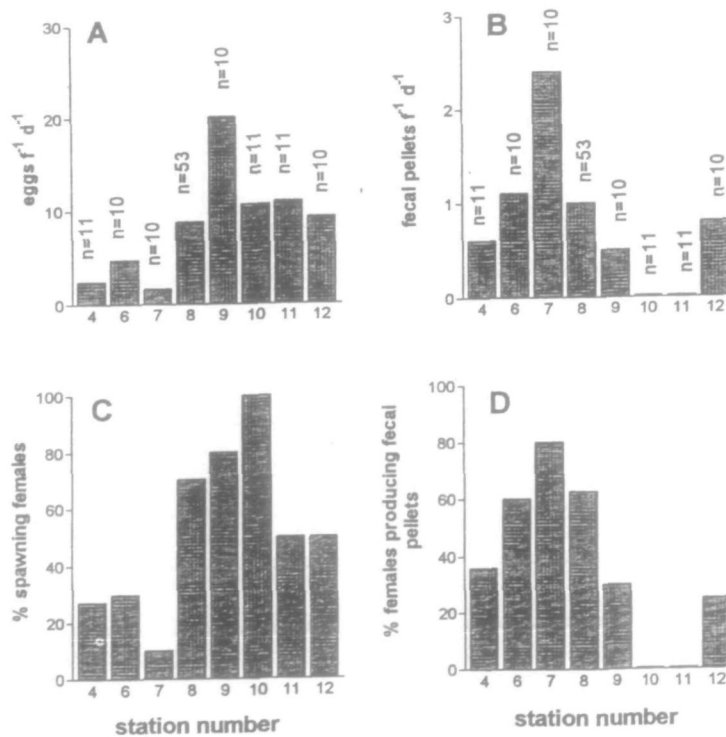**Fig. 3.** *Calanus simillimus*. Egg production (**A**), fecal pellet production (**B**), percentage of spawning females (**C**) and percentage females producing fecal pellets (**D**) after 24 h incubation experiments. *n* is the number of females.

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Table I. Egg and fecal pellet (fp) production rates over time for *C.simillimus* females fed excess food from net phytoplankton hauls

Days	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Eggs	fp	Eggs	fp	Eggs	fp	Eggs	fp	Eggs	fp
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	8	4*	0
3	0	1	0	2	0	0	0	0	0	0
4	0	0	0	3	0	0	0	0	0	0
5	0	0	0	29	0	1	0	0	0	0

*Abnormal eggs.

By this time, unhatched eggs showed strong abnormal cellular divisions. Normal, freshly spawned *C.simillimus* eggs were characterized by an elevated fertilization envelope which surrounded the developing embryo and a distinct lipid-like 'eyespot' at the center of the dense cytoplasm (Figure 4A). In unhatched eggs, this spot was often fragmented into several granules (Figure 4C) and the developing embryo was clearly asymmetrical and divided abnormally (Figure 4E). Many of the hatched nauplii showed strong anatomical deformities in the shape and length of the body, and number of feeding appendages (Figure 4D and F), compared to normal nauplii (Figure 4B). None of these deformed nauplii survived for more than 24 h in the laboratory.

Both egg and naupliar viability were very low at all stations sampled (Figure 5). Mean (± SD) egg mortality was 72.5 ± 21.2% of the total production, reaching up to 100% in some cases (S12); mean naupliar mortality was much lower (11.7 ± 6.7%). Together, total mortality ranged from 60 to 100% with a mean value of 81.8 ± 16.1%. Such dramatically high rates have never been reported for sub-Antarctic regions and may contribute to the rapid decline in the population at the end of summer.

Discussion

Several factors are known to affect the physiological mortality of copepod eggs and nauplii in temperate areas. Among these, remating success has been the most studied with results confirming that species of the genus *Calanus* require a single insemination to fertilize the total eggs produced by a single female (Marshall and Orr, 1972; Runge, 1984). More recent studies have shown that low egg viability may also depend on an inadequate diet related to concentrations of fatty acids and, in particular, to the polyunsaturated fatty acids in the food (Kleppel and Burkhart, 1995; Jónasdóttir and Kiørboe, 1996) or to the presence of deleterious anti-mitotic compounds blocking normal embryonic development (Poulet et al., 1994). The latter compounds, suspected in at least 16 diatom species screened until now (Ban et al., 1997), are accumulated in the gonads in a dose-dependent manner, inducing up to 100% egg mortality within 5–10 days of continuous feeding (Chaudron et al., 1996). However, in the present study, females had stopped feeding or were feeding at very low rates, as inferred by the low number
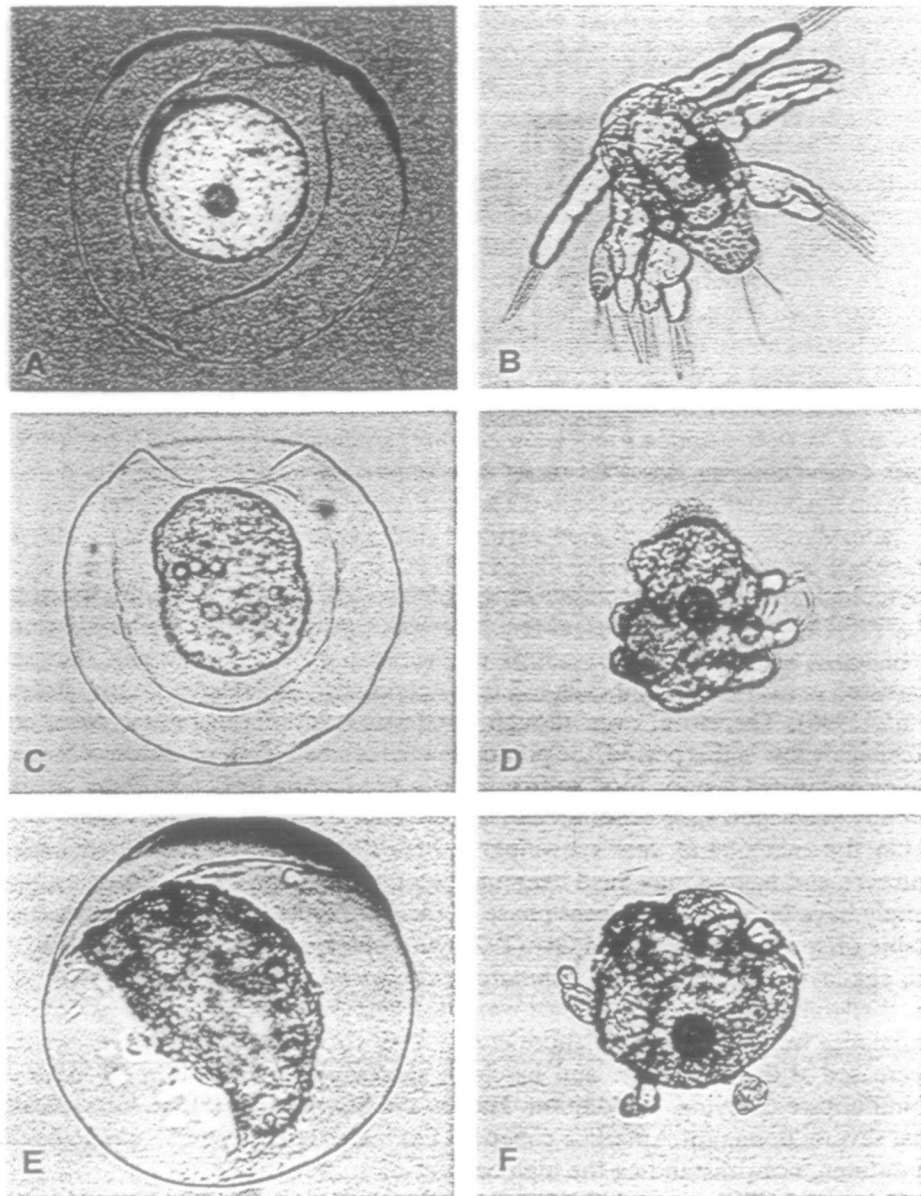
Fig. 4. *Calanus simillimus*. Normal (**A**) and abnormal (**C–E**) newly spawned eggs. The mean diameter of normal eggs is 310 μm (including the fertilization membrane). Normal (**B**) and abnormal (**D–F**) hatched nauplii. The mean length of normal nauplii is 236 μm.

of fecal pellets produced (0.8 fp female^{-1} day^{-1}). Females also did not resume feeding even after 5 days of exposure to dense quantities of ambient phytoplankton cells. At the time, Chl concentrations in the central and Paso Ancho areas of the Straits ranged between 0.3 and 1.0 μg Chl *a* l^{-1} (Figure 2) with

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■ % naupliar mortality
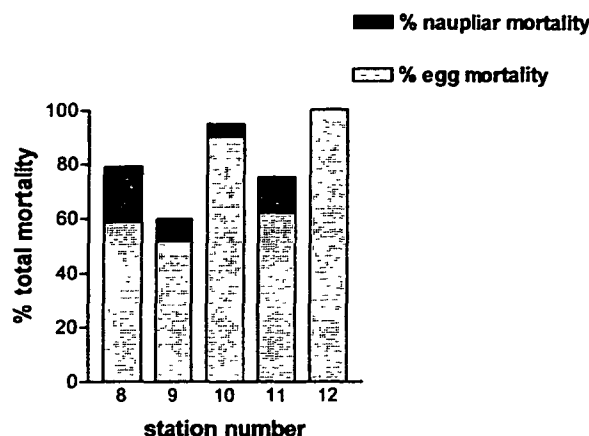
▭ % egg mortality

Fig. 5. *Calanus simillimus*. Percentage egg and naupliar mortality.

phytoplankton cell concentrations ranging from 10 to 30 × 10^4 cells l^{-1} (Iriarte *et al.*, 1996). Most of the phytoplankton, however, consisted of small-sized diatoms (<10 μm), which constituted >50% of the bulk of the population; the rest consisted mainly of coccoid cells (<5 μm) and microflagellates (<10 μm) (Iriarte *et al.*, 1996). Therefore, even though phytoplankton concentrations were not excessively low in this period, the predominance of small phytoplankton cells may have constituted a poor food source for egg production in *C.simillimus*, as has already been shown for other species of the genus *Calanus* (Runge, 1985).

On the other hand, low spawning activity and/or production of abnormal embryos and nauplii may have been unrelated to nutritive effects. Females may simply have been in a senescent phase of their life cycle and too old to produce viable offspring. Marshall and Orr (1972) also observed that towards the end of the egg-laying period, *Calanus finmarchicus* eggs were less healthy. Fewer eggs hatched and many went opaque or were laid with defective membranes. Similar anomalies were reported by Ianora *et al.* (1996) for *Acartia clausi*. Egg viability decreased with female age and hatching success was <50% after 20 days of continuous egg laying. In addition, Hagen and Schnack-Schiel (1996) reported that several dominant Antarctic copepods contained spent or semi-spent gonads in autumn, notwithstanding the high content of lipid reserves.

Calanus simillimus populations over the South Georgia shelf break in November and December were dominated by CVs, but also by younger stages (CI–CIII) or adults (Ward and Shreeve, 1995). These authors reported that 72% of the females spawned eggs during the 24 h incubation period as compared to 48% in this study. Assuming that the *C.simillimus* populations of the South Georgia shelf and Magellan areas have similar biological life cycles, the data suggest a progressive diminution in the number of spawning females with time in sub-Antarctic areas. Also, the high mortality of embryos and nauplii suggests that very few younger stages are recruited into the plankton by the beginning of the austral

autumn. The overwintering population consists only of CVs that have matured after the summer and that constitute >90% of the *C.simillimus* population in this period. These individuals do not undergo diel migrations in our study area and are mainly concentrated in deeper waters in the central areas of the Straits (>100 m).

The present study is one of the first to report high egg and naupliar mortalities for polar regions. The mortality of *C.simillimus* eggs and nauplii ranged from 60 to 100%, with mean values of 81.8% in March–April. High egg mortality (30–65%) was also recently reported by Burkhart and Kleppel (1998) for several Antarctic species from the Weddell Sea in November–December, indicating that low hatching success may also occur earlier in the year. Much lower annual values of ~30% egg mortality have been reported for *Acartia tonsa* (Ambler, 1985), *Centropages typicus* (Ianora *et al.*, 1992), *Temora stylifera* (Ianora and Poulet, 1993) and *Calanus helgolandicus* (Pond *et al.*, 1996; Laabir *et al.*, 1997) in sub-temperate regions. However, values as high as 80% egg mortality have been recorded for *C.helgolandicus* in the English Channel in certain periods of the year (Laabir *et al.*, 1997), similar to values reported here for the Magellan Straits.

In conclusion, our results indicate that hatching success may be an important factor limiting the population growth of species not only in temperate but also in polar areas, and that the causes of high egg mortality may not only be related to food characteristics, but also to other biotic factors such as failure to feed and/or female age and physical well-being, as suggested in this study.

Acknowledgements

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