

# Temperature and female size effects on egg production of *Calanus chilensis*: Laboratory observations

Efectos de la temperatura y tamaño de las hembras sobre la producción de huevos de *Calanus chilensis*: observaciones en laboratorio

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## ABSTRACT

Three experiments under laboratory condition were conducted to study the effect of temperature and female size, measured as prosome length and dry weight, on egg production and egg viability of *Calanus chilensis* Brodsky captured from the Bay of Mejillones, northern Chile. In the first experiment egg spawning was monitored every 2 hours in females kept in individual vials at five constant temperatures, namely 9, 12, 15, 18 and 20° C. In a second experiment, temperature was constant, 15° C. Finally, a third experiment was conducted at three constant temperatures, 12, 15 and 20° C under excess of food to determine temperature effects on egg production for a more extended period of time. As food, a monoculture of *Isochrysis galbana* Parke was used for the first experiment and phytoplankton collected from the same Bay cultured in F2 medium was used for the second and third experiments. In the first experiment clutch size (CS), defined as the total number of eggs laid at once within a 2 h period, ranged between 5 and 84 eggs female<sup>-1</sup>. In most cases all the eggs were able to hatch and those that did not hatch were assumed as nonviable. CS was positively correlated to temperature, but only 2 out of 12 females were able to lay eggs at the lowest temperature (9° C), whereas at the highest temperature (20° C) CS tended to decline. There was a lack of synchrony in egg laying and no temperature effect on the number of nonviable eggs was detected. However a significant positive correlation between CS and the number of nonviable eggs was found ( $F_{1,25} = 4.67, P < 0.05$ ). In the second experiment CS was highly variable,  $36.2 \pm 18.67$  (mean  $\pm$  S.D.) and not associated with either female length ( $F_{1,26} = 1.86, P > 0.05$ ), or with female dry weight ( $F_{1,26} = 0.026, P > 0.05$ ). Finally from the third experiment, egg production on a daily basis, was drastically reduced at 20° C, while differences between 12° C and 15° C were not detected. Our results agree with previous studies indicating that egg production correlates positively with temperature. However, there appears to be an "optimal temperature range", within which maximal clutch sizes and daily egg production rates are attained. This temperature range coincides with that observed at the upper 10 m layer in the study area. This suggests that maximal egg production rates of this species would only take place at the upper layer. On the other hand, sharp population declines, such as that observed during "El Niño" 1991-92, may have been caused by abrupt changes in water temperature giving rise to a poor recruitment, because of depleted egg production taking place at temperatures greater than 20° C.

**Key words:** *Calanus*, egg production, temperature, body size.

## RESUMEN

Se realizaron tres experimentos en condiciones de laboratorio para estudiar el efecto de la temperatura y tamaño de las hembras, medido como longitud del prosoma y peso seco individual, sobre la producción y viabilidad de huevos de *Calanus chilensis* Brodsky capturados en la Bahía de Mejillones, norte de Chile. En el primer experimento el desove de huevos se inició con exceso de alimento y se registró cada 2 h por cerca de 30 h, a través de observaciones de las hembras mantenidas a 5 temperaturas nominales, 9, 12, 15, 18 y 20° C. En el segundo experimento la temperatura se mantuvo constante en 15° C. Finalmente, en un tercer experimento se utilizaron tres temperaturas, 12, 15 y 20° C para determinar el efecto de la temperatura sobre la producción de huevos en un período de tiempo más extendido. Como alimento se utilizó un monocultivo de *Isochrysis galbana* Parke para el primer experimento y fitoplancton colectado desde la misma bahía y cultivado en medio f/2 para el segundo y tercer experimento. En el primer experimento, el tamaño de la puesta de huevos (CS), definido como el total de huevos puestos a la vez, en un período de 2 h, fluctuó entre 5 a 84 huevos hembra<sup>-1</sup>. En la mayoría de los casos todos los huevos pudieron eclosionar y aquellos que no lo hicieron se definieron como no viables. CS estuvo positivamente correlacionado a la temperatura, aunque sólo 2 de 12 hembras fueron capaces de poner huevos a baja temperatura (9° C), mientras que a alta temperatura (20° C) CS mostró una tendencia a declinar. También existió una falta de sincronía en la puesta de huevos y la temperatura no afectó en forma significativa el número de huevos no viables. Sin embargo, se obtuvo una correlación significativa entre CS y el número de huevos no viables ( $F_{1,25} = 4.67, P < 0.05$ ). En el segundo experimento, CS fue muy variable,  $36.2 \pm 18.67$  (media  $\pm$  D.E.) y no estuvo asociado con la talla de la hembra ( $F_{1,26} = 1.86, P > 0.05$ ) ni con el peso seco de las hembras ( $F_{1,26} = 0.026, P > 0.05$ ). Finalmente en el tercer experimento, la producción de huevos en un ciclo diario, se redujo drásticamente a 20° C, mientras que entre 12° C y 15° C no se detectaron diferencias ( $P > 0.05$ ). Estos resultados concuerdan con estudios previos que describen la correlación positiva entre la producción de huevos y la temperatura. Sin embargo, parece existir un "rango óptimo de temperatura", dentro del cual el tamaño de la puesta de huevos y las producciones diarias alcanzan sus valores máximos. Tal rango de temperatura coincide con aquél observado en la capa de agua de 10 m en el área de estudio. Esto sugiere que la producción máxima de huevos ocurriría solamente en la capa de agua superficial. Por otra parte, una disminución drástica de las poblaciones de esta especie, tal como aquélla observada durante "El Niño" 1991-92, pudo haber sido causada por cambios abruptos en temperatura, dando origen a un pobre reclutamiento, debido a una baja en la producción de huevos en condiciones de temperatura superior a 20° C.

**Palabras clave:** *Calanus*, producción de huevos, temperatura, tamaño corporal.

## INTRODUCTION

The copepod *Calanus chilensis* Brodsky appears to be a key component of the Chile-Peru coastal upwelling ecosystem. This typical herbivorous copepod (Boyd et al. 1980) is very abundant (Heinrich 1973), and likely a major source of food for fish populations in the area.

Recent work has shown that this species may reproduce continuously in coastal waters of northern Chile throughout the year (Escribano & Rodriguez 1994) and may also show an exponential-type growth in natural condition (Escribano & Rodriguez 1995). These authors pointed out that continuous production of new cohorts of this species may be an indication of non-limiting food condition for population growth throughout seasons. Indeed this coastal zone appears to be highly productive at most times of the year, as shown by some measurements of phytoplankton biomass in the area, with levels that may exceed  $40 \mu\text{g l}^{-1}$  Chlorophyll-a (Rodriguez et al. 1991, Boyd & Smith 1980). Under such conditions reproduction may occur throughout the year. Thus egg production rates may primarily limit population growth of this species. In this context, information on the role played by environmental factors in regulating the production and hatching success of eggs seems very relevant to ascertain the productive potential of this species.

Egg production in marine and freshwater copepods may depend on various factors, such as food concentration and food quality (Arnott et al. 1986, Ianora & Poulet 1993), body size (Durbin et al. 1992), reproductive condition (Ianora et al. 1989) and temperature (Sekiguchi et al. 1980). Additionally the contribution of egg production to copepod recruitment may be limited by production of nonviable eggs (Ianora & Poulet 1993). Such eggs are either unfertilized, or simply fail to develop to hatching. Very little is known about the factors that may cause production of nonviable eggs. Even although Ianora and Poulet (1993) have recently shown that diatoms may strongly influence production of viable eggs, presence of nonviable eggs has often been viewed as anomalous (e.g. Parrish & Wilson 1978).

From the literature it is evident that much attention has been paid to the role of food quantity and quality on copepod egg production, but the effect of temperature on production of viable and nonviable eggs has not been deeply investigated. Sekiguchi et al. (1980) found a positive relationship between egg production and temperature, which presumably applies in nature above threshold food levels. Furthermore, some authors (Sekiguchi et al. 1980, McLaren & Leonard 1995) have sustained the hypothesis that egg production rates are equivalent to the growth rate of developmental stages, under condition of exponential, temperature-dependent growth rates. This suggests that egg production may be associated with temperature within a range that occurs in the water layer where egg laying takes place. Indeed phytoplankton levels do not explain the variation in egg production of *Calanus finmarchicus* Gunnerus in the Gulf of St. Lawrence (Ohman & Runge 1994) and food quality may be more related to the number of viable eggs rather than to the total number of eggs (Ianora & Poulet 1993).

Temperature is currently viewed as a key variable in controlling copepod growth and production (e.g. Davis 1987, McLaren et al. 1989; and Huntley & Lopez 1992 for review). As mentioned above, temperature may increase egg production, but this relationship is not well-known for *Calanus* species. Moreover the estimates of egg production in *Calanus* have often used short-term incubation of groups of animals, such that the individual responses due to size differences, or genetic variation within population (e.g. Marcus & Alatalo 1989) have not been evaluated. Individual variation might explain some of the variability in egg production rates during the annual cycle.

In this work we study the influence of temperature and female size on egg production and hatching success of individual females. We will not attempt to analyze any food effects on egg production, provided that animals are exposed to sufficiently high food levels to induce production of eggs and they experience similar conditions, such that their individual responses are comparable. Nevertheless, a description of field conditions will

be given as an attempt to explain observed responses in the laboratory.

#### METHODS

In a first experiment (Experiment I), females *Calanus chilensis* were sorted from zooplankton samples obtained during September 1994 from three points located at the Bay of Mejillones, Chile (Fig. 1). Zooplankton was captured using a 250  $\mu\text{m}$  Nansen-type net, vertically hauled from nearly 60 m to surface. Samples were immediately diluted on deck in a 68 l cooler and transported to the laboratory within 2 h. At the sampling locations vertical profiles of temperature, salinity and dissolved oxygen were obtained from near bottom (70 m) to surface using a Seabird-19 CTD. Water samples at 5 depths, near bottom, 18, 12, 10, 5 and 0 m, were also obtained to estimate chlorophyll-a concentra-

tion and to identify and count phytoplankton cells. Chlorophyll-a was estimated through spectrophotometry (Strickland & Parsons 1972), while phytoplankton cells were identified and counted under an inverted microscope.

At the laboratory, inside a 9° C coldroom, about 60 adult females were sorted from the zooplankton samples. In order to allow recovering from sampling and handling these females were kept overnight in a 2 l jar containing filtered seawater.

Five nominal temperatures were used for Experiment I: 9, 12, 15, 18 and 20° C. Temperature was set by using 15 l circulating baths equipped with thermostats and placed inside the 9° C coldroom. In each bath, 30 ml plastic vials were partly immersed to obtain the desired temperature. There were small differences of temperature between the baths and the vials, thus we only considered the values from the vials. The medium was

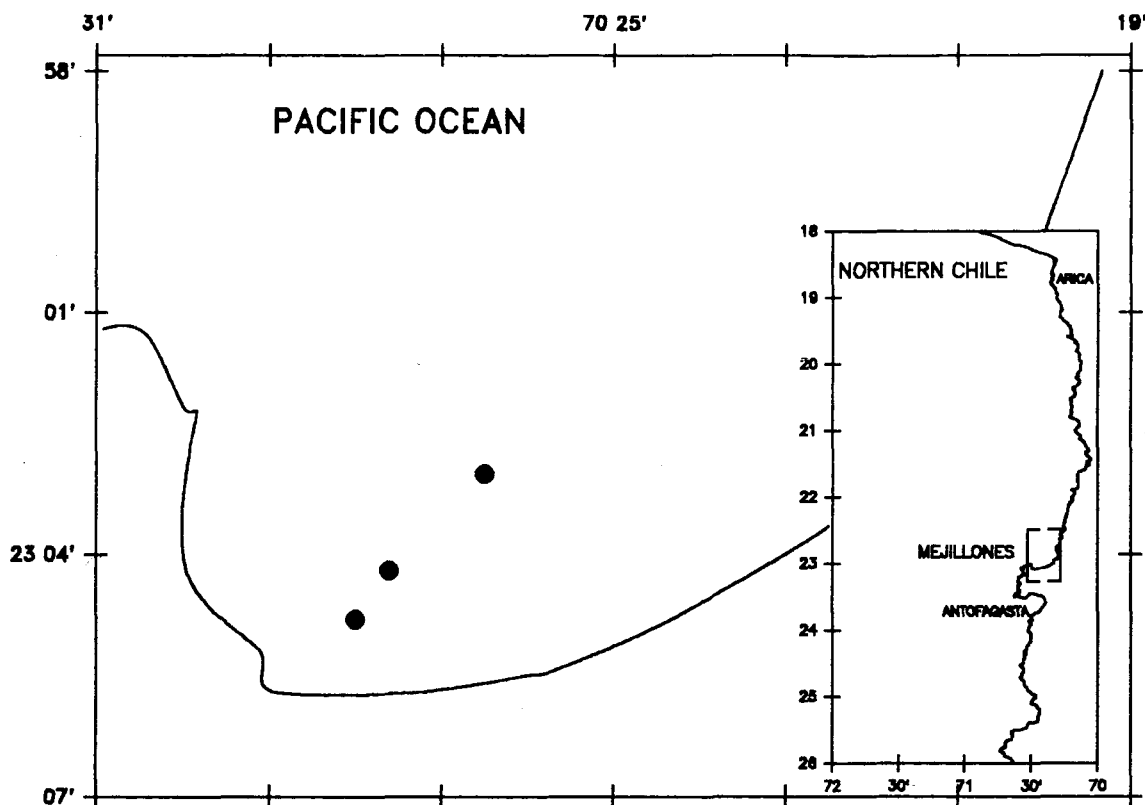


Fig. 1: Map showing the location of Bay of Mejillones and the three points from which females *Calanus chilensis* were captured along with oceanographic data to perform laboratory experiments on egg production.

Mapa mostrando la localización de la Bahía de Mejillones y los tres puntos desde los cuales se capturaron hembras de *Calanus chilensis* junto a información oceanográfica para realizar experimentos de laboratorio sobre producción de huevos.

prepared by using overnight aerated seawater, previously filtered with Millipore (0.45  $\mu\text{m}$ ) membranes. A unique medium was prepared for the five temperatures using *Isochrysis galbana* cultured in f/2 medium. The food concentration in the medium was estimated as  $1.3 \times 10^5$  cells  $\text{ml}^{-1}$ . This is a mean value from two aliquotes obtained at the beginning of the experiment. Twelve females for each nominal temperature were individually placed into the vials. From the start of the experiment and every 2 h thereafter each female was observed and the eggs counted. Observations were made under a dissecting microscope inside the coldroom using a cold and low intensity illumination. An observation for a single female did not take longer than 2 minutes and no detectable changes in vial temperature were registered. Production of fecal pellets was observed, but not quantified to verify if feeding had taken place. Temperatures in the vials and in the glass container were also checked every 2 h. Once a female had laid eggs, she was removed from the vial and the eggs counted. Thus, the total number of eggs, laid at once by a female (i.e. within 2 hours), was here defined as clutch size. We assumed that frequency of observations prevented significant loss of eggs through potential cannibalism. Only a single clutch for female was allowed. Prosome lengths of females were then measured to the nearest 0.01 mm using a calibrated micrometer-eyepiece. They were rinsed for fractions of seconds with distilled water, placed in very small pre-weighed aluminum pans and dried at 70° C in an oven. Constant weight was obtained within a few hours, but in order to standardize drying time, they were all dried for 12 h. Females were weighed for at least 3 readings using a Denver 250D microbalance to the nearest 0.01 mg.

In order to avoid excessive manipulation of the vials, altering temperature and disturbing the eggs or animals, there was no change of medium, or adding of food, and no information on food quality or quantity was obtained after the start of the experiment. However, once the females had been removed, about half of the remaining media was carefully pipetted out from the bottom of the vials and replaced with fresh filtered

seawater, which was maintained in each bath at the corresponding temperature. From this point the eggs were counted and observed every 2 h until hatching. At hatching much care was taken to count the remaining eggs, discarding any empty capsules. The observations continued until any remaining eggs started to disintegrate indicating that they were nonviable. No attempts were made to count swimming nauplii, but their behavior and motility were considered as an indication of quality of the medium. Light condition in the coldroom was at low intensity using a 12:12 h dark: light period.

The second experiment (Experiment II) was carried out in October 1994. For this, females were sorted at the same manner as in Experiment I from zooplankton samples captured at the same sampling sites at Bay of Mejillones (Fig. 1). About 50 females were incubated individually in the 30 ml vials at a constant temperature of 15° C in the coldroom. In this case, as an attempt to better simulate a natural situation, the females were fed with phytoplankton assemblages obtained a week before the experiment started from the same sampling site at Bay of Mejillones. For this, phytoplankton was collected using a 64  $\mu\text{m}$  net vertically hauled from 20 m to surface. The sample was diluted at the field and brought to the laboratory in a 10 l cooler. At the laboratory the sample was sieved through a 153  $\mu\text{m}$  mesh to remove zooplankton and cultured in f/2 medium (Sunda & Guillard 1976). This culture after a few days reached a stable composition dominated by the diatoms *Navicula cryptocephala* Kütz, *Rhizosolenia fragilissima* Bergon, *Skeletonema* spp. and at least three unidentified species of phytoflagellates at a total concentration of about  $6 \times 10^5$  cells  $\text{ml}^{-1}$ . For the experiment the animals were fed with this culture by adding 5 ml to each vial, resulting a phytoplankton concentration of  $1.7 \times 10^4$  cells  $\text{ml}^{-1}$  in the vials.

The females were observed every 2 h until they laid eggs, after which they were removed from the vials, measured and weighed, as described above. The eggs were counted for each vial and not further monitored, since the interest was focused on the effect of female size on clutch size, but not on hatching success.

A third experiment (Experiment III) was performed in April 1995. This experience was designed to verify temperature effects on egg production for a more extended period of time. Because in the experiments I and II only one burst of eggs per female was allowed (defined above as clutch size), egg production, in this case, was defined as the total daily production of eggs per female, giving the possibility that more than one burst of eggs may have occurred during the 24 hours period. For this, 3 temperatures were used: 12.1, 15.5 and 20.0° C. In this experiment five 500 ml glass jars were used for each temperature, containing 5-6 females each. As in Experiment II, phytoplankton from Bay of Mejillones was used as food. In this case, a culture of freshly-caught phytoplankton collected in March 1995 was initiated in f/2 medium. This culture was kept for several weeks in f/2 medium and 12:12 h under continuous light. The phytoplankton composition during the experiment was predominated by *Navicula cryptocephala*, *Nitzschia* spp. and unknown species of phytoflagellates. The concentration of phytoplankton cells in this culture ranged between  $3 \times 10^4$  and  $1 \times 10^5$  cells ml<sup>-1</sup> during the experiment. This culture was thus used to feed the animals every day. Once the experiment started the jars were observed two times a day. At each observation, the medium was partly replaced (60%) by fresh one made out of filtered seawater (0.45 µm) containing the phytoplankton culture at final concentrations that were not measured, but in amounts sufficiently high to obtain a brown-wish color of the media. The eggs were counted and removed twice a day by pipetting from the bottom of jars.

In order to test temperature effects on clutch size, on the time required for egg spawning and on the number of nonviable eggs the data were first tested for homogeneity of variance among temperatures using Barlett test (Wilkinson 1990), significant heterogeneity of variance was overcome by log-transforming the data. After data transformation a one-way Anova was applied. Since individual responses were subject to different temperature treatments, no attempts were made to test normality of the data, assuming that they became nearly nor-

mal by log-transformation. The multiple comparisons Tukey test was also applied to compare clutch sizes among temperatures and the non-parametric Friedman test to compare daily egg production at different temperatures. Finally, linear regressions were used to establish any functional relationships between body length, dry weight and clutch size, or daily production of eggs.

## RESULTS

### *Field conditions*

Vertical profiles of temperature, water density, dissolved oxygen and chlorophyll-a concentration for the three sampling dates, September 1994, October 1994 and April 1995, are shown in Fig. 2.

In September 1994 the temperature in the water column ranged between 17° C and 13° C from surface to bottom. The high stratification of the water column is evident from Fig. 2b, possibly causing accumulation of phytoplankton biomass within the first 10 m. The oxygenation of water is also limited to the 20 m layer (Fig. 2c). The phytoplankton species at densities of 150 cells ml<sup>-1</sup> near the surface were composed by a very rich variety of the diatoms *Leptocylindrus danicus* Cleve, *Eucampia cornuta* (Cleve) Grunow, *Thalassiosira aestivalis* Gran et Angst, *Chaetoceros lacinosus* Schütt and *Chaetoceros constrictus* Gran, among other species, while the chlorophyll concentration reached values of nearly 10 µg l<sup>-1</sup> near the surface (Fig. 2d). Analysis of one of the sample replicates suggested that the *C. chilensis* population at that time was having an intense productive period. All developmental stages from copepodite CI to adult were present, although most were in the older stages CV and adult males and females. In October the water column was colder (15° C at surface and 13° C near the bottom) and with less stratification (Fig. 2b). The density profile indicated a more mixed column than that of September (Fig. 2b) and the very low oxygen levels near surface (Fig. 2c) suggest that an upwelling process was taking place. Phytoplankton levels reached values of about 300 cells ml<sup>-1</sup> near the surface and it was dominated by

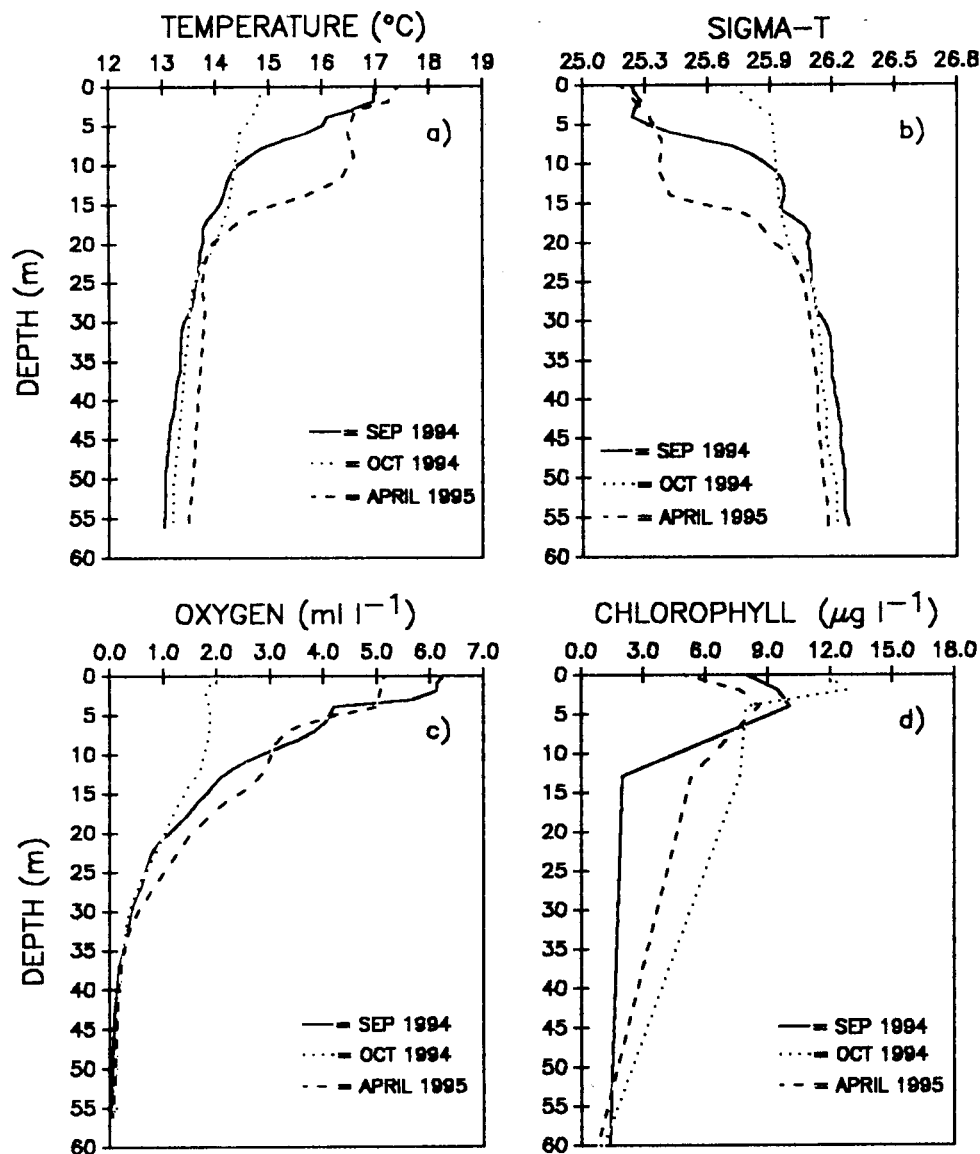


Fig. 2: Vertical profiles of temperature (a), water density (b)(Sigma-T), dissolved oxygen (c) and chlorophyll concentration (d) at three different times of the year (1994-95) in Bay of Mejillones.

Perfiles verticales de temperatura (a), densidad del agua (b)(Sigma-T), oxígeno disuelto (c) y concentración de clorofila (d) en tres diferentes épocas del año (1994-95) en la Bahía de Mejillones.

large diatoms of the genera *Coscinodiscus*, *Asterionella* and *Thalassiosira*. The phytoplankton biomass was  $12 \mu\text{g Chla l}^{-1}$  near the surface. The *C. chilensis* population at that time was dominated by early stages CII and CIII, although there were also adult females and males. In April 1995 the water temperature ranged between 16 and  $14^\circ\text{C}$  from surface to bottom (Fig. 2a). Water column stratification was similar to that of Sep-

tember 1994 and the phytoplankton species reached levels of  $65 \text{ cells ml}^{-1}$  near the surface and they were dominated by *Chaetoceros* spp., *E. cornuta* and *Rhizosolenia fragilisima*. Chlorophyll concentration reached maximal levels of  $8 \mu\text{g l}^{-1}$  in surface waters. Analysis of zooplankton samples indicated that *C. chilensis* population at this time of the year was mostly represented by late stages CV and adult males and females,

suggesting that intense reproduction was taking place.

#### Temperature and clutch size

Table 1 summarizes the results for Experiment I. This experiment, designed to test temperature effects on clutch size, on the number of nonviable eggs and on the time required to initiate egg laying (Te), lasted for about three days. It was initially expected that egg laying would occur at the first day. However, after 24 hours of observation some females did not produce eggs and were not apparently feeding, because no fecal pellets could be seen in the vials. Such females were discarded after 3 days. For instance, at the lowest temperature (12° C) only 2 females produced eggs. At the other temperatures, egg production began soon after feeding and the number of egg-producing females was variable, not showing any trend with temperature.

The one-way ANOVA applied to log-transformed data revealed significant effects of temperature on clutch size, but not on Te and on the number of nonviable eggs (Table 2). However a significant correlation between clutch size and the number of nonviable eggs was found ( $F_{1,25} = 4.67$ ,  $P < 0.05$ ).

The relationship between temperature and clutch size is positive and well described by a linear regression on log-transformed data ( $F_{1,25} = 7.93$ ,  $P < 0.01$ ). This relationship is illustrated in Fig. 3. However, it should be kept in mind that at the lowest temperature only two females were able to lay eggs. At

the highest temperature (20° C), on the other hand clutch size shows a tendency to decline (Fig. 3), suggesting a potential inhibition of egg production at high temperature. Indeed a Tukey test for multiple comparisons indicated that the mean clutch size at 20° C was significantly smaller than the mean value obtained at 18° C ( $q_{9,2} = 3.20$ ,  $p < 0.05$ ).

#### Female size and clutch size

Among copepod species egg production, as number eggs female<sup>-1</sup> day<sup>-1</sup>, increases with female size (Kjørbe & Sabatini 1994). This relationship has not been examined within species. Our Experiment I revealed no correlation between clutch size and female size, measured either as prosome length ( $F_{1,25} = 1.58$ ,  $P > 0.05$ ), or as body dry weight ( $F_{1,25} = 1.34$ ,  $P > 0.05$ ). However, temperature might have obscured a size effect on clutch size. To avoid such interference in Experiment II temperature was kept constant. Therefore here we only consider results from Experiment II.

Experiment II was run for about 30 h and no measure of food quantity, or quality was made after the experiment started. A high production of fecal pellets, however indicated that feeding was intense. A total number of 28 females laid eggs within the 30 h period. For all these animals dry weight and prosome length were obtained.

As expected, dry weight was much more variable than length,  $199.4 \pm 33.67 \mu\text{g}$  and  $2.51 \pm 0.085 \text{ mm}$  (mean  $\pm$  S.D. for weight and length respectively). Size ranges were

TABLE 1

Clutch size of *Calanus chilensis* under laboratory condition (see Methods) from Bay of Mejillones, northern Chile. Time was assumed as the time taken for each female to lay eggs, while a nonviable egg was defined as one unable to reach hatching after 3 days

Tamaño de la puesta de huevos de *Calanus chilensis* en condiciones de laboratorio (ver Métodos) desde Bahía Mejillones, Norte de Chile. Time corresponde al tiempo que requiere cada hembra para poner huevos, mientras que un huevo no viable se definió como aquél incapaz de eclosionar después de 3 días

Temperature (°C)	N° of Females	Clutch Size Mean $\pm$ S.D.	Time (h) Mean $\pm$ S.D.	N° of Nonviable
9.6	2	23 $\pm$ 7.8	19 $\pm$ 18.4	2.5 $\pm$ 3.56
12.5	5	17 $\pm$ 6.8	12 $\pm$ 9.3	1.8 $\pm$ 1.13
15.1	9	47 $\pm$ 12.3	9 $\pm$ 7.0	12.0 $\pm$ 16.26
17.8	4	57 $\pm$ 19.3	8 $\pm$ 2.3	1.7 $\pm$ 2.23
20.2	7	42 $\pm$ 18.7	5 $\pm$ 1.9	14.9 $\pm$ 24.59

TABLE 2

One-way ANOVA to test temperature effects on clutch size (eggs female<sup>-1</sup>), on the time required to initiate egg laying (Te), and on the number of nonviable eggs of *Calanus chilensis* from Bay of Mejillones, northern Chile.  
df is degrees of freedom

ANOVA de una vía para probar los efectos de la temperatura sobre el tamaño de la puesta de huevos (huevos hembra<sup>-1</sup>), sobre el tiempo requerido para iniciar la puesta de huevos (Te) y sobre el número de huevos no viables en *Calanus chilensis* de la Bahía Mejillones, Norte de Chile.  
df son los grados de libertad

Variable	Barlett Test ( $\chi$ )	df	SS	F	P
Clutch size	1.53 n.s.	4	4.76	7.90	0.009
Te	4.55 n.s.	4	2.93	1.16	0.354
Nonviable eggs	8.26 n.s.	4	6.17	0.89	0.485

2.28-2.70 mm and 110.0-266.0  $\mu\text{g}$  for prosome length and dry weight respectively. The length-weight regression on log transformed variables was,

$$\ln L = 2.09 + 3.47 \ln W$$

$$r = 0.65$$

where L = prosome length (mm) and W = individual dry weight ( $\mu\text{g}$ ). The slope 3.47 is not significantly different from 3 (t-test,  $P > 0.05$ ).

The number of eggs per females, i.e. clutch size, was highly variable,  $36.2 \pm 18.67$  (mean  $\pm$  S.D.) and not associated with either female length ( $F_{1,26} = 1.86$ ,  $P > 0.05$ ), or female weight ( $F_{1,26} = 0.026$ ,  $P > 0.05$ ). Such lack of correlation is evident from Fig. 4.

#### Long-term effects of temperature

Experiment III lasted 10 days, time at which egg production became very low, or ceased completely. After 10 days the phytoplankton culture became predominated by flagellates species and this may have caused poor feeding conditions for egg production. Egg production reached maximal rates in the first 2 days at the three temperatures (Fig. 5), while at 20° C egg production was much lower. Daily maximal rate were obtained at 12° C (nearly 30 eggs female<sup>-1</sup> day<sup>-1</sup> in average). This temperature also showed the most sustained daily production (Fig. 5). The non parametric Friedman test indicated that at

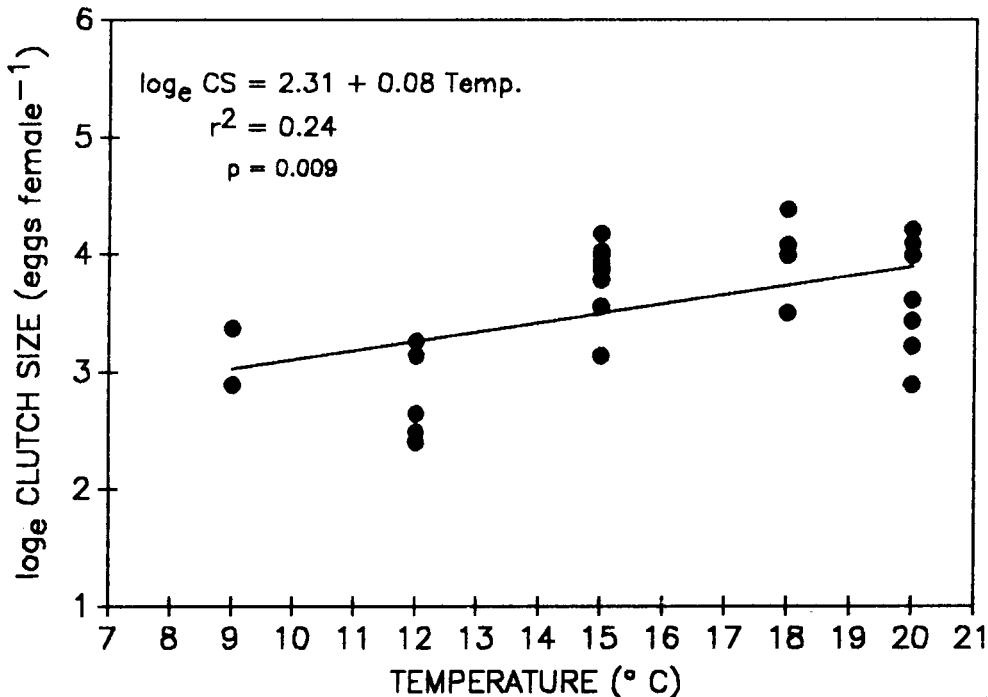


Fig. 3: Clutch size as a function of temperature in *Calanus chilensis* in laboratory condition (see Methods). CS = clutch size.

El tamaño de la puesta de huevos en función de la temperatura en *Calanus chilensis* en condiciones de laboratorio (ver Métodos). CS = Tamaño de la puesta de huevos.



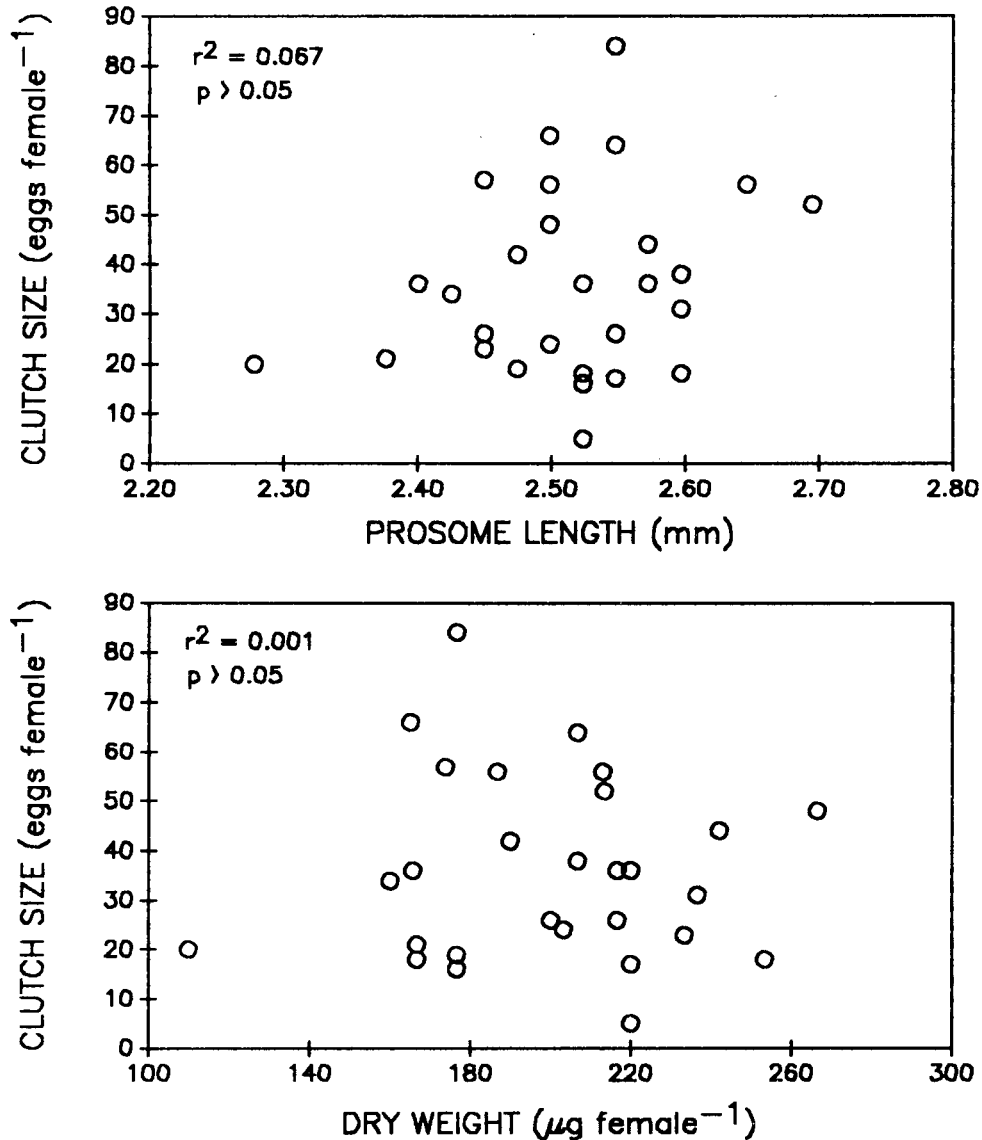


Fig. 4: Clutch size as a function of female size in *Calanus chilensis* under laboratory condition (see Methods). Body size was measured as prosome length (mm) and as individual dry weight ( $\mu\text{g}$ ).

El tamaño de la puesta de huevos en función del tamaño de hembras en *Calanus chilensis* en condiciones de laboratorio (ver Métodos). El tamaño corporal se midió como longitud del prosoma y como peso seco individual.

20° C daily egg production was significantly reduced (Friedman statistic = 9.95,  $p < 0.001$ ) respect to the other two temperatures. Although egg production between 12° C and 15° C was not significantly different (Friedman statistic = 2.50,  $p > 0.05$ ).

#### DISCUSSION

Among the factors that may influence the rate of egg production in marine copepods,

much attention has been paid to food quantity (e.g. Durbin et al. 1983, Arnott et al. 1986, Jonasdottir 1989) and food quality (e.g. Donaghay 1985, Ianora & Poulet 1993). Both, field and laboratory studies have often concluded that egg production rates are highly dependent on food concentration (e.g. Peterson & Kimmerer 1994, Kiørbe & Nielsen 1994) and that food quality may exert a significant influence on egg production (e.g. Donaghay 1985) and on egg

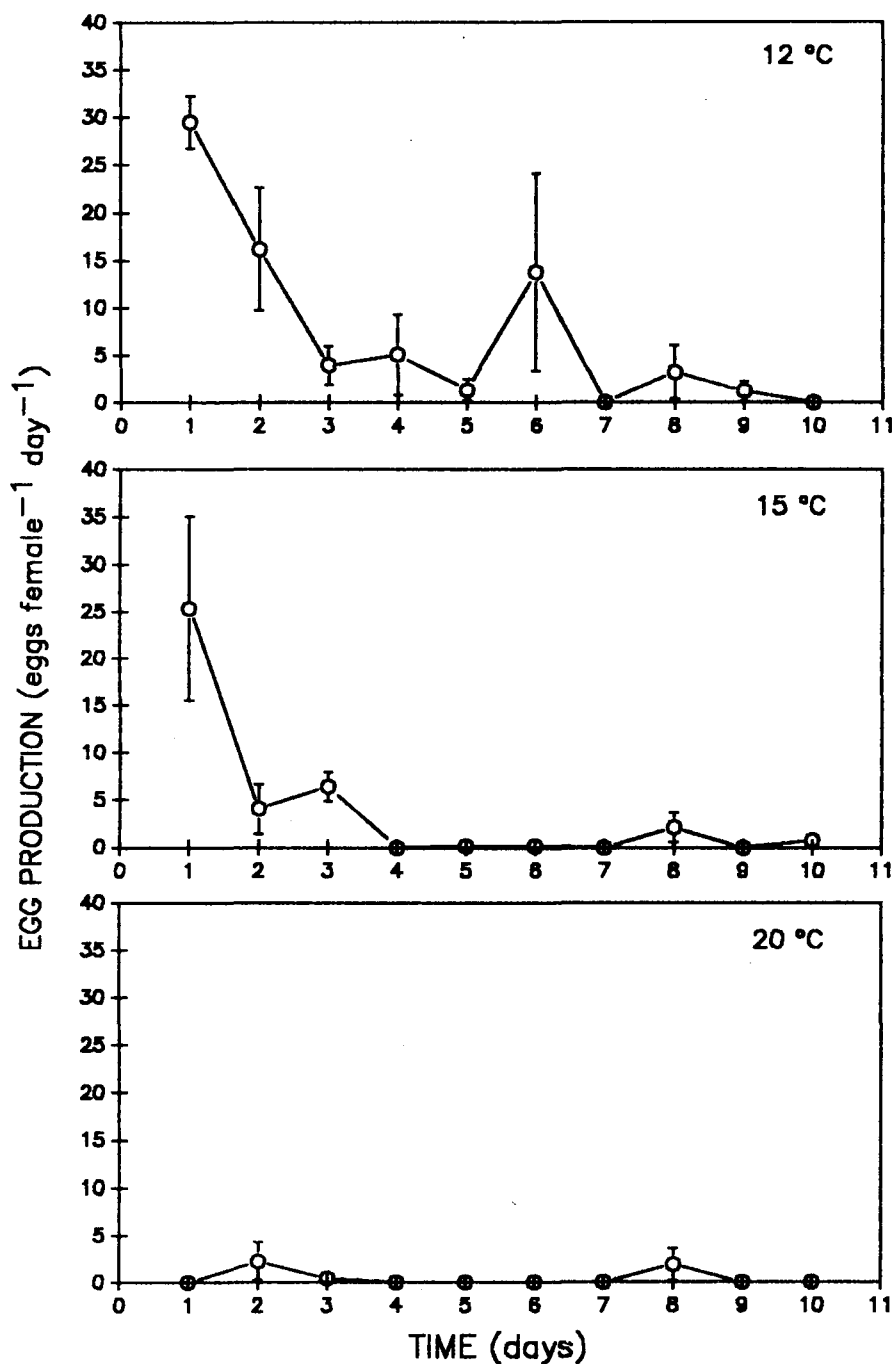


Fig. 5: Daily egg production rate of *Calanus chilensis* at three temperatures in laboratory conditions. Vertical bars are standard deviations from the mean.

Tasa diaria de producción de huevos en *Calanus chilensis* a tres temperaturas en condiciones de laboratorio. Las barras verticales representan la desviación estándar desde el valor promedio.

hatching success (e.g. Ianora & Poulet 1993, Poulet et al. 1995). By contrast, the influence of temperature on egg production rates has received little attention (e.g. Sekiguchi et al. 1980, Uye 1981), despite the recognition

that temperature is major factor controlling physiological rates of copepods (e.g. Huntley & López 1992).

McLaren and Leonard (1995) argue that daily rates of egg production are temperature

dependent in well-fed females of *Calanus* species. Thus, under potential food-saturated levels, which might be the case for *Calanus chilensis* (Escribano & Rodriguez 1994, 1995), clutch size (as defined above) and daily production rates of eggs would correlate positively with ambient temperature. However, the temperature range where such correlation operates appears rather narrow and it depends on the temperature to which animals are exposed in field conditions. For instance, in our Experiment I at low temperature (9.6° C) clutch size was not only smaller, but also fewer females laid eggs when compared to the other temperatures. At the highest temperature (20° C) clutch size again became smaller, suggesting that between 15° C and 18° C there was an "optimal temperature range" for greater clutch sizes. This range coincides with that of the upper 10 m layer at Bay of Mejillones (Fig. 2a). In this layer egg production may also be favoured by well-oxygenated waters and high concentration of phytoplankton (Fig. 2c, Fig. 2d). The highest temperature (20° C) does not hold the positive correlation between clutch size and temperature. The inhibition effect of high temperature is also reflected in greatly depressed egg production rates observed at 20° C in Experiment III (Fig. 5). It is necessary to stress that clutch size in our experiments does not represent the total potential capacity of females to produce eggs on a daily basis. This is important, because production of eggs may occur in bursts of eggs laying, with lack of synchrony among females (Plourde & Runge 1993), giving raise to a large variance in egg laying. Lack of synchrony in egg laying may depend on the stage of the ovarian cycle of females (Tester & Turner 1990), which in turn depends upon prior environmental conditions. In fact, as shown in Experiment I, there was much unexplained variation in the time required to initiate egg laying within and between temperatures. Also it is possible that food availability, at least in terms of phytoplankton concentration and species variety, was not limited in field conditions at the time of capture, although in laboratory condition the adjustment to achieve full reproductive capacity may take longer than 24 h (McLaren & Leonard 1995). This

potential interference, however, did not appear to affect the response to experimental temperature.

The influence of female size on clutch size was studied in *Pseudocalanus* species by Corkett and McLaren 1969, who suggested that larger females would be able to produce more eggs. Runge (1984) reached a similar conclusion for *Calanus pacificus* Brodsky. However, in our Experiment II, at constant temperature, we found no size effect on clutch size. According to McLaren and Leonard (1995) the positive relationship between female size and clutch size would only arise when considering a sufficiently wide range of sizes. Even although the females for our Experiment II came from a single sample obtained during the Spring, their size ranges, in terms of length and weight, were as wide as those described by Escribano and Rodriguez (1995) for the same species on a year-round cycle near Bay of Mejillones. Perhaps a size-related effect on clutch size is associated to genetic variation among different generations. Genetic variation among generations has been reported for other copepod species (Marcus & Alatalo 1989). Seasonal size-related effects of females on clutch size has been described in *Daphnia* species (Berverovic et al. 1990), but such effects require further studies in marine copepods.

The presence on nonviable eggs may be more related to feeding conditions of females (e.g., Ianora & Poulet 1993). Hatching success may be inhibited when females are fed by diatoms, instead of alternate food, such as microzooplankton (Poulet et al. 1995). In our Experiment I the variation in the number of nonviable eggs could not be explained by temperature, or food quality, since they all experience the same food type. In this respect, it is interesting to note that the number of nonviable eggs was positively correlated to clutch size. Thus, even although a large clutch size may be an advantage for population growth, this may be accompanied by poor survival of eggs. Nevertheless, we cannot discard the possibility that egg survival could have been affected by egg density in the small vials rather than by differences in egg quality.

Even although we were only focused in examining temperature effects on egg

production and egg viability, there may be questions about the kind of other conditions our females were subject during the experiments, and if such conditions may have affected our results. In this respect, our three experiments may not be comparable to each other, not only because experimental conditions were different, but also because the females came from different cohorts. The most critical condition might have been the type and amount of food they experienced. We do not know if under our food conditions females reached their full reproductive capacity. Clutch size in our experiments varied widely between 5 and 84 eggs female<sup>-1</sup>. As an example, daily production rates of eggs in *C. finmarchicus* show a range of 11 to 45 eggs female<sup>-1</sup> d<sup>-1</sup> from the Gulf of St. Lawrence (Ohman & Runge, 1994). The same species in other areas shows a narrower range, 13 - 29 eggs female<sup>-1</sup> d<sup>-1</sup> (Runge 1985) and 12-14 eggs female<sup>-1</sup> d<sup>-1</sup> (Hirche 1990). Ohman & Runge (1994) also reported a possible time lag for maximum rates to take place, such that values up to 60 eggs female<sup>-1</sup> d<sup>-1</sup> were later observed at the same area. Clearly there is much variability in clutch size and there is no previous information of this type for *C. chilensis*, but our data in *C. chilensis* fall within the range reported for *C. finmarchicus*, suggesting that experimental conditions were adequate for full reproductive capacity.

Size of container might be another experimental condition affecting production of eggs. In using small (30 ml) vials in Experiments I and II we were able to make more frequent observations at the individual level, without introducing much interference from handling and sharp changes in temperature. Small volume may however prevent adequate oxygenation and animal motility. Nevertheless females kept in small vials produced more eggs per individual than those in the larger 500 ml jars.

For a natural situation, our experimental results predict that under conditions of low temperature (< 12° C), or high temperature (> 20° C), egg production would be reduced and hence maximal rates are only attained within the upper 10 m layer. For instance, Escribano and Rodriguez (1994) described a drastic reduction of the *C. chilensis* popula-

tion during the time when a warm water mass was present due to El Niño 1991-92. At that time there was no explanation on what type of role the rise of temperature could have played on affecting the population abundance. Water temperature was near 22° C at the upper layer off Antofagasta, while the usual surface temperature reaches its maximal value up to 20° C in late summer. The abnormal 22° C may have certainly reduced egg production at the upper layer causing a much lower copepod recruitment for the next generations.

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#### LITERATURE CITED

- ARNOTT GH, GW BRAND & LC KOS (1986) Effect of food quality and quantity on the survival, development and egg production of *Gladioferens pectinatus* Brady (Copepoda: Calanoidea). Australian Journal of Marine and Freshwater Research 37: 467-473.
- BERVEROVIC R, K BIKAR & W GELLER (1990) Seasonal variability of the embryonic development time of three planktonic crustaceans: dependence on temperature, adult size, and egg weight. Hydrobiologia 203: 127-136.
- BOYD CM, SL SMITH & TJ COWLES (1980) Grazing patterns of copepods in the upwelling system off Peru. Limnology and Oceanography 25: 583-596.
- CORKETT CJ & IA McLaren (1969) Egg production and oil storage by the copepod *Pseudocalanus* in the laboratory. Journal of Experimental Marine Biology and Ecology 3: 90-105.
- DAVIS CS (1987) Components of the zooplankton production cycle in the temperate ocean. Journal of Marine Research 45: 947-983.
- DONAGHAY PL (1985) An experimental test of the relative significance of food quality and past feeding history to limitation of egg production of the estuarine copepod *Acartia tonsa*. Archive Hydrobiologique Beihlein 21: 235-245.
- DURBIN EG, AG DURBIN, TJ SMAYDA & PG VERITY (1983) Food limitation of production by *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnology and Oceanography 28: 1199-1213.
- DURBIN EG, AG DURBIN & RG CAMPBELL (1992) Body size and egg production in the marine copepod *Acartia hudsonica* during a winter-spring diatom bloom in Narragansett Bay. Limnology and Oceanography 37: 342-360.
- ESCRIBANO R & L RODRIGUEZ (1994) The life cycle of *Calanus chilensis* Brodsky in Bay of San Jorge, Antofagasta, Chile. Hydrobiologia 292/293: 289-294.

- ESCRIBANO R & L RODRIGUEZ (1995) Seasonal size variation and growth of *Calanus chilensis* Brodsky in northern Chile. *Revista Chilena de Historia Natural* 68: 373-382.
- HEINRICH AK (1973) Horizontal distribution of copepods in the Peru current region. *Oceanology* 13:97-103.
- HIRCHE H-J (1990) Egg production of *Calanus finmarchicus* at low temperature. *Marine Biology* 106: 53-58.
- HUNTLEY M & MDG LOPEZ (1992) Temperature dependent growth production of marine copepods: a global synthesis. *American Naturalist* 140: 201-242.
- IANORA A & SA POULET (1993) Egg viability in the copepod *Temora stylifera*. *Limnology and Oceanography* 38: 1615-1626
- IANORA A, MG MAZZOCHI & P MASCELLARO (1989) The reproductive biology of the planktonic copepod *Temora stylifera*. *Marine Biology* 101: 187-194.
- JONASDOTTIR SH (1989) Effects of food concentration on egg-Xproduction rates of two species of *Pseudocalanus*: laboratory observations. *Journal of Experimental Marine Biology and Ecology* 130: 33-43.
- KIØRBE T & M SABATINI (1994) Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. *Journal of Plankton Research* 16: 1353-1366.
- KIØRBE T & TG NIELSEN (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 1. Copepods. *Limnology and Oceanography* 39: 493-507.
- MARCUS NH & P ALATALO (1989) Conditions for rearing *Calanus finmarchicus* (Gunnerus 1770) (Copepoda, Calanoidea) through multiple generations in the laboratory. *Crustaceana* 57:101-103.
- McLAREN IA & A LEONARD (1995) Assessing the equivalence of growth and egg production of copepods. *ICES Journal of Marine Sciences* 52: 397-408.
- McLAREN IA, MJ TREMBLAY, CJ CORKETT & JC ROFF (1989) Copepod production on the Scotian Shelf based on life-history analyses and laboratory rearings. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 560-583.
- OHMAN MD & JA RUNGE (1994) Sustained fecundity when phytoplankton resources are in short supply: omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. *Limnology and Oceanography* 39: 21-36.
- PARRISH KK & DF WILSON (1978) Fecundity studies in *Acartia tonsa* (Copepoda: Calanoidea) in standardized culture. *Marine Biology* 46: 65-81.
- PETERSON WT & WJ KIMMERER (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. *Limnology and Oceanography* 39: 1594-1605.
- PLOURDE S & JA RUNGE (1993) Reproduction of the planktonic copepod *Calanus finmarchicus* in the lower Gulf of St Lawrence estuary. relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. *Marine Ecology Progress Series* 102: 217-227.
- POULET SA, M LAABIR, A IANORA & A MIRALTO (1995) Reproductive response of *Calanus helgolandicus*. I. Abnormal embryonic and naupliar development. *Marine Ecology Progress Series* 129: 85-95.
- SEKIGUCHI H, IA McLaren & CJ CORKETT (1980) Relationship between growth rate and egg production in the copepod *Acartia clausi hudsonica*. *Marine Biology* 58: 133-138.
- STRICKLAND JDH & TR PARSONS (1972) A Practical handbook of seawater analysis. Fisheries Research Board of Canada. Bulletin 167, 310 pp.
- SUNDA W & R.R.L. GUILLARD (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *Journal of Marine Research* 34: 511-529.
- RODRIGUEZ L, V MARIN, M Farias & E OYARCE (1991) Identification of an upwelling zone by remote sensing and in situ measurements. *Mejillones del Sur Bay (Antofagasta-Chile)*. *Scientia Marina* 55: 467-473
- RUNGE JA (1984) Egg production of the marine planktonic copepod, *Calanus pacificus* Brodsky: laboratory observations. *Journal of Experimental Marine Biology and Ecology* 74: 53-66.
- RUNGE JA (1985) Egg production rates of *Calanus finmarchicus* in the sea off Nova Scotia. *Ergebener Limnology* 21: 33-40.
- TESTER PA & JT TURNER (1990) How long does it take copepods to make eggs? *Journal of Experimental Marine Biology and Ecology* 141: 169-182.
- UYE S-I (1981) Fecundity studies of neritic calanoid copepods *Acartia clausi* Giesbrecht and *A. steueri* Smirnov: a simple empirical model of daily egg production. *Journal of Experimental Marine Biology and Ecology* 50: 255-271.
- WILKINSON L (1990) SYSTAT: The system for statistics. Evanston, IL: SYSTAT, Inc. 468 pp.