

Egg production rates of *Calanus helgolandicus* females reared in the laboratory: variability due to present and past feeding conditions

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ABSTRACT: Four egg production experiments were performed under controlled conditions on *Calanus helgolandicus* females from laboratory cultures with a known history of feeding conditions. Two of the experiments were run with females from a cohort reared at a low concentration of *Prorocentrum micans* ($77.5 \mu\text{g C l}^{-1}$, Cohort L). One of the egg production experiments was run at the same low food concentration (females called LL) and the other one at high food concentration (females LH). Another 2 experiments were performed with females from a cohort reared at a high *P. micans* concentration ($278 \mu\text{g C l}^{-1}$, Cohort H). One of these experiments was run at the same high food level (females HH) whereas the other one was run at the low food concentration (females HL). These 4 experiments allowed us to estimate the influence of both present and past feeding conditions on the egg production rates of the females. The catabolism of the internal resources in terms of carbon (lipid) and nitrogen (protein) of the females was more important for those held at the low concentration and it also varied with the origin of the females. Females L, which had been food-limited during their growth (LL and LH), were apparently deficient in nitrogen since they lost twice as much carbon as nitrogen, whereas females H (HH and HL) drew equally on their carbon and nitrogen resources. After 7 to 10 d, the egg production of females L was significantly lower than that of females H, at both food concentrations (LL < HL and LH < HH). To explain these results, we propose that females L had a lower assimilation efficiency than females H and that, even if they had a higher specific ingestion rate at the beginning of the experiment, they were not able to compensate for metabolic deficiencies (e.g. in protein). Our results indicate that the feeding history of the females may be important in explaining the egg production values found in the field since *in situ* food conditions are unlikely to remain optimal during an entire generation.

KEY WORDS: *Calanus helgolandicus* · Female · Egg production rate · Food concentration · Feeding history

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INTRODUCTION

In situ egg production is increasingly used to estimate the growth rates of planktonic copepods and

these growth rates are often extrapolated to all the stages of the species (Kiørboe & Nielsen 1994, Hay 1995, Calbet & Agusti 1999, Irigoien et al. 2000a). Egg production is a relatively simple measurement to make on cruises compared to the estimation of juvenile growth (Peterson et al. 1991). However, to fully understand the results, it is important to keep in mind all the factors that may influence egg production measure-

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ments. Environmental factors (mostly temperature, food concentration and food quality) measured at the same time as spawning are those which are the most frequently reported in the studies to explain variation among egg production rates found at different times or in different areas (Plourde & Runge 1993, Hay 1995, Calbet & Agusti 1999, Niehoff et al. 1999, Harris et al. 2000, Irigoien et al. 2000a).

During the last decade, some authors have suggested that the past environmental conditions could also be important in understanding the *in situ* variation of physiological rates (Båmstedt 1988, Båmstedt & Tande 1988, Donaghay 1988, Huntley 1988, Attwood & Peterson 1989, Lopez 1991, Huntley & Lopez 1992). These authors have pointed out the necessity of studying the influence of past conditions in more detail; however, very few studies have been carried out since. To estimate such an effect on the female egg production rate, 2 kinds of experiments can be performed in the laboratory. First, short-term history can be assessed by modifying environmental conditions during egg production experiments and comparing the results with controls where conditions remain stable. A certain number of studies have been carried out following this protocol (Runge 1984, Frost 1985, Attwood & Peterson 1989, Nival et al. 1990, Carlotti & Hirche 1997, Hirche et al. 1997, Båmstedt et al. 1999). In most of the cases, food concentration was modified during the experiment in order to mimic the field situation in which the females do not encounter homogeneous food concentration but rather patchily distributed food.

Second, to estimate experimentally the influence of past conditions, the egg production rate of females can be measured in environmental conditions different from those used during their juvenile growth. Indeed, this scenario probably corresponds to that most frequently found in the field since the environmental conditions often change over 1 population generation

time. The problem raised in our study is to know whether the egg production rate is simply the consequence of recent environmental conditions encountered by the females or if it depends also on their own body characteristics (size, biochemical contents), which are the result of their past growth history. Such investigations, which are rare in the literature (Rey et al. 1999), require a lengthy experiment in which the environmental parameters are controlled from hatching to the spawning of the resultant adult females. Our experiments were done on *Calanus helgolandicus*, a dominant copepod species in the North Atlantic, reared in the laboratory under different food concentrations. The egg production rates as well as other physiological body characteristics were measured individually for 20 d on females with different growth histories.

MATERIALS AND METHODS

Origin and fertilization of the females. The *Calanus helgolandicus* females used in our experiments came from 2 cohorts L (low food level) and H (high food level) reared as described in Rey-Rassat et al. (2002, this volume). Briefly, each of these cohorts was raised from eggs spawned within 12 h by wild females collected from Stn L4 (50° 15' N, 4° 13' W), which is approximately 10 km off Plymouth, western English Channel (Table 1). These eggs were then transferred into 15 l glass beakers filled with a suspension of *Prorocentrum micans* adjusted to 200 cell ml⁻¹ every 12 h, up to Day 10 (Day 0 is defined as the time when 50% of the eggs were spawned). Subsequently, 3500 individuals (mostly at the copepodite stage CI) were transferred to a 150 l polyethylene tank filled with a suspension of *P. micans*. The 2 cohorts isolated individually in a 150 l tank were finally reared at 2 different *P. micans*

Table 1. *Calanus helgolandicus* fed on *Prorocentrum micans*. Summary of the nutritional conditions of the females during their past growth and during the 4 spawning experiments run with the Females LL (i.e. low food when growing, low food when spawning), LH, HH and HL. During all their life, the females were fed on *P. micans* but at different cell concentrations (cell ml⁻¹; checked every 12 h). The day $t = 0$ is defined as the time when 50% of the eggs were spawned (the eggs were spawned within 12 h). Tf corresponds to the time when the first fertilized females were found in the tanks. In Tank L, the 40 females required for the spawning experiments were found on Days 44 and 45, and in Tank H, the 40 females were found between Days 29 and 34 (the geometric mean is 32)

Process:	Growth			Spawning			
Stage:	Nauplii		Copepodites	Adults			
Place:	15 l beaker		150 l tank	280 ml spawning beaker			
Time (d):	0	10	Tf	Tf	Tf+10	Tf+15	Tf+20
Expts: LL			50 cell ml ⁻¹ (L)	50 cell ml ⁻¹ (L)	60 cell ml ⁻¹ (L)		
LH	200 cell ml ⁻¹			180 cell ml ⁻¹ (H)		240 cell ml ⁻¹ (H)	
HL			180 cell ml ⁻¹ (H)	50 cell ml ⁻¹ (L)	60 cell ml ⁻¹ (L)		
HH				180 cell ml ⁻¹ (H)		240 cell ml ⁻¹ (H)	

concentrations, a low (50 cell ml⁻¹, Cohort L) and a high one (180 cell ml⁻¹, Cohort H).

Because we wanted to follow the egg production rates of the females, we needed fertilized females at the end of both cohort developments. However, fertilization of *Calanus* sp. females in the laboratory is difficult to achieve because males are rarely found in *Calanus* cultures (Peterson 1988, Irigoien et al. 2000c) and also because the mating success under rearing conditions is rather low (Tsuda & Miller 1998), which contrasts with rearing performed in *in situ* mesocosm enclosures (Hygum et al. 2000). In one of our cohorts (Cohort L), no males were found and we were forced to introduce wild males isolated from samples collected at Stn L4 once most of the CVs had moulted into females (50 males on Day 36 and 100 males on Day 40). The first fertilized females were found around 10 d either after the introduction of males in the case of Cohort L or after the appearance of the first adults in the case of Cohort H. However, in both cohorts, the percentage of the fertilized females was very low (~10%). In the case of Cohort L, the fertilized females used for the experiments were found on Days 44 and 45 and in the case of Cohort H on Days 29 to 34 (the geometric mean is 32). This means that the females from Cohort L were older than those from Cohort H at the start of the experiments. This comes from the fact that the appearance of the females in the Cohort L was later than in the Cohort H (due to slow development of the Cohort L, see Rey-Rassat et al. 2002) and that the collection of wild males (required to fertilize the females L) had been delayed due to a problem with plankton collection. The first fertilized females found in both cohorts were used for egg production experiments, the following ones were used to run ingestion experiments.

Egg production experiments. Fertilized females collected from both Tanks L and H were held individually in 280 ml spawning cylinders made of Plexiglas with 400 µm mesh false bottoms suspended in 400 ml beakers filled with a *Prorocentrum micans* suspension (Table 1). Twenty replicates from Cohort L were incubated at the same low concentration L (Females LL), and 20 others at the high concentration H (Females LH). In a similar way, 20 females from Cohort H were incubated at concentration H (Females HH), and 20 others at concentration L (Females HL).

The dinoflagellate *Prorocentrum micans* (26.6 µm equivalent spherical diameter) used in our experiment was grown in a 10 l semi-continuous culture using f/2 medium under a 12:12 h light:dark cycle (for more details see Rey-Rassat et al. 2002). The cell concentration was estimated by 3 cell counts made with a Coulter Multisizer, fitted with a 100 µm orifice tube. *P. micans* was chosen because of its motility and good

nutritional value. During experiments we tested how the egg production rate of the females responded to slight variation of the food supply and, for that purpose, we increased the food concentration as follows (see also Table 1): (1) for the low concentration L, from 50 to 60 cell ml⁻¹, 10 d after the first experimental day; and (2) for the high concentration H, from 180 to 240 cell ml⁻¹, 15 d after the first day.

All the females were maintained at a constant temperature of 15°C, which also corresponds to the temperature that they encountered during their growth.

Each day, each cylinder containing 1 female was quickly placed in a new beaker with fresh food. Eggs and fecal pellets produced during the previous 24 h were counted under a dissecting microscope. After 20 d of experiments, each female was first isolated on a GF/C filter in order to determine its prosome length, oil sac volume and the stage of gonad maturity. Then, each female was transferred to an aluminum cup and stored at -25°C for later carbon and nitrogen analysis.

To have an idea of the condition of the females when the egg production experiments were started, we also determined the prosome length, the oil sac volume and the carbon and nitrogen content of the fertilized females that were used in the ingestion experiments.

Hatching success and ingestion rates. The hatching success of the eggs was estimated by collecting the eggs laid within 24 h by each female for the 4 different categories (LL, LH, HH and HL) and by incubating them at 15°C in Petri dishes filled with filtered seawater. The hatching success was measured after 72 h by counting the dead eggs (all the abnormal nauplii were considered dead). This estimate of the hatching success was made 10 d after the start of each experiment (or the day after, if the female had not spawned at this time).

The ingestion rates of the fertilized females were measured in 4 experiments corresponding to the 4 categories of females considered in the egg production experiments. Groups of 4 females were placed in 2.5 l bottles filled with a suspension of *Prorocentrum micans* (either low or high concentration). Four replicates with females and 4 controls without animals were used for each experiment. The 8 bottles were incubated on a rotating wheel (0.2 rpm) for 24 h. The cell concentration at the beginning and end of the experiments was measured using a Coulter Multisizer. Filtration and ingestion rates were calculated following Frost (1972).

Data analysis. The wax ester content was estimated from the volume of the oil sac (for details see Rey et al. 1999). The volume (in ml) was transformed into wax ester (in gC) by (1) multiplying it by the density of 0.86 g ml⁻¹ (Sargent & Henderson 1986), (2) multiplying it by the ratio of the carbon content in wax ester

of 0.78 (from Table 2.3 of Sargent & Henderson 1986), and (3) dividing it by 1.44, a conversion factor coming from the fact that the wax ester content estimated from the oil sac volume was found to be 1.44 higher than that estimated from a calibrated Iastroscan, in Miller et al. (1998, from their Fig. 6). The stage of gonad maturity of the females was determined based on criteria described in Runge (1987). Reproductive index was calculated as the ratio of actively spawning females (in gonadal development stages 4 to 7) to the total number of females (Runge 1987, Plourde & Runge 1993).

The female specific egg production rate g_f was calculated using the following equation:

$$g_f = W_{\text{eggs}}/W_{\text{female}} \quad (1)$$

where W_{eggs} is the total weight (in μgC or μgN) of the eggs produced within 24 h and W_{female} is the weight of the female (in μgC or μgN). The chemical weight of a *Calanus helgolandicus* egg was considered to be 0.362 μgC and 0.071 μgN (Rey et al. 2001). These values were very close to those found by Pond et al. (1996, their Table 1). We also wanted to calculate the structural egg production rate, which can be estimated using the structural weight of the female (i.e. the total weight minus the weight of wax ester and the weight of the gonad) in Eq. (1), rather than the total weight of the female. As described by McLaren & Leonard (1995), a female with a gonad full of ripe oocytes, ready to spawn, is much heavier than a female that has just laid its eggs, whereas the production of both these females is the same. The spawning capacity of both these females depends on their structural weight. To solve this problem, we calculated the structural weight (in μgC) of our females by using the allometric relationship established by Rey-Rassat et al. (2002):

$$\text{Structural weight} = 2.1329 \times 10^{-8} \times \text{Length}^{2.7925} \quad (2)$$

Length is expressed in μm . This relationship was obtained by subtracting the mass of wax ester (in μgC) from the total body mass of carbon of all copepodite stages of *Calanus helgolandicus* (from CI to CV). A similar relationship was used by McLaren & Leonard (1995) to calculate the female structural weight in *C. finmarchicus*. SDs for specific egg production rates were calculated according to the formula given by Takahashi & Bienfang (1983) for a ratio (as the specific egg production rate is actually a ratio). This was not done in the case of the structural egg production rate because structural weights were not measured values but estimated values from length.

A general linear model (GLM) was built using SYSTAT 7.0 to test (1) whether the past feeding conditions had an effect on the egg production regarding the food condition used during the incubation and (2) whether this food treatment effect varied over the course of the 20 experimental days.

$$\text{Egg production} = \beta_0 + (\beta_1 \times \text{PIF}) + (\beta_2 \times \text{IF}) + [\beta_3 \times (\text{PIF} \times \text{IF})] + [\beta_4 \times (\text{PIF} \times \text{IF} \times \text{time})] + \epsilon \quad (3)$$

where β is the coefficient of interaction, PIF is the pre-incubation food treatment, IF is the incubation food treatment, time is the number of experimental days and ϵ is error. The significance level of the interaction terms (PIF \times IF) and (PIF \times IF \times time) enabled the Questions (1) and (2) to be tested. A similar model was considered for fecal pellet production.

RESULTS

Body characteristics of the adults

The females from Cohort L were always significantly lighter and shorter than the females from H (Table 2).

Table 2. *Calanus helgolandicus*. Body characteristics of the fertilized females estimated at days close to the beginning of the spawning experiments (Initial) and at the end of the experiments (Final). All estimates are indicated as the mean \pm SD. Tests were done to determine the significant differences between 2 means (Z-tests for the length and the wax ester, and Kolmogorov-Smirnov tests for the carbon and nitrogen content). p: probability that means are equal; s or ns: the difference is significant or not at the 5% level; n: number of individuals; t: day when the individuals are collected ($t = 0$ is the time when 50% of the eggs were spawn)

Time	Length (μm)	Wax ester (μgC)	Carbon (μgC)	Nitrogen (μgN)
Initial				
Females L	2387 \pm 92	1.6 \pm 7.5 (n = 30; t = 51)	77.6 \pm 14.3	11.7 \pm 2.1 (n = 27; t = 51)
Females H	2589 \pm 93	11.4 \pm 16.5 (n = 30; t = 42)	132.5 \pm 29.9	24.3 \pm 1.3 (n = 5; t = 42)
Test	p = 0.00 s	p = 0.00 s	p = 0.00 s	p = 0.00 s
Final				
Females LL	1928 \pm 75	0 (n = 20; t = 64 or 65)	43.3 \pm 12.4	9.0 \pm 3.1 (n = 19; t = 64 or 65)
Females LH	1911 \pm 88	0 (n = 20; t = 64 or 65)	64.5 \pm 10.9	14.7 \pm 3.5 (n = 17; t = 64 or 65)
Test	p = 0.50 ns	–	p = 0.00 s	p = 0.00 s
Females HH	2090 \pm 65	~0 (n = 20; 49 < t < 54)	83.9 \pm 17.5	17.4 \pm 5.4 (n = 18; 49 < t < 54)
Females HL	2097 \pm 62	~0 (n = 20; 49 < t < 54)	54.8 \pm 11.2	11.4 \pm 3.5 (n = 20; 49 < t < 54)
Test	p = 0.73 ns	–	p = 0.00 s	p = 0.00 s

Under different incubation conditions, females with the same origin had significantly different carbon and nitrogen contents, even if their prosome lengths were statistically the same (Table 2). The females from Cohort H which were incubated at the low food level (Females HL) had a much lower weight than those which remained at the high concentration (Females HH). Inversely, the weight of the Females LH was much higher than those of the Females LL. The wax ester had been consumed in all the categories of females and only a few tiny oil droplets were visible in some of the females from Cohort H. When the initial body characteristics of females were compared with those at the end of the experiments, their carbon and nitrogen content as well as their wax ester content was found to have greatly decreased. However, in 1 case (Females LH) there was an increase in the nitrogen content. Interestingly, the prosome length had also considerably decreased during experiments, which indicates the relative elasticity of the exoskeleton. In the future, this phenomenon needs to be checked by considering a greater number of individuals (>20).

Egg and fecal pellet production versus time

The mean egg and fecal pellet production versus time was calculated for Females HH and LH (Fig. 1) and Females LL and HL (Fig. 2). The statistical model (Eq. 3 in 'Materials and methods') was used to analyze these data. In this analysis, the egg production and the egestion measured on Day 1 of each experiment were not considered because, for Females LH and HL, these values were the results of the food conditions encountered the day before (i.e. when these females were still at the food concentration used for their growth).

The significant model (coefficients of all terms were significant with $p < 0.05$) was found to be: Egg production = $-15.28 + (17.19 \times IF) + [4.03 \times (PIF \times IF)] - [0.09 \times (PIF \times IF \times \text{time})]$. Some conclusions can be pointed out from this model: (1) the coefficient of the term considering the incubation food concentration (IF) was significant and high, i.e. IF was the major effect to explain the egg production variability; (2) the coefficient of the interaction term (PIF \times IF) between IF and the pre-incubation food concentrations (PIF) was significant, i.e. the effect of the past feeding conditions on the egg production depended on the feeding condition used during the incubation. In other words, for each IF, the egg production of the females resulting from the 2 PIF was not the same; it happened to be higher for the females from the high PIF (in high IF: HH > LH; in low IF: HL > LL). This clearly indicates the effect of the past feeding conditions of the females on the egg production; and (3) the coefficient of the interaction term (PIF \times IF \times time) was significant, i.e. for each IF, the effect of past feeding conditions of the females on their egg production was time-dependent. The egg production decreased obviously with time in females LL and LH, whereas it did not in females HH and HL.

In the case of the fecal pellet production, the significant model ($p < 0.05$) was: Fecal pellet production = $-16.73 + (4.06 \times PIF) + (31.35 \times IF) - [0.13 \times (PIF \times IF \times \text{time})]$. Both variables PIF and IF had a significant effect on the fecal pellet production, but the interaction between both terms was not significant. In fact this latter result certainly derives from the fact that for both IFs the fecal pellet production of females resulting from the low PIF (Females LL and LH) became lower than that of females from the high PIF after some days of incubation (as was the case for the egg production), but then it increased again when the IF con-

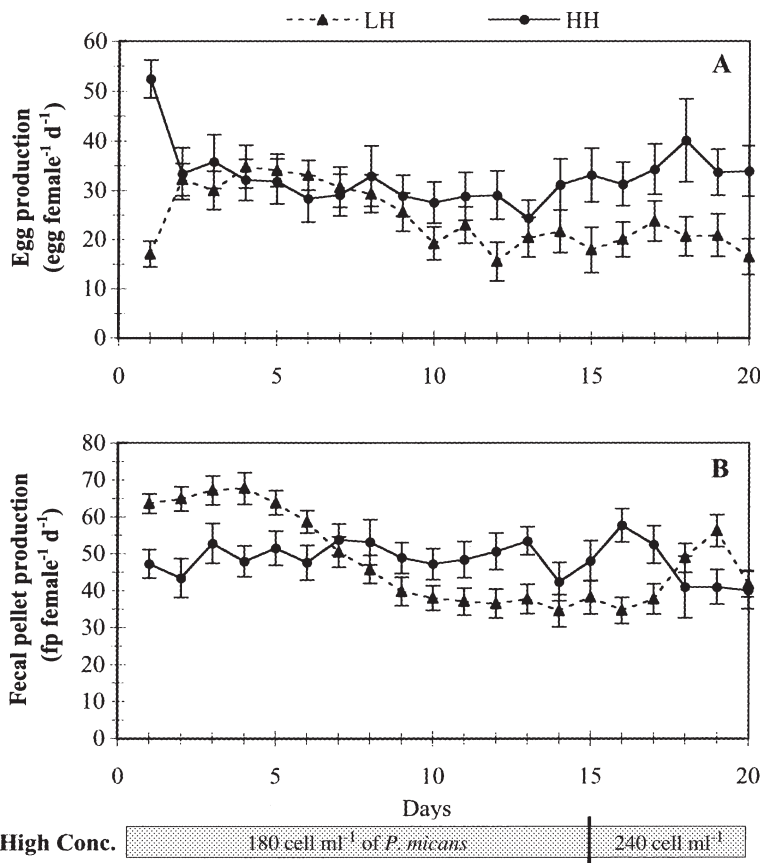


Fig. 1. *Calanus helgolandicus*. Mean egg (A) and fecal pellet (B) production with time during both spawning experiments run at the high *Prorocentrum micans* concentration (LH and HH). The high concentration increased slightly at Day 15. Vertical bars indicate SD

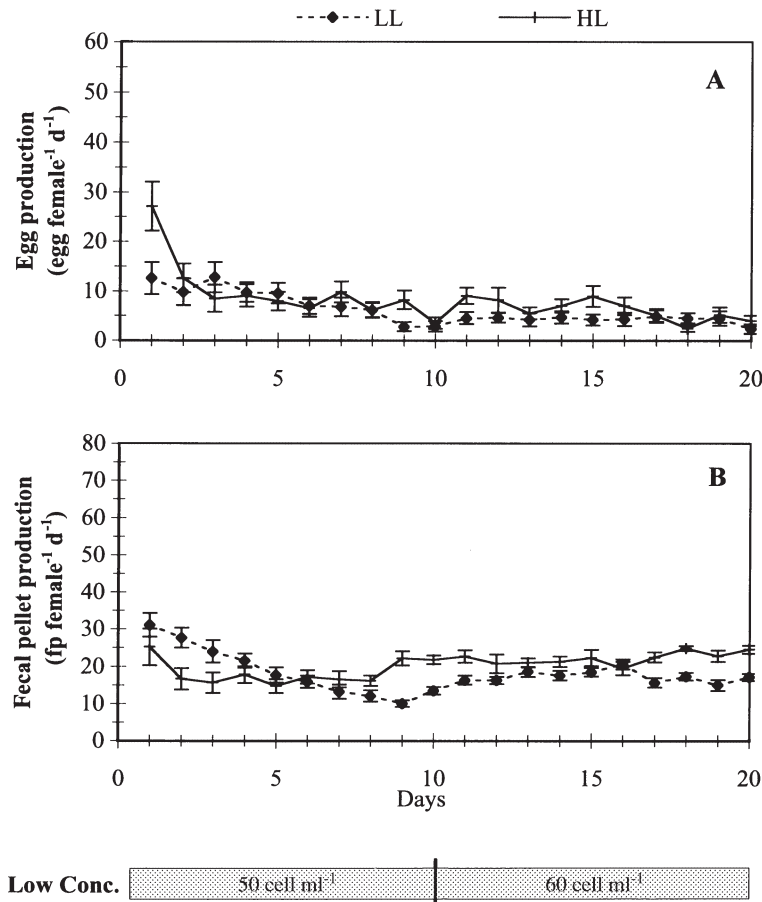


Fig. 2. *Calanus helgolandicus*. Mean egg (A) and fecal pellet (B) production with time during the 2 spawning experiments run at the low *Prorocentrum micans* concentration (LL and HL). The low concentration increased slightly at Day 10. Vertical bars indicate SD

centration was slightly increased (at Days 15 and 10 for the high and low IF, respectively), which was not the case for the egg production. Indeed, the effect of time on the fecal pellet production, regarding the PIF and IF concentrations, was apparent in the model with the interaction term (PIF \times IF \times time).

Gonadal stage at the end of the experiments

The reproductive index (RI), which is the frequency of the females with mature gonads (Fig. 3), was significantly different among the 4 categories of females (χ^2 -test, $p < 0.05$). The highest RI was found for the females that were maintained at the high concentration (HH, LH). In terms of past feeding conditions, it was always the females resulting from Cohort H that had the highest RI (HH > LH, HL > LL); however, these differences were not significant (HH > LH, $p = 0.15$; HL > LL, $p = 0.26$). When we looked at the females which

had spawned (at least 5 eggs) during the last 2 d of each experiment, we found that the majority had mature gonads when experiments ended (55, 73, 88 and 100% for Females LL, HL, LH and HH, respectively) but others (45, 18 and 6% for Females LL, HL and LH, respectively) had gonads which had already reverted to an immature stage. We also found 2 females with a senescent gonad. Concerning the females which had not spawned or produced <5 eggs during the last 2 experimental d, their gonads were still immature when the experiments were stopped, but in some cases their gonads had switched to a mature stage at the end of the experiments (14 and 33% for Females LL and HL).

Relationships between the female body characteristics and egg production rate

In each of the experiments, we investigated the possible relationships between the different measurements made on the females (length, weight, egg production rate, egestion). We considered the different linear regressions between 2 of the variables (Table 3). There was a significant relation ($p < 0.05$) between carbon content versus the nitrogen content. The females which had the highest C:N ratio were those which belonged to Cohort H, correspond-

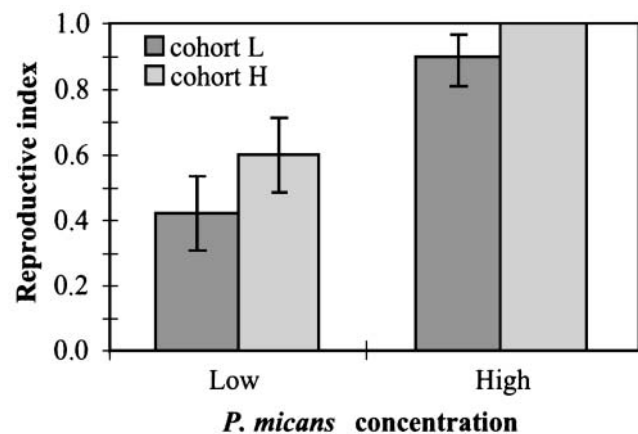


Fig. 3. *Calanus helgolandicus*. Reproductive index of the females determined at the end of the spawning experiments run at the high and low concentration. The reproductive index indicates the proportion of the females with a ripe gonad (see Runge 1987). Vertical bars indicate SD calculated for frequencies (note that for females HH, SD = 0 since all the females were mature)

ing to copepods which still had some oil droplets. The regression between carbon (or nitrogen) content versus the prosome length was significant for the females that were incubated at the high concentration (LH, HH). When we established logarithmic regressions between the carbon or nitrogen content and the size, the slopes of the regression represented the exponents of the allometric relationship relating the weight to the size. We found that the females incubated at the same concentration (low [LL, HL] or high [HH, LH]) had very similar relationships. The egg production rate was always strongly related to egestion but with different slopes each time. In contrast, egg production rate was never related to the prosome length of the females and only occasionally to the carbon and nitrogen content.

Table 3. Relationship between 2 measurements (the egg production, the fecal pellet production [pf]) or different body characteristics of a female, i.e. its prosome length (size), its carbon (C) and nitrogen (N) content, made on the females of each experiment (LL, LH, HH or HL). We also looked at the relationship found with the females held at the same concentration (Females LL+HL and HH+LH). *t*-tests are done to determine whether the slopes of each of the linear relationships are significant. If the slope is found significantly different from 0 at the 5% level, its value is indicated in the table. Otherwise, ns (not significant) is reported

	LL	LH	HH	HL	LL+HL	HH+LH
Slope C/N	3.75	2.17	2.57	2.78	–	–
Slope C/size	ns	0.09	0.05	ns	0.057	0.114
Slope N/size	ns	0.023	0.169	ns	0.012	0.024
Slope ln(C)/ln(size)	ns	2.79	4.25	ns	2.44	3.05
Slope ln(N)/ln(size)	ns	2.75	7.05	ns	2.4	2.68
Slope egg/C	ns	–0.187	ns	ns	ns	0.144
Slope egg/N	0.01	–0.0087	ns	0.01	ns	ns
Slope egg/size	ns	ns	ns	ns	ns	ns
Slope egg/pf	0.62	0.24	0.69	0.43	–	–

Table 4. *Calanus helgolandicus*. Mean egg and fecal pellet production found in each experiment (number of eggs or faecal pellets (Fp) $\text{fem}^{-1} \text{d}^{-1}$), by taking into account either the first values of production (between Days 2 and 5), or those found at the end of the experiments (between Days 17 and 20). Values obtained on Day 1 were not considered because they did not reflect the food conditions used during the spawning experiments. CV: coefficient of variation

Females	Days	n	Eggs $\text{fem}^{-1} \text{d}^{-1}$	CV (%)	Fp $\text{fem}^{-1} \text{d}^{-1}$	CV (%)
LL	2 to 5	80	10.4	104.8	22.7	58.1
	17 to 20	68	4.1	123.5	16.2	47.7
LH	2 to 5	80	32.7	50.2	65.8	30.0
	17 to 20	50	20.6	68.6	46.3	49.5
HH	2 to 5	80	33.3	64.8	48.8	48.8
	17 to 20	75	35.5	72.3	43.6	59.7
HL	2 to 5	80	9.5	116.6	16.1	51.7
	17 to 20	75	4.1	125.5	23.6	42.7

Specific egg production rate and hatching success

The specific egg production rate was calculated at the beginning (initial rate) and at the end (final rate) of each experiment using Eq. (1) (see 'Materials and methods') since the time interaction term in the egg production model ($\text{PIF} \times \text{IF} \times \text{time}$) was found to be significant. The values of egg production used to estimate the initial and final rates were those found between Days 2 to 5 and 17 to 20 (Table 4). Concerning the weight of the females, we used their initial and final weights (Table 2). In view of the overlapping of the SDs, the only difference that is obvious among specific egg production rates is that rates found at the low food concentration were lower than those found at the high concentration, both in terms of carbon and nitrogen.

To determine the structural egg production rate, the structural weight of the females was estimated using Eq. (2) (Table 5). Gonad weight was then deduced by considering the total weight minus the structural weight and the weight of the wax ester, which means that no statistics were feasible to compare gonad weights because they were not measured directly. However, it is remarkable to see how coherent the results were for gonad weight and egg production. Indeed, gonads were the heaviest in females held in or coming from high food concentration (Females HH, HL at the beginning and HH, LH at the end). Females HH, which were apparently producing the same number of eggs at the beginning and end of experiments, were also those maintaining almost the same gonad weight during the experiments (Table 5). Also, females coming from Cohort L had lower gonad weight at the end of experiments than that of females from cohort H ($\text{LH} < \text{HH}$, $\text{LL} < \text{HL}$), as was the case for the egg production (Table 4). Gonad weight was quite important since it varied between 11.5 and 48.6 μgC , which is a bit higher than that estimated for *Calanus finmarchicus* females by McLaren & Leonard (1995) (~30 μgC) and that provided by the model of Carlotti & Hirche (1997) (5 to 30 μgC). Because of the substantial difference between the total and the structural weight of the females, the specific egg production rates calculated from the struc-

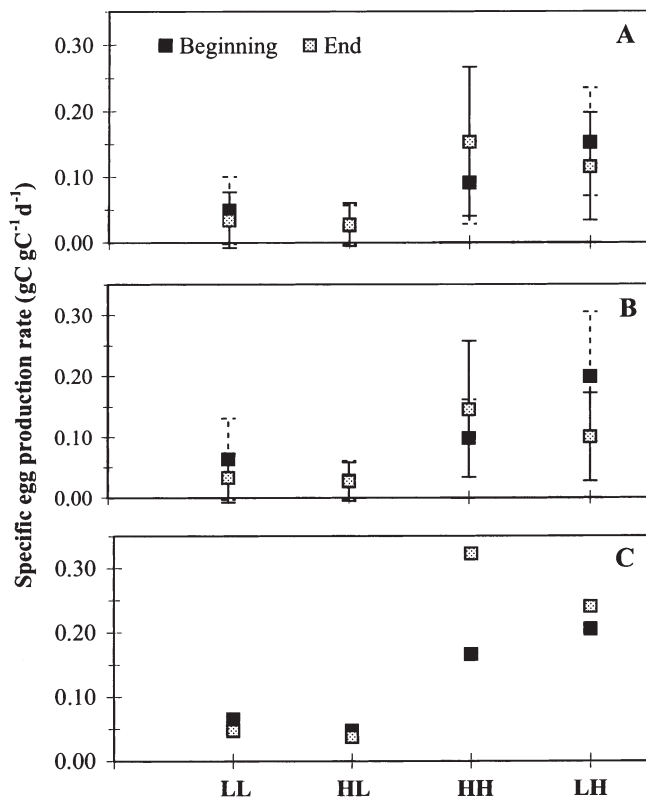


Fig. 4. *Calanus helgolandicus*. Specific egg production rate of the different categories of females ($\mu\text{gC } \mu\text{gC}^{-1} \text{d}^{-1}$) calculated at the beginning and end of each experiment. The rates are calculated from (A) carbon content, (B) nitrogen content and (C) structural weight of the females. Vertical bars are the standard deviation calculated for ratio (formula given by Takahashi & Bienfang 1983; dashed line: beginning, solid line: end)

tural weight were markedly higher than those found from the total carbon weight (Fig. 4).

The hatching frequency of the eggs laid by each category of females during 1 d of the experiment is presented in Fig. 5. The hatching frequency was high in all experiments varying between 0.88 and 0.95. However, when we compared the frequencies obtained in each experiment, we found that they were significantly different (χ^2 -test, $df = 3$, $p < 0.05$), probably because the hatching frequency for the females held and raised at the low concentration (LL) was significantly lower than the other.

Ingestion rate and gross egg production efficiency

The ingestion rate measured at the beginning of the experiments was expressed either in terms of carbon or nitrogen (Table 6). The differences between the ingestion rates of the females incubated at the same concentration but from different cohorts were not significant.

Table 5. *Calanus helgolandicus*. Structural weight of the females (in μgC) at the beginning and at the end of each experiment. The structural weight was calculated from the relation established by Rey-Rassat et al. (2002; see the text). The gonad weight is the total carbon weight minus the structural weight and the lipids (lipids are only present in Females HL and HH at the beginning of experiments as shown in Table 2)

Females	Period	Structural weight	Gonad weight
LL	Beginning	57.8	18.24
	End	31.8	11.49
LH	Beginning	57.8	18.24
	End	31.0	33.46
HH	Beginning	72.5	48.63
	End	39.9	44.05
HL	Beginning	72.5	48.63
	End	40.2	14.57

However, when we consider the ingestion rate per unit of the female weight (specific ingestion rate), we found that the values of the females from Cohort L were higher (74 to 112%) than for the Females H (LH > HH and LL > HL).

We also estimated the gross egg production efficiency of the females by considering the initial specific egg production rates (Fig. 4) divided by the specific ingestion rates (i.e. the ingestion rate divided by the initial weight of the females). Differences among gross egg production efficiencies were too low to be considered significant, but we can stress the fact that those calculated from the carbon data were always lower than those estimated on the basis of nitrogen.

DISCUSSION

Influence of actual food concentration on females

The actual nutritional conditions during the incubation of the females for egg production experiments

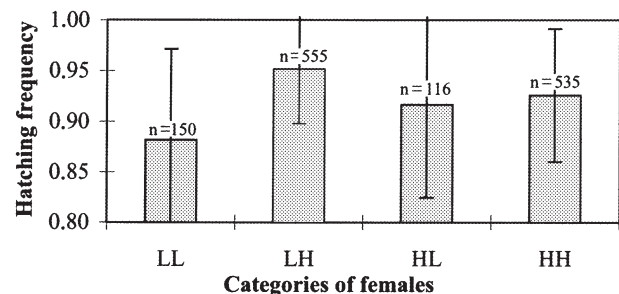


Fig. 5. *Calanus helgolandicus*. Frequency of hatching success calculated with the 4 categories of females (LL, LH, HL, HH). Vertical bars indicate SD. n: total number of eggs (hatched or not) considered for each category of females

strongly influenced their physiology. The females incubated at the low food concentration (Females LL and HL) produced fewer eggs than those at the high concentration (HH and LH). This phenomenon is well known for the genus *Calanus* (Marshall & Orr 1952, Frost 1985, Runge 1985, Peterson 1988, Hirche et al. 1997) even if it has never been studied in *C. helgolandicus* specifically. Moreover, despite the generally high hatching success, we found lower values for the eggs laid by the females held at the low concentration, which is in good agreement with Guisande & Harris (1995). All these results can be directly related to the actual feeding of the females since the ingestion and egestion rates were much lower at the low concentration. *C. helgolandicus* is 1 species of the genus *Calanus* which can not spawn without a food supply, in contrast to *C. hyperboreus* or *C. glacialis*, which can produce eggs using only their stored lipid (Hirche & Kattner 1993). In our study, it was clear that *C. helgolandicus* females also used internal body resources during experiments, and their loss in body carbon and nitrogen was greater for the females held at the low concentration (LL, HL, average of 46 %) than for those at the high concentration (HH, LH, average of 14 %). These internal resources were used to support different metabolic costs (egg synthesis included), which are impossible to evaluate separately. However, because the gonad of the females at low food concentration often reverted to an immature stage after laying eggs, these females may have to spend more energy (i.e. draw on their own body resources) to mature their gonad again before the next spawning event, compared with females at high food concentration, which remain mature between 2 spawning events (Runge 1984, Niehoff & Hirche 1996, Niehoff et al. 1999).

Since a decrease in weight occurred for both females held at high and low food concentration, it may be that

the experimental conditions induced stress and increased the metabolic costs. In fact, this decrease is in contradiction with Hirche (1990) and Hirche & Kattner (1993), who found no decrease and even an increase of the female body carbon after 77 d of spawning experiments at excess food. However, these differences in results might come from the use of a different species (*Calanus finmarchicus* and *C. glacialis*) and temperature ($\leq 0^{\circ}\text{C}$). The fact that in Hirche (1990) and Hirche & Kattner (1993) the female weight was lower at the start of the experiments than at the end can also be due to the fact that females may have been food-limited at the time of collection or that they may have recently entered the stage, since, according to Rey-Rassat et al. (2002), the weight of females increases during the first days of the adult lifetime.

The gross egg production efficiency (GEE) measured at the beginning of the experiments and expressed in terms of carbon was always lower (17 to 22 %) than the GEE in terms of nitrogen (25 to 31 %). This suggests that the conversion of the egg production rate in terms of carbon into ingestion rate (by using the standard value of GEE 30 %) has to be considered with caution. Peterson (1988) found, for *Calanus marshallae* females, the GEE to be between 22 and 28 % for food concentrations of 38 and 438 $\mu\text{gC l}^{-1}$, respectively. For this same relationship (GEE vs food), Båmstedt et al. (1999) found contradictory results because some indicated no significant relationship (their Fig. 9), whereas others suggested a positive one (their Fig. 12). In our case, the GEE was rather similar at both concentrations with, somehow, higher values at the highest concentration (HH > HL and LH > LL). More studies need to be performed to understand what the exact influence of food on the GEE is. Moreover, the GEE of the females was found to be lower than almost all the gross growth efficiency measured for each copepodite stage from the Cohorts H and L (Rey-Rassat et al. 2002). The decrease of the efficiency through the stages was also reported by Paffenhöfer (1976). The amount of carbon ingested and transferred to the eggs appears to be less important than for the somatic growth of the copepodites. This could come from the fact that the metabolism of the females is certainly higher than that of the copepodite stages because of higher energetic costs related to the functioning and the maintenance of the gonad.

Influence of past feeding history on females

The past feeding history of the females held at the low or high concentration during their spawning also strongly influenced a number of aspects of their physiology (Fig. 6).

Table 6. *Calanus helgolandicus*. Ingestion rate in terms of carbon and nitrogen (μgC or $\mu\text{gN ind.}^{-1} \text{d}^{-1}$) and gross egg production efficiency (GEE) of the females in the 4 experiments. The ingestion rate of the females incubated at the same high or low concentration is not found significantly different at the 5 % level (Z-tests)

Females		Ingestion rate	GEE
LL	C	20.5 ± 3.8	0.184
	N	2.80 ± 0.52	0.266
LH	C	54.6 ± 9.5	0.217
	N	7.46 ± 1.29	0.312
HH	C	65.1 ± 6.9	0.185
	N	8.90 ± 0.94	0.266
HL	C	20.1 ± 4.4	0.171
	N	2.74 ± 0.60	0.247

Weight

In females from Cohort H, the loss in body carbon during experiments was similar to the loss in nitrogen (35% for Females HH and 55% for Females HL), which was not the case in Females L that lost twice as much carbon as nitrogen. Results obtained for the Females H can be related to those of Mayzaud (1976), who showed that CV of *Calanus finmarchicus* were able to alternate between protein and lipid catabolism when needed. This was not the case for copepods without lipid storage such as *Acartia* sp., which was forced to draw on its protein resources until reaching the lethal protein threshold (Ikeda 1974, Mayzaud 1976). Our Females H, which catabolized the same relative amount of carbon and nitrogen, were using both their proteins and lipids to satisfy their energetic requirements. On the contrary, our Females L were certainly deficient in nitrogen components (e.g. in their protein resources) from the very beginning of the experiments, and they could not draw on these resources (Mayzaud 1976) as they would have been too close to the critical content in protein right from the beginning of the experiments. This would explain that, in the presence of an excess of food, the nitrogen content of the Females LH was the only one which increased during the experiments in order to rebuild the protein capital which had become depleted.

Egestion

Females from Cohort L had, for at least 5 d, a much higher fecal pellet production than females from H (Figs. 1 & 2). Interestingly, very similar results were obtained by Rey et al. (1999). These authors found that the egestion of *Calanus finmarchicus* females from a cohort reared under limiting food conditions was higher than that of females from a cohort reared under better food conditions; this phenomenon lasted for the 15 experimental d. However, the ingestion measured at the beginning of our spawning experiments was not influenced by the origin of the females. All these results suggest that the number of fecal pellets (fp) produced per unit of food ingested varied with the origin of the females (1.1 to 1.2 fp for the Females LL and LH, 0.8 to 0.7 for the Females HL and HH). There are 2 possible explanations for this: (1) The females from

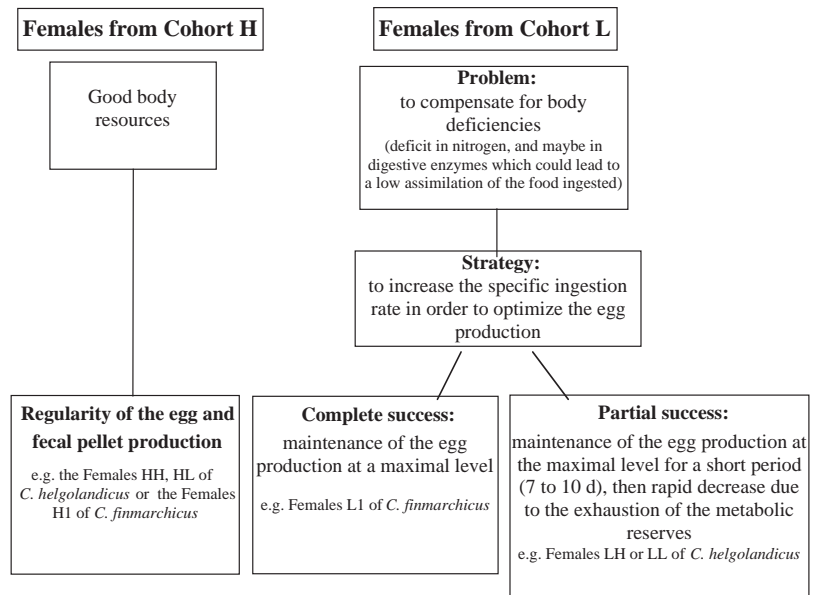


Fig. 6. Conceptual schema which synthesizes the influence of the past feeding history of the females of *Calanus helgolandicus* and *C. finmarchicus* (see Rey et al. 1999) on their egg and fecal pellet production

Cohort L produced shorter (or less dense) fecal pellets than females from H, but the total volume of fecal pellets produced by Females L and H was the same. Since we did not measure the fecal pellet volume, it is impossible to determine if this happened. However, we assumed this was not the case because Rey et al. (1999) found no significant difference between the volume of the fecal pellets produced by females from cohorts reared under high and low food conditions (see also Båmstedt et al. 1999). (2) The assimilation efficiency of Females L was initially lower than that of Females H (Fig. 6). This hypothesis would be in good agreement with the results of Mayzaud & Conover (1976), Hirche (1981) and Tande & Slagstad (1982), who showed a positive relationship between the nutritional conditions in the field and the digestive enzyme content of copepodites CV or females of *C. finmarchicus* and *C. helgolandicus*. Therefore, the enzymatic content of the copepods held at low concentration (as in Cohort L) would be lower than that of those at the high concentration (Cohort H). However, we have to acknowledge that other authors have found inverse relationships (Hassett & Landry 1983). Because we did not measure the enzymatic activity of the females in our experiments, it is impossible to make a definite conclusion. Nevertheless, we can note that the second hypothesis (i.e. low assimilation and low enzymatic activity for Females L) would be compatible with the fact that Females L were initially deficient in protein, as has been assumed previously.

Ingestion

As we said before, ingestion rates were independent of the origin of the females. However, when considering the ingestion rate per unit of the female weight (specific ingestion rate), we found that the values of the females from Cohort L were higher (74 to 112%) than for the females of Cohort H. Such results can be related to those of Runge (1980), Hassett & Landry (1983, 1990) and Frost (1985), who showed that the ingestion rates of *Calanus pacificus* females increased after they had been starved or submitted to very low food concentration for a certain period. High specific ingestion rates would permit the females from Cohort L to compensate for their low assimilation efficiency in order to optimize their spawning (Fig. 6).

Egg production rate

The females held at the same food concentration initially had the same egg production, whatever their origin. However, after a certain time into the experiment (7 to 10 d), the egg production of females from Cohort L decreased and became significantly lower than that of Females H (LL < HL and LH < HH). This decrease is obviously related to smaller clutch size and longer intervals between clutch deposition as well as to the fact that a certain number of the Females L stopped spawning, and at the end of the experiments their gonads were found to be at an immature or senescent stage. The past feeding history of the females seems also to influence the hatching success since the eggs of Females LL had the lowest hatching success (LL < LH, HH, HL). Following the previous paragraphs, we can assume that Females L could not maintain a maximal egg production rate because of the exhaustion of their metabolic reserves. By comparison with our Females L, the *Calanus finmarchicus* females coming from the Cohort L1 (reared at low food) in Rey et al. (1999) did not decrease their spawning during the 15 d of the experiment, which suggests that they may have succeeded in compensating for their initial deficiency (Fig. 6). The difference between the spawning vs time of our Females L and those (L1) described in Rey et al. (1999) certainly comes from the fact that the food condition was more limiting in the present case (average of $80 \mu\text{gC l}^{-1}$) than in Rey et al. ($430 \mu\text{gC l}^{-1}$), i.e. that the body deficiency was much greater in this study.

Even if *Prorocentrum micans* has been recognized as a nutrient of high quality (Hitchcock 1982, Rey et al. 2001), the mono-specific diet during all the development may have induced a nutritive deficiency. However, it is important to underline that the egg production values measured at the high concentration

coincide perfectly with the maximal ones reported in laboratory and field studies on the same species (Gill & Harris 1987, Bautista et al. 1994, Pond et al. 1996, Laabir et al. 1998, Biegala et al. 1999, Irigoien et al. 2000a).

Some authors suggest that copepods such as Females L are in fact rarely found in the field because they would be rapidly eliminated from the population (Huntley & Lopez 1992, Aksnes 1996). It is certainly true that the lifetime in the case of the Females L could be shorter than that of the females which grew under optimal food conditions. However, it is rather difficult to imagine that females which grow under limiting food conditions are so rare in the field. They may represent a large part of the total females because the food conditions are unlikely to remain optimal during an entire generation. The patchy distribution of the food or mismatch between growth and phytoplankton blooms may also contribute to changes in the growing conditions of the females. Strong seasonal differences in the C:N ratio of the females have been indicated by different authors suggesting differences in the growing conditions of the females (Tande 1982, Pond et al. 1996, Irigoien et al. 1998, 2000b). Therefore, it is likely that in the numerous field studies in which egg production was not found to be maximal considering the food and the temperature conditions, the feeding history of the females may have influenced their spawning (Bautista et al. 1994, Pond et al. 1996, Laabir et al. 1998, Irigoien et al. 2000a). These results indicate that the variability in the egg production rates found in field studies has to be interpreted cautiously, because it can be related not only to present or recent past conditions (food quantity, quality and temperature), but also to the growth history of the female.

CONCLUSION

In our experiments, the influence of the past and present feeding conditions on the egg production of *Calanus helgolandicus* females was investigated and we can conclude that: (1) the egg production was supported by the amount of food ingested but also by catabolism of body carbon and nitrogen resources; this catabolism was more important for females incubated at the low food concentration (LL, HL) than those at the high food concentration (HH, LH); (2) the catabolism also varied with the past feeding history of the females; while the percentage of loss of carbon during experiments was equal to that of nitrogen for females from Cohort H (i.e. HH and HL), twice as much carbon as compared to nitrogen was lost from females of Cohort L (LL and LH) suggesting that Females L could not draw as much on their protein resources because of

an initial deficiency; and (3) the egg production of Females L after 7 to 10 d of experiments was significantly lower than that of Females H, at both food concentrations (LL < HL and LH < HH); in other words, the past feeding history of females had a long-term effect on their egg production. Females L will experience a more rapid exhaustion of their internal resources compared with Females H, and this will contribute to finally reducing their total fecundity.

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