

Comparison of *in situ* egg production rate in *Calanus finmarchicus* and *Metridia longa*: discriminating between methodological and species-specific effects

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ABSTRACT: A comparison of the *in situ* egg production rate (EPR) in *Calanus finmarchicus* and *Metridia longa* was conducted using freshly collected adult females (C6f), individually incubated in 45 ml dishes with and without egg separators at 6°C for 24 h. Incubations with egg separators resulted in significantly greater clutch size and *in situ* EPR in *M. longa* than without egg separation, while no significant effect was measured in *C. finmarchicus*. The use of egg separators resulted in lower hatching success and naupliar viability (%), an effect more pronounced in *M. longa*. A highly significant effect of food on egg removal was observed in *M. longa*, with C6f removing up to 85 % of their eggs when the ambient algal biomass at capture was less than 50 mg chl *a* m⁻², suggesting an effect of the feeding 'history' of C6f. In the absence of cannibalism on the eggs, the combined effect of smaller clutch size and longer spawning interval resulted in a lower EPR in *M. longa* by a factor of 4 to 5 relative to *C. finmarchicus*. Based on egg and C6f weight, the weight-specific EPR (growth) in *M. longa* was half that in *C. finmarchicus*. *M. longa* produced eggs at a rate similar to *C. finmarchicus* only when C6f were incubated at a higher temperature (+4°C). These results are discussed in the context of the paradox between the low EPR and high abundance in *Metridia* species and the different life history strategies developed by *Metridia* and *Calanus* species.

KEY WORDS: *In situ* egg production rate · *Metridia longa* · *Calanus finmarchicus* · Egg separation · Species comparison · Reproductive strategy

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INTRODUCTION

The sub-arctic *Calanus finmarchicus* and the arctic *Metridia longa* are key components of the plankton community in the Lower St. Lawrence Estuary (LSLE) and in the northwest Gulf of St. Lawrence (GSL; Plourde et al. 2002, Harvey et al. 2005). The *in situ* egg production rate (EPR) in *C. finmarchicus* has been measured on a routine basis since 1991 at a monitoring station in the LSLE (Plourde & Runge 1993). The method of incubating individual adult females (C6f) in petri dishes in filtered seawater (FSW) as described by Runge & Roff (2000) has been used since 1993 to describe individual variability in clutch size and body size (Plourde et al. 2001). Females were individually

incubated in 45 ml petri dishes, and eggs were counted only once at the end of the 24 h incubation; this approach was chosen because clutch size was consistent with those obtained during incubations with multiple counts and egg removal, indicating that no significant cannibalism occurs during incubation (Runge & Plourde 1996, Runge & Roff 2000, Plourde et al. 2001). Using the same incubation technique, EPR in *M. longa* was measured less frequently but resulted in strikingly lower clutch sizes and EPR than in *C. finmarchicus* (S. Plourde unpubl. data), although both appeared greater than the few measurements previously published for other *Metridia* species (Buskey & Stearns 1991, Calbet & Irigoien 1997). However, some large clutches (>60 eggs) that were observed occasionally

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and a gonadal morphology and maturation process similar in *Metridia* spp. and in *C. finmarchicus* (Niehoff 2007) suggest that *M. longa* shows potential for greater EPR and that egg cannibalism during incubation could potentially alter the measurement of *in situ* EPR in this species.

The detrimental effect of cannibalism and/or egg destruction during incubation for the measurement of *in situ* EPR in the genus *Metridia* has been recently reported. Hopcroft et al. (2005) pointed out the paradox between the high abundance of *Metridia* spp. and their low *in situ* EPR. Using individual incubations with 50 ml containers equipped with egg separators, they measured much higher *in situ* EPR in *M. pacifica* and *M. okhotsensis* in the Gulf of Alaska than with other techniques without egg separation (10 ml multi-well plates or bottles). The authors argued that *Metridia* spp. showed equivalent weight-specific EPR as other calanoids and concluded that methodological flaws allowing egg cannibalism and destruction of abnormal and fragile eggs by females during incubations mainly caused this paradox between the low EPR and high abundance in *Metridia* species. Additionally, greater egg hatching success without egg separators was attributed to C6f eating/destroying unhealthy eggs during incubation (Hopcroft et al. 2005). Using the same incubation set-up, Halsband-Lenk (2005) similarly observed greater *in situ* EPR in *M. pacifica* in Dabob Bay, Washington, with egg separation than in 15 ml multi-well plates; the rates were much lower than in *C. pacificus* because of lower spawning frequency (Pierson et al. 2005). However, the use of different container types (volumes) in these studies precludes any firm conclusion about the effect of egg separation in the measurement of the reproductive rate in *Metridia* species.

Our study was conducted to test the null hypothesis based on the conclusions of Hopcroft et al. (2005) that *Metridia longa* produces eggs at the same rate as *Calanus finmarchicus* in the GSL. As secondary objectives, we also explored the effects of potential methodological bias in measuring *in situ* EPR in these copepods. In addition to egg hatching success and viability, *in situ* EPR was measured concurrently in both species. Egg cannibalism during incubations was estimated by comparing clutch size obtained with and without egg separation using the same container, and a cannibalism index related to phytoplankton biomass was developed. Finally, we tested the potential effect of using egg separators on egg hatching success and viability. The implications of our results are discussed in relation to the life history characteristics of *M. longa* and the capacity of this species to achieve high population abundance despite its lower reproductive rate.

MATERIALS AND METHODS

Field sampling. Zooplankton was collected at a station (340 m depth) located 20 km north of Rimouski in the central LSLE, which was visited weekly from May to November in 1998 (see Plourde et al. 2001) and 2005. The basic sampling protocol included a CTD-12 (Applied Microsystems) profile from the surface to 250 m, collection of water with 5 l Niskin bottles at 8 depths (0, 5, 10, 15, 20, 25, 35, and 50 m) for the measurement of chlorophyll *a* (chl *a*) biomass, and sampling of zooplankton with a 1 m diameter (ratio 1:4), 333 μm mesh ring net fitted with a restricted-flow cod-end from 320 m to the surface. In 2005, zooplankton was sampled in 2 different depth layers (0 to 100 and 100 to 320 m) with a 0.75 m diameter (ratio 1:5), 202 μm mesh ring net equipped with a General Oceanic closing device. The catch was diluted into 4 l jars filled with 0.2 μm FSW. Samples were consistently collected between 08:00 and 12:00 h, and zooplankton was maintained at 5 to 6°C in coolers during transport to the laboratory at the Maurice-Lamontagne Institute (Mont-Joli, Québec, Canada; see Plourde et al. 2001 for laboratory analysis details).

***In situ* EPR.** C6f in good condition were selected under a binocular microscope from the integrated catch (1998) or from live samples collected in the 0–100 and 100–320 m layers (2005). Most of the *Calanus finmarchicus* C6f were sorted from the 0–100 m samples. Because *Metridia longa* C6f were seldom found in this layer, they were all sorted from samples collected in the 100–320 m layer. Usually 30 to 40 C6f of each species were incubated individually in 45 ml petri dishes filled with 0.2 μm FSW for 24 h in the dark at 6°C. This basic protocol combines both the need for sufficient replication and a relatively large volume for these large-bodied copepods (Runge & Roff 2000). The incubation temperature was chosen because it grossly reflected the 'average' temperature experienced by C6f migrating from the warmer surface layer (0 to 25 m) at night and cooler deep water during the day in the LSLE (*C. finmarchicus*: 75 to 100 m; *M. longa*: 175 to 225 m; S. Plourde unpubl. data). As *C. finmarchicus* typically lay 1 clutch d^{-1} at this temperature (Hirche et al. 1997), eggs were counted only once at the end of the 24 h incubation; these counts were assumed to represent distinct clutches, which was confirmed by the observation of the developmental state of the eggs. EPR was estimated by the product of the mean clutch size and spawning frequency. Prosome length (PL) of spawning C6f was measured on a dissecting microscope equipped with a micrometer (1 unit = 0.039 mm) at 25 \times .

Egg hatching success and viability to naupliar Stage 2 (N2) were assessed in both species at 6°C. Eggs were left untouched in the dishes after being counted at the

end of the 24 h incubations. Following an additional incubation of 96 h allowing the development of eggs to N2 at 6°C (McLaren et al. 1969, Campbell et al. 2001), the number of unhatched eggs and malformed and dead nauplii on the bottom of the dishes was recorded. After the addition of 2 ml of acetic acid (white vinegar) to the dishes in order to 'kill' the free swimming nauplii, the total number of nauplii was determined. Egg hatching success was estimated from the difference between clutch size and the total number of nauplii, while the difference between clutch size and the number of healthy nauplii represented the egg viability to N2.

Effect of cannibalism and temperature. The effect of egg cannibalism by C6f during the incubation was quantified on 7 occasions in *Calanus finmarchicus* in 1998 and 14 times in 2005 in *Metridia longa*. *In situ* EPR was measured concurrently with the routine individual 24 h incubations in 45 ml petri dishes with and without egg separators (mesh size = 333 µm). The egg separators were held about 2 mm above the bottom of the dishes and caused only a slight decrease in the incubation volume. The ratio between the mean clutch size without and with egg separators was used as an index of cannibalism, where a value of 0 corresponds to the disappearance of 100% of the eggs.

On 6 different occasions in the treatment at 6°C without egg separation, we estimated a rough egg removal rate by *Metridia longa* C6f in order to explore the effect of using a larger incubation volume (45 ml instead of 15 ml, see Halsband-Lenk 2005). Eggs laid were counted after 15 to 16 h in addition to the count at the end of the 24 h incubation. In total, 57 clutches were surveyed. Since the mean clutch size observed in the treatment with egg separation was used as the initial value (Time 0), a coarse egg removal rate (% of initial clutch size h⁻¹) was estimated by linear regression between the mean egg number (calculated from 3 to 13 clutches depending on the occasion) and time assuming synchrony in egg laying (Halsband-Lenk 2005, Hopcroft et al. 2005).

We tested the effect of incubating *Metridia longa* C6f at a surface temperature typical of the summer-autumn period such as described by Hopcroft et al. (2005) in comparison to our approach using a temperature representing a rough average between the day and night depths. The effect of temperature on the EPR in *Calanus finmarchicus* is well described (Runge & Plourde 1996, Hirche et al. 1997), but little is known about its effect on the *in situ* EPR in *M. longa*. Using egg separation, *M. longa* C6f were incubated on 10 occasions at a temperature typical of the upper layer in summer and early autumn (10°C) in the LSLE concurrently with incubations at 6°C.

Weight-specific clutch size and EPR. Carbon weight-specific clutch size and *in situ* EPR were estimated

using dry weight (DW) against PL relationships and CHN analysis (S. Plourde & P. Joly unpubl. data; see Runge & Plourde 1996 and Runge et al. 2006 for technical details). In *Calanus finmarchicus*, DW (µg) was estimated from PL (µm) using a relationship based on individuals collected in the LSLE from May to November in different years (n = 427, p < 0.0001):

$$\log DW = (2.231 \times \log PL) - 5.18 \quad (1)$$

C6f carbon weight (µg) was estimated using a carbon content of 49% of DW (S. Plourde & P. Joly unpubl. data). An egg carbon content of 0.223 µg C egg⁻¹ was used (Runge & Plourde 1996), similar to the estimation from egg diameter and an egg carbon weight of 0.14 ng C µm⁻³ (Kiørboe & Sabatini 1994). In *Metridia longa*, DW (µg) was estimated from a relationship based on a small number of C6f collected in late spring-early summer (n = 36, p < 0.01):

$$\log DW = (3.573 \times \log PL) - 9.759 \quad (2)$$

This relationship resulted in similar DW estimates (lower by only 5 to 8%) than that established by Hopcroft et al. (2005) based on a limited number of 'lipid-rich' *M. pacifica* and *M. okhotensis* (n = 16, p < 0.0001), the latter being similar in size to *M. longa*. A carbon content of 48% of DW was applied (Groenvik & Hopkins 1984, Conover & Huntley 1991). We estimated the egg carbon weight in *M. longa* from its diameter (175 µm; Kiørboe & Sabatini 1994), resulting in an egg carbon weight of 0.393 µg C.

Statistical analysis. A Mann-Whitney *U*-test (unpaired comparisons) was used to compare results among experimental treatments, because most of the variables did not meet the conditions for applications of parametric statistical tests. Statistical analyses were performed using the STATView 5.0.1 (SAS Institute) statistical package. A regression model that showed the best fit to the data was fitted using SigmaPlot 9.0 software.

RESULTS

Clutch size and egg cannibalism

The use of egg separators induced a difference in clutch size in *Metridia longa* but not in *Calanus finmarchicus* (Fig. 1). In *C. finmarchicus*, there was no effect of egg separation on clutch size (Mann-Whitney *U*-test, p > 0.05; 1998 data); the overall average clutch size was 90 eggs (1998 and 2005 data pooled; Fig. 1A,B). In *M. longa*, the frequency distribution of clutch sizes was skewed heavily toward small clutches without egg separation compared to the egg separation treatment (Fig. 1C,D). Consequently, mean clutch

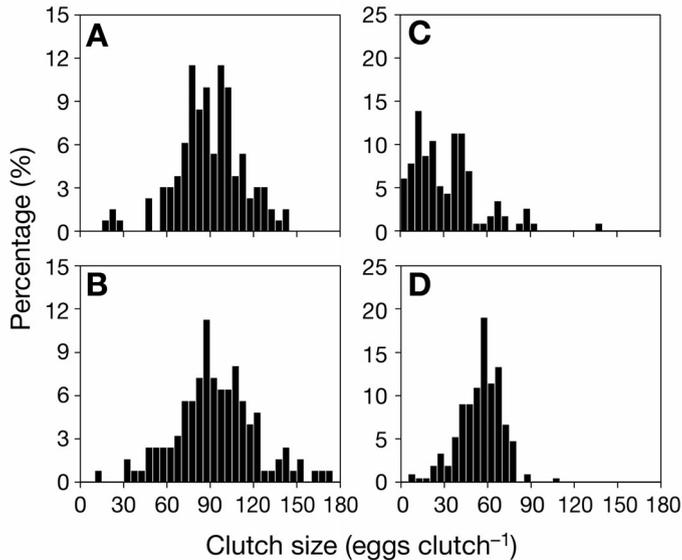


Fig. 1. *Calanus finmarchicus* and *Metridia longa*. Frequency distribution of clutch size (A,C) without and (B,D) with egg separators in (A,B) *C. finmarchicus* in 1998 and (C,D) *M. longa* in 2005

size was significantly larger in the egg separator treatment, with a mean of 54 eggs compared to 32.6 eggs without egg separation (Mann-Whitney *U*-test, $p < 0.0001$).

Temperature (6 and 10°C) had no discernable effect on clutch size in *Metridia longa* (Mann-Whitney *U*-test, $p > 0.05$). Clutch size was not related to PL in *M. longa* without egg separation, but showed a significant relationship when egg separators were used (pooled data at 6 and 10°C; Fig. 2A,B). Clutch size in *C. finmarchicus* was related to PL using all data collected in 1998 and 2005 (Fig. 2C). Despite the great variability, the slope was highly significant ($p < 0.0001$) in both species.

The similar clutch size with and without egg separation in *Calanus finmarchicus* resulted in a lack of a relationship between its cannibalism index (fraction of eggs removed) and ambient chl *a* biomass; clutches laid without egg separation represented 97% of those with egg separation (Fig. 3). However, the points below 100 mg chl *a* m⁻² (mean = 7%) suggested the potential for some cannibalism in this species during periods of low food availability. Cannibalism was obviously more important in *Metridia longa*, which consistently removed 38% of its eggs at high food concentration, while this species removed an increasing fraction of the clutches below a threshold of 50 mg chl *a* m⁻² to a maximum of 85% (Fig. 3). A survey of egg number over time in several clutches indicated that *M. longa* C6f removed eggs at 3% of the initial clutch size h⁻¹, resulting in an average total egg removal of 72% in the 45 ml dishes (Fig. 4). Therefore, clutch size and EPR in

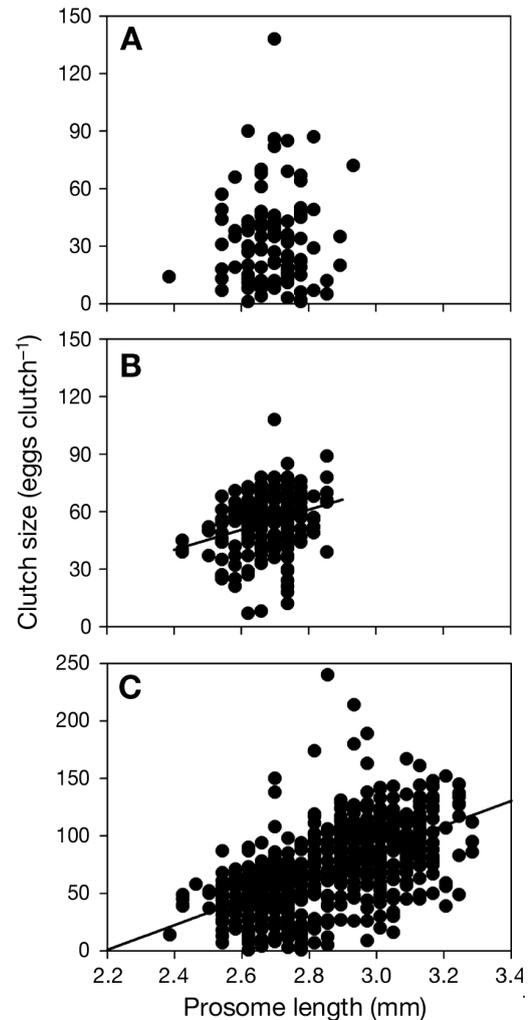


Fig. 2. *Calanus finmarchicus* and *Metridia longa*. Relationship between clutch size and C6f prosome length in *M. longa* in 2005 (A) without and (B) with egg separation and in *C. finmarchicus* in 1998 and 2005 (C; all data together). Data fitted to a linear regression model. (A) No significant relationship ($p < 0.05$). (B) $y = 52.4x - 85.6$, $r^2 = 0.08$, $p < 0.0001$. (C) $y = 107.9x - 236.6$, $r^2 = 0.36$, $p < 0.0001$

M. longa measured without egg separators were not considered in further analyses and species comparisons.

Clutch size averaged on each sampling occasion in 2005 was significantly related to ambient algal biomass in both species, being limited below 60 to 70 mg chl *a* m⁻² (Fig. 5). A much lower maximum clutch size of 55 eggs was determined from the regression in *Metridia longa* than in *Calanus finmarchicus* (101 eggs; Fig. 5A). When species-specific egg and C6f carbon masses were considered, *M. longa* showed a greater weight-specific maximum clutch size (15% body C clutch⁻¹) than *C. finmarchicus* (11%; see regressions in Fig. 5B).

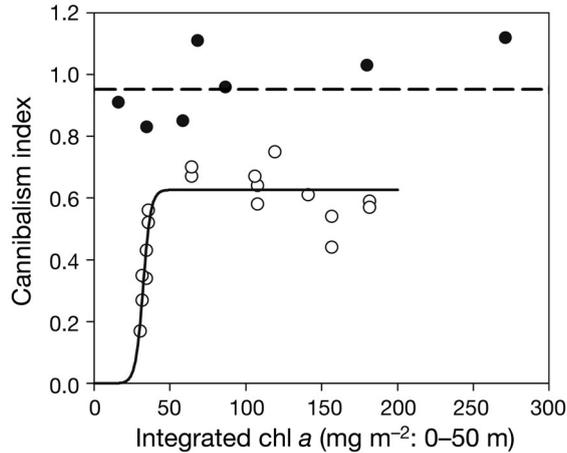


Fig. 3. *Calanus finmarchicus* and *Metridia longa*. Cannibalism index in *C. finmarchicus* in 1998 (●) and *M. longa* in 2005 (○) in relation to the integrated chl *a* standing stock (mg chl *a* m⁻²). See 'Materials and methods' for details on the calculation of the cannibalism index. *M. longa*: data fitted to a sigmoidal regression model (best fit), where $y = 0.63/[1 - e^{-[(x - 32.4)/2.5]}]$, $r^2 = 0.77$, $p < 0.0001$. *C. finmarchicus*: dashed line indicates average of all observations

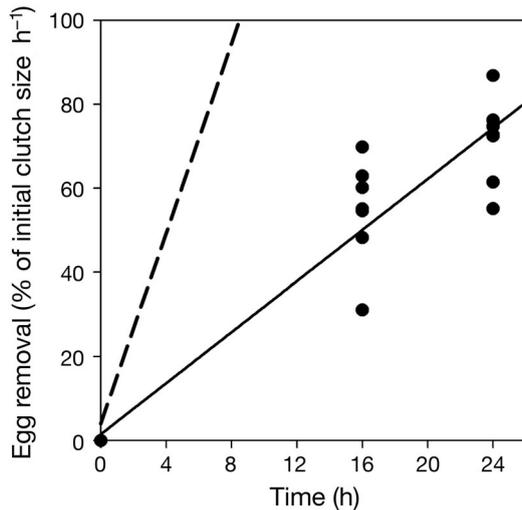


Fig. 4. *Metridia longa*. Egg removal rate by C6f in 2005. Each point represents the mean of 3 to 13 observations (total = 57). Data fitted to a linear regression model, where $y = 3.03x + 1.49$, $r^2 = 0.91$, $p < 0.0001$. Dashed line: egg removal rate (11% of initial clutch size h⁻¹) observed in *M. pacifica* in 15 ml multi-wells (adapted from Fig. 5b in Halsband-Lenk 2005)

Spawning frequency and *in situ* EPR

Spawning frequency in *Calanus finmarchicus* and *Metridia longa* in 2005 showed a similar response to algal biomass, but maximum spawning frequency determined from regressions was species-specific. While 71% of the *C. finmarchicus* C6f laid eggs at 6°C at non-limiting phytoplankton biomass (>70 mg

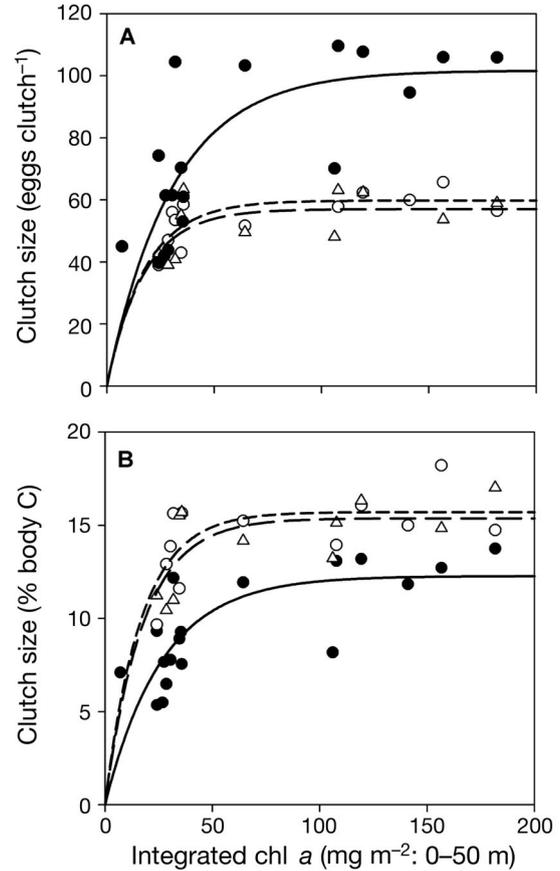


Fig. 5. *Calanus finmarchicus* and *Metridia longa* in 2005. (A) Clutch size and (B) weight-specific clutch size in *C. finmarchicus* (●) at 6°C and in *M. longa* at 6°C (○) and 10°C (Δ) in relation to the integrated chl *a* standing stock. Data fitted to an Ivlev regression model (best fit). (A) *C. finmarchicus*: $y = 101.7 \times (1 - e^{-0.032x})$, $r^2 = 0.57$, $p < 0.001$; *M. longa* 6°C: $y = 59.8 \times (1 - e^{-0.056x})$, $r^2 = 0.56$, $p < 0.01$; *M. longa* 10°C: $y = 57.0 \times (1 - e^{-0.058x})$, $r^2 = 0.37$, $p < 0.05$. (B) *C. finmarchicus*: $y = 12.3 \times (1 - e^{-0.038x})$, $r^2 = 0.48$, $p < 0.01$; *M. longa* 6°C: $y = 15.7 \times (1 - e^{-0.06x})$, $r^2 = 0.46$, $p < 0.05$; *M. longa* 10°C: $y = 15.3 \times (1 - e^{-0.056x})$, $r^2 = 0.48$, $p < 0.05$

chl *a* m⁻²), *M. longa* showed a much lower maximum spawning frequency of 30% at 6°C and 51% at 10°C (Fig. 6A). Spawning frequency in *M. longa* at 6°C without (not shown) and with egg separation was similar ($p > 0.05$). Overall, the spawning frequency in this species represented 36% (6°C) and 62% (10°C) of the fraction of *C. finmarchicus* that produced eggs at 6°C (slope of linear regressions, Fig. 6B).

In situ EPR in *Calanus finmarchicus* and *Metridia longa* in 2005 were significantly related to ambient chl *a* biomass, with EPR being food-limited below a phytoplankton biomass of 60 to 70 mg chl *a* m⁻² (Fig. 7A). However, the small number of data points in this range of algal biomass precluded the determina-

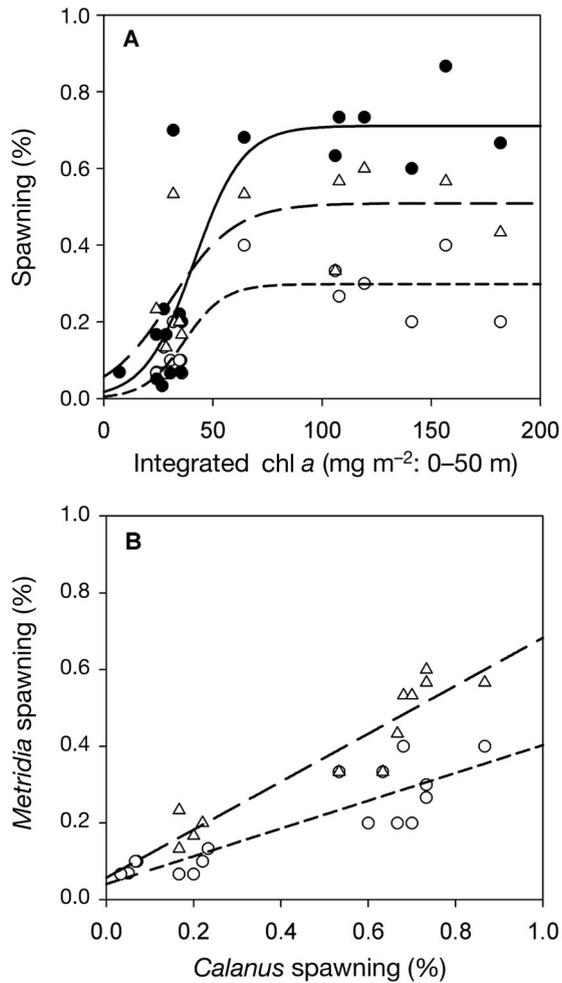


Fig. 6. *Calanus finmarchicus* and *Metridia longa* in 2005. (A) Spawning frequency in *C. finmarchicus* (●) at 6°C and in *M. longa* at 6°C (○) and 10°C (Δ) in relation to the integrated chl a standing stock. (B) Spawning frequency in *M. longa* at 6°C (○) and 10°C (Δ) in relation to spawning frequency in *C. finmarchicus*. (A) Data fitted to a sigmoidal regression model (best fit). (A) *C. finmarchicus*: $y = 0.71/[1 - e^{-(x-41.3)/11.2}]$, $r^2 = 0.76$, $p < 0.0001$; *M. longa* 6°C: $y = 0.30/[1 - e^{-(x-36.9)/9.1}]$, $r^2 = 0.72$, $p < 0.0001$; *M. longa* 10°C: $y = 0.51/[1 - e^{-(x-30.5)/14.8}]$, $r^2 = 0.50$, $p < 0.05$. (B) Data fitted to linear regression. *M. longa* 6°C: $y = 0.36x + 0.04$, $r^2 = 0.77$, $p < 0.0001$; *M. longa* 10°C: $y = 0.62x + 0.06$, $r^2 = 0.88$, $p < 0.0001$

tion of a more precise species-specific food concentration threshold. Because of its lower clutch size and spawning frequency, *M. longa* produced eggs at a lower rate than *C. finmarchicus*, with 17 and 29 eggs female⁻¹ d⁻¹ at 6 and 10°C compared to 71 eggs female⁻¹ d⁻¹ in *C. finmarchicus* at 6°C (Fig. 7A). *M. longa* showed a maximal weight-specific EPR at 10°C (8% body C d⁻¹), similar to *C. finmarchicus* at 6°C (8.5% body C d⁻¹), but a much lower maximal EPR (4.5% body C d⁻¹) at 6°C (Fig. 7B). EPR (eggs female⁻¹ d⁻¹) in *M. longa* represented, respectively, 20 and 31 %

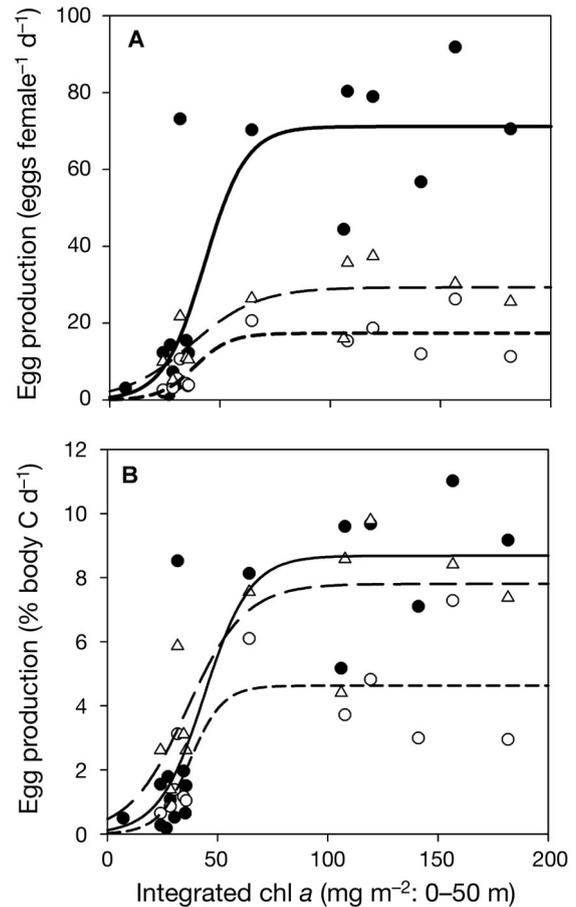


Fig. 7. *Calanus finmarchicus* and *Metridia longa* in 2005. (A) *In situ* EPR and (B) weight-specific *in situ* EPR in *C. finmarchicus* (●) at 6°C and in *M. longa* at 6°C (○) and 10°C (Δ) in relation to the integrated chl a standing stock. Data fitted to sigmoidal regression model (best fit). (A) *C. finmarchicus*: $y = 71.2/[1 - e^{-(x-43.0)/9.4}]$, $r^2 = 0.72$, $p < 0.0001$; *M. longa* 6°C: $y = 17.4/[1 - e^{-(x-38.4)/8.0}]$, $r^2 = 0.68$, $p < 0.01$; *M. longa* 10°C: $y = 29.3/[1 - e^{-(x-37.1)/15.1}]$, $r^2 = 0.64$, $p < 0.05$. (B) *C. finmarchicus*: $y = 8.7/[1 - e^{-(x-44.0)/10.3}]$, $r^2 = 0.73$, $p < 0.0001$; *M. longa* 6°C: $y = 4.6/[1 - e^{-(x-37.7)/7.3}]$, $r^2 = 0.61$, $p < 0.05$; *M. longa* 10°C: $y = 7.8/[1 - e^{-(x-36.2)/13.1}]$, $r^2 = 0.67$, $p < 0.05$

of that in *C. finmarchicus* at 6 and 10°C, while its weight-specific EPR corresponded to 45 and 71% of the weight-specific EPR in *C. finmarchicus* (slope of linear regression, Fig. 8).

Hatching success and naupliar viability

Egg hatching success and viability to N2 was consistently lower with egg separation in both species (Fig. 9). In *Calanus finmarchicus* (1998), hatching success was, respectively, 81 and 74% without and with egg separation (Mann-Whitney *U*-test, $p < 0.05$), while *Metridia longa* (2005) eggs showed a highly signifi-

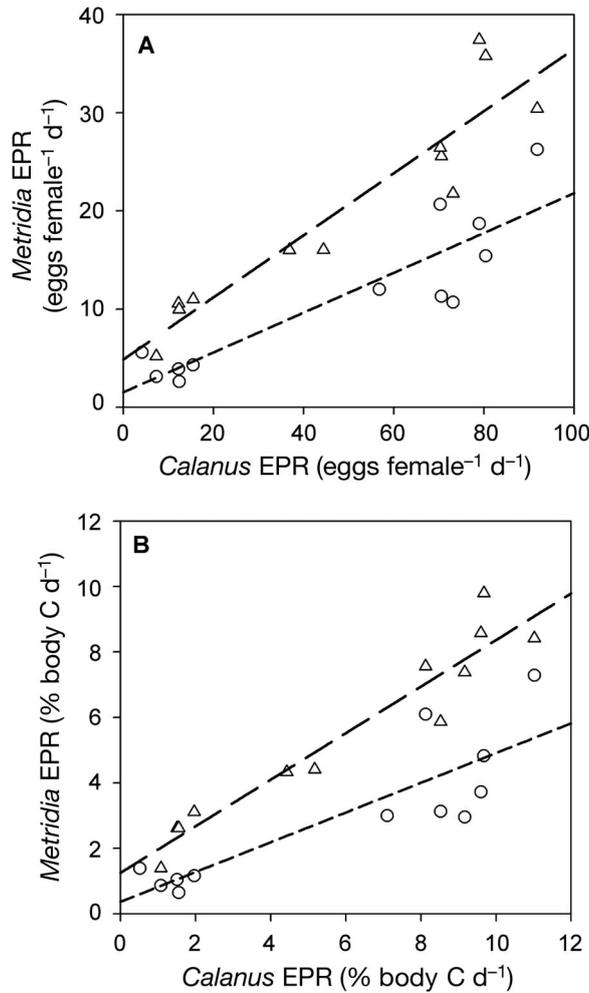


Fig. 8. *Calanus finmarchicus* and *Metridia longa* in 2005. Relationship between *in situ* EPR in *M. longa* at 6°C (O) and 10°C (Δ) and *in situ* EPR in *C. finmarchicus* at 6°C. (A) *In situ* EPR and (B) weight-specific *in situ* EPR. Data fitted to a linear regression model. (A) *M. longa* 6°C: $y = 0.20x + 1.53$, $r^2 = 0.79$, $p < 0.0001$; *M. longa* 10°C: $y = 0.31x + 4.86$, $r^2 = 0.87$, $p < 0.0001$. (B) *M. longa* 6°C: $y = 0.45x + 0.36$, $r^2 = 0.73$, $p < 0.001$; *M. longa* 10°C: $y = 0.71x + 1.25$, $r^2 = 0.92$, $p < 0.0001$

cantly lower hatching success (29%) with egg separation than without (51%; Mann-Whitney *U*-test, $p < 0.0001$). The pattern in egg viability to N2 mirrored these differences but with lower values (Fig. 9).

DISCUSSION

This study confirmed the importance of egg cannibalism during the measurement of *in situ* EPR in species of the genus *Metridia* (Halsband-Lenk 2005, Hopcroft et al. 2005). However, the use of a unique type of container to test the effect of egg separators on

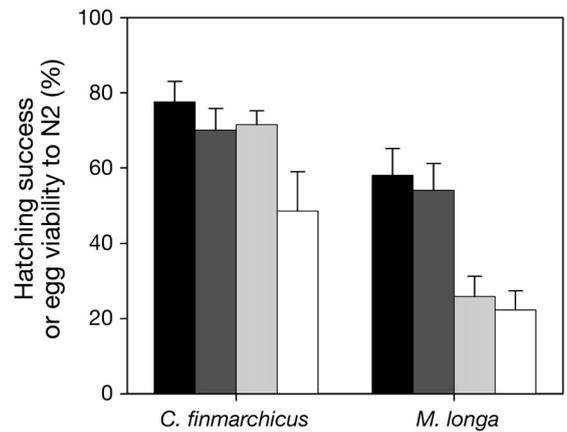


Fig. 9. *Calanus finmarchicus* and *Metridia longa*. Hatching success and egg viability to N2 in *C. finmarchicus* in 1998 and *M. longa* in 2005. Black (hatching) and dark gray bars (viability): without egg separation. Light gray (hatching) and white bars (viability): with egg separation. Error bars: 95% confidence intervals

the measurement of EPR concurrently in *Calanus finmarchicus* and *M. longa* allowed us to control for other potential effects and directly compare reproductive rates in both species.

EPR measurements: method

In their studies, Hopcroft et al. (2005) and Halsband-Lenk (2005) compared small 15 ml multi-well plates (no egg separation) to larger 50 ml containers used with egg separators for individual incubations of *Metridia pacifica* and *M. okhotensis*, whereas the same 45 ml dishes were used in both treatments in our study on *M. longa*. Both clutch size and EPR without egg separation in *M. longa* in 45 ml dishes were greater than in *M. pacifica* and *M. okhotensis* using 15 ml multi-wells or bottles (see Table 2 in Hopcroft et al. 2005, Fig. 4 in Halsband-Lenk 2005). In fact, the mean clutch size in *M. longa* without egg separation under non-limiting food concentration represented 62% of that with egg separation, a proportion much greater than reported for *M. pacifica*. *M. longa* showed an egg removal rate of 3% of the initial clutch size h^{-1} (Fig. 4), representing roughly one-third of the removal rate (11%) estimated for *M. pacifica* in Dabob Bay (Fig. 4) (Halsband-Lenk 2005). Although we cannot rule out species-specific differences in behavior, this difference in egg removal rate corresponds to the difference in volume between the 45 ml dishes and 15 ml multi-wells. The use of a large volume (45 ml) apparently reduced the effect of cannibalism in *M. longa*, indicating that a smaller volume (15 ml) is inadequate for relatively large-bodied copepods such as *M. pacifica* and *M. okhotensis* (Runge

& Roff 2000). This positive effect of larger incubation volume on clutch size and *in situ* EPR was further supported by the similar spawning frequency obtained with and without egg separation in *M. longa*, indicating that C6f were unable to remove/destroy all eggs during the 24 h incubation. Therefore, container volume undoubtedly represents an additional factor explaining the large difference observed in clutch size and EPR between treatments with and without egg separation in Halsband-Lenk (2005) and Hopcroft et al. (2005).

The highly significant relationship between the cannibalism index in *Metridia longa* and ambient algal biomass suggests an important influence of the feeding history during the few days prior to their capture on this behavior. Greater cannibalism at low phytoplankton biomass could have been induced by the low food availability during the few days prior to capture, a 'hunger' effect commonly observed in calanoid copepods (e.g. Runge 1980). Although *M. longa* C6f are considered to be more omnivorous/carnivorous than herbivorous, and their ability to feed on eggs of other species of copepods has been shown (Haq 1967, Conover & Huntley 1991, Campbell et al. in press), this significant relationship (in addition to that between EPR and chl *a*, see below) would suggest that chl *a* biomass could adequately be used as a food proxy for the omnivorous *M. longa* in the LSLE.

Although the ability of *Calanus* species to eat their own eggs and early naupliar stages during feeding experiments in bottles has been demonstrated (Landry 1981, Bonnet et al. 2004, Basedow & Tande 2007), this cannibalistic behavior in *C. finmarchicus* was likely hindered in the petri dishes. *C. finmarchicus* C6f mostly swam on their backs when on the bottom of dishes, meaning that they were rarely observed with their mouthparts in contact with the dishes; in contrast, *Metridia longa* were observed predominantly with their mouthparts in contact with the bottom of the dishes, sweeping the surface while swimming (S. Plourde & P. Joly pers. obs.). Additionally, *M. longa* C6f exhibit different swimming behavior and in general, much greater swimming activity than *C. finmarchicus* (Hirche 1987), suggesting a greater potential for encounters with eggs. These differences in behavior would explain the striking difference in cannibalism levels during incubation in 45 ml dishes among these species and reinforces the importance of using egg separation in the measurements of *Metridia* EPR (Halsband-Lenk 2005, Hopcroft et al. 2005).

Egg hatching success and viability to N2 were consistently lower with egg separation in both species, a result also obtained for other *Metridia* species (Halsband-Lenk 2005, Hopcroft et al. 2005). We suggest that freshly laid eggs could be damaged by sinking through the egg separator mesh, causing abnormal

development and death. Freshly laid copepod eggs are fragile, as their membranes are not yet formed (Marshall & Orr 1972). The greater negative effect of egg separation on hatching success and egg viability to N2 in *M. longa* could also be explained by the greater proportion of abnormal eggs in *Metridia* relative to *Calanus*, at least during some period of the year (Halsband-Lenk 2005, Hopcroft et al. 2005, Pierson et al. 2005). We believe this is a more likely explanation than C6f only destroying unhealthy eggs while swimming (Hopcroft et al. 2005), although the likelihood of this occurring would be greater in the small 15 ml multi-wells than in the larger 45 ml dishes. An additional cause of the species-specific difference in hatching success and egg viability to N2 could be that *M. longa* eggs are in general more fragile than *Calanus finmarchicus* eggs and are more affected by their contact with the bottom of the dishes (Halsband-Lenk 2005).

Comparison of *in situ* EPR in *Calanus finmarchicus* and *Metridia longa*

Without the effect of cannibalism, *Metridia longa* produced eggs at 20% the rate of *Calanus finmarchicus* under the same conditions, similarly to other studies in which *in situ* EPR in *M. pacifica* and *C. pacificus* was measured concurrently (Halsband-Lenk 2005, Pierson et al. 2005). This conclusion holds even when the greater egg/female weight ratio in *M. longa* was considered, resulting in the maximal weight-specific EPR representing 45% of that in *C. finmarchicus*. This lower EPR mainly resulted from the lower spawning frequency of *M. longa* relative to *C. finmarchicus*. A low spawning frequency could reflect the continuous recruitment of immature C6f in a population characterized by the production of successive generations such as in the case of *M. pacifica* (Batchelder 1985). However, *M. longa* generally shows a typical 1 yr life cycle with a clear cohort production and development during which nearly 100% of the C6f show mature gonads during the main reproductive period (Tande & Groen-vik 1983). In this situation, the spawning frequency reflects the spawning interval, i.e. the time taken for a C6f to lay consecutive clutches. Therefore, the maximal spawning frequency of 0.30 (determined statistically from regression, Fig. 6A) observed at 6°C would correspond to a spawning interval of ca. 3 d, reflecting the growth rate in *M. longa* C6f. In comparison, 71% of *C. finmarchicus* laid eggs at 6°C under non-limiting food concentrations in 2005, while this species is capable of laying 1 clutch d⁻¹ (100% spawning frequency) at 6°C (Hirche et al. 1997).

Our results contradict the conclusion that *Metridia pacifica* and *M. okhotensis* showed *in situ* EPR similar

to other calanoid species (although these data were not shown, see Hopcroft et al. 2005), but are similar to *in situ* EPR measured concurrently in *M. pacifica* and *Calanus pacificus* in Dabob Bay (Halsband-Lenk 2005, Pierson et al. 2005). In addition to the type of containers used, some methodological differences between Hopcroft et al. (2005) and our study could explain this discrepancy. Firstly, *in situ* EPR in species showing important diel vertical migrations such as *Metridia* spp. (Hays 1995, Hays et al. 1995, Falkenhaus et al. 1997) could have been overestimated by incubation at surface temperatures. Switching temperature during vertical migrations could induce physiological costs in zooplankton, as individuals experiencing switching temperatures exhibited growth rates below the average of growth at both temperatures (Reichwaldt et al. 2005). Although our approach did not reveal such an effect, our incubations conducted at a temperature roughly equivalent to that averaged between the daytime (1 to 4°C) and nighttime (7 to 10°C) habitats in summer likely better reflected the conditions experienced by C6f over a 24 h cycle. *M. longa* showed an important increase (+70%) in EPR at 10°C relative to 6°C, a response to temperature somewhat similar to *Calanus finmarchicus* (Runge & Plourde 1996), resulting in a similar maximal weight-specific EPR in *M. longa* at 10°C and *C. finmarchicus* at 6°C (Fig. 7B). These results would suggest that incubations performed at surface temperatures likely overestimated the *in situ* EPR in *M. pacifica* and *M. okhotensis*, especially during the summer–autumn period in the Gulf of Alaska (temperature of 10 to 12°C; Coyle & Pinchuk 2005, Hopcroft et al. 2005). Secondly, Hopcroft et al. (2005) collected *Metridia* C6f in the upper 50 m, which would not have encompassed the entire copepod population (Batchelder 1985, Coyle & Pinchuk 2005). Some evidence indicates that mature C6f are located closer to the surface at night to lay eggs compared to C6f that are not fully ripe, as observed in *C. pacificus* (Dabob Bay, J. Runge pers. comm.) and in *C. finmarchicus* on Georges Bank (J. Runge & S. Plourde unpubl. data). Therefore, collecting only surface-dwelling C6f could have resulted in an overestimation of the spawning frequency and consequently, the *in situ* EPR of the population.

In situ EPR in *Metridia longa* responded to food similarly to *Calanus finmarchicus*, with a very good fit with chl *a* biomass. Food concentration affected both clutch size and spawning frequency in *C. finmarchicus* and *M. longa*. Historically, body size and temperature have been considered the most influential causes of variation in clutch size and spawning frequency, respectively (Runge 1985, Runge & Plourde 1996, Hirche et al. 1997, Gislason 2005). The detrimental effect of food limitation on clutch size has been demon-

strated only in recent years in *C. finmarchicus* (Runge & Plourde 1996, Niehoff 2004). Additionally, the significant relationships between EPR and phytoplankton suggest that phytoplankton biomass would represent a good proxy for heterotroph microplankton and metazoan prey (Runge et al. 2006). The high degree of correlation between the EPR in the 2 species suggested that the reproductive period of *M. longa* in the LSLE in 2005 closely followed that of *C. finmarchicus*.

Paradox of low EPR and high abundance in *Metridia longa*

Fitness in zooplankton is achieved through a variety of adaptations and strategies (Aksnes & Giske 1990). In copepods, reproductive output (EPR) is one of the key elements, but other adaptations/behaviors such as reproductive strategy, diel and ontogenetic vertical migration, swimming and feeding behaviors, and overwintering strategies would contribute to the overall fitness of a species. This is best illustrated by the similar fitness (abundance) achieved by species showing contrasting reproductive rates, such as the egg-carrying *Pseudocalanus* spp. (low daily EPR) and the broadcast spawner *Calanus* spp. (high daily EPR). By carrying their eggs, *Pseudocalanus* greatly reduce egg mortality relative to *Calanus*, which would compensate for their lower reproductive rates (Ohman et al. 2002, Eiane & Ohman 2004). Additionally, *Pseudocalanus* spp. nauplii appear less vulnerable to predation than *Calanus* spp. nauplii because of their different swimming behavior (Sell et al. 2001).

We would argue that entire life cycle adaptations and strategies must be considered in explaining the paradox between the 'low' *in situ* EPR in *Metridia* species and their high abundance (good fitness). When fitness and recruitment (achieved abundance) are addressed, the number of eggs produced must be considered, not the weight-specific EPR (indices of secondary production). Specific EPR at 6°C in *M. longa* represented only 20% of that in *Calanus finmarchicus*, implying that other characteristics of its life history would allow *M. longa* to maintain high abundance in the St. Lawrence system. *M. longa* generally shows greater amplitude in diel vertical migrations, a markedly different swimming behavior, and greater activity levels and respiration rates than *C. finmarchicus* (Conover & Corner 1968, Hirche 1987, Conover & Huntley 1991, Falkenhaus et al. 1997). Additionally, *Metridia* species do not overwinter, but rather stay active during the winter period, a strategy radically different from the 'diapause state' observed in *C. finmarchicus* and *C. pacificus* (Hopkins et al. 1984, Conover & Huntley 1991, Ohman et al. 1998). These

characteristics suggest that the 'basic' metabolism in *M. longa* is in fact greater than in *C. finmarchicus*. Since weight-specific ingestion rates in *M. longa* are roughly similar to those in *Calanus* spp. (Campbell et al. in press and references therein), its greater basic metabolism would result in a lower 'growth efficiency' than in *C. finmarchicus*. Although the mortality pattern in *Metridia* species is unknown, these differences in behavior and strategies could induce an overall lower mortality during the whole development than in *Calanus*. Therefore, the life-cycle strategy in *M. longa* would have evolved toward investing less in reproduction and expending more energy in order to minimize mortality, allowing this species to achieve 'fitness' similar to *C. finmarchicus*.

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