
The mystery of *Microsetella*: combination of sac- and broadcast-spawning in an Arctic fjord

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Abstract:

Different life-history stages of the pelagic harpacticoid *Microsetella norvegica* were sampled in a Greenland fjord, to investigate how this slowly growing species can achieve high abundances at low temperatures. We expected low but continuous reproduction coupled with a low mortality, but observed the opposite: a short reproductive period with high estimated weight-specific egg production and egg mortality, and indication of a life-history strategy combining the advantages of egg carrying with egg production rates independent of temperature.

Keywords: Arctic ; *microsetella norvegica* ; mortality ; reproduction ; sac-spawning

Broadcast and sac-spawning copepods have typically different maximum fecundity and egg mortality rates as well as different egg hatching times (e.g., Kiørboe and Sabatini, 1995; Bunker and Hirst, 2004). It has been suggested that the high egg production and short hatching time of eggs in broadcast spawners would be an adaptation to high mortality of freely spawned eggs, while sac-spawners could afford a low feeding and fecundity due to the low mortality of carried eggs (Kiørboe and Sabatini, 1994). Further, sac-spawner fecundity is more tightly coupled to temperature than broadcast spawner fecundity (Bunker and Hirst, 2004), due to the temperature-dependence of egg hatching (see Ward and Hirst, 2007). It has been suggested that the effect of temperature on egg hatching could even control the distribution of sac-spawning copepods, as reproduction in low temperatures could be too slow to compensate for the mortality losses (Ward and Hirst, 2007).

All comparisons between the two life-history strategies of copepods include an unquestioned assumption that sac-spawning copepods carry their eggs until hatching, and that spawning of a new egg-sac is limited by hatching of the previous one. From this follows two assumptions, namely that the maximum egg production rate of sac-spawning copepods is controlled by egg hatching time, thus temperature, and that the egg mortality equals female mortality (with the exception of eggs passing unharmed through the gut of the predator; e.g., Flinkman *et al.*, 1994). However, a few observations suggest that egg-sacs can be released before hatching and that a new egg-sac can be produced before the previous one has hatched (Devreker *et al.*, 2012) – a combination which could contribute to a higher reproductive rate than typically expected for sac-spawning copepods.

Life-history strategies of pelagic harpacticoids are likely to resemble those of other sac-spawning copepods, as the disadvantages and advantages of broadcast vs. sac-spawning should not be connected to the copepod species / groups (Kiørboe and Sabatini, 1994). The few studies on pelagic harpacticoids seem to confirm this. Uye *et al.* (Uye *et al.*, 2002) observed relatively low reproduction and growth rates of the pelagic harpacticoid *Microsetella norvegica*, and suggested that their high abundances in **Sea of Japan** would be due to low predation mortality. Similarly, reproduction rates of benthic harpacticoids appear low, and their embryonic development times are strongly connected to temperature and long (Lee *et al.*, 1985; Santos *et al.*, 1999).

Although most species of pelagic harpacticoids are not very widely distributed in the open ocean, *Microsetella norvegica* is an exception. This species can obtain high abundances from subtropical to arctic areas (Uye *et al.*, 2002; Arendt *et al.*, 2013), in temperatures ranging from a few to >20 °C. We wanted to study the discrepancy between a high abundance and low reproduction of *Microsetella norvegica*, and its apparent disconnection to temperature. As a study location, we chose a fjord in west Greenland, where this species can be extremely abundant,

although the annual peak surface temperatures rarely exceed 6 °C (e.g., Smidt, 1979; Arendt *et al.*, 2013).

We sampled every 7 to 14 days from March to August 2010 at four stations, along a transect spanning the length of the 25 km long fjord branch Kapisigdlit (Table I). All stations had a similar seasonal succession of hydrography, with well-mixed, cold (≤ 2 °C) and saline (≥ 33) water layer until late April, when stratification was established at ca. 10 m depth. The environmental conditions during the reproductive season at 0-50 m encompassed temperature of 4.4 ± 1.1 °C, salinity of 31.6 ± 1.4 and chl-*a* of 0.90 ± 0.95 $\mu\text{g L}^{-1}$ (mean \pm SD in May-August at all sampling stations). On every cruise, vertical distribution of salinity and temperature were recorded using a Seabird CTD, and at station 4, water samples were taken for chl-*a* analysis (Riisgard *et al.*, submitted). Zooplankton were sampled throughout the study using a Hydrobios Multinet (type Mini, opening 0.25 m²) equipped with 50 μm mesh nets, or using a WP-2 net with a 50 μm mesh size equipped with a non-filtering cod-end. Although zooplankton were mostly sampled in discrete water layers (Table I), in the present study we only use depth-integrated abundances. All nets were hauled with a speed of 0.2-0.3 m s⁻¹. Samples were immediately preserved in buffered formalin (4% final concentration).

Approximately 400 individuals were counted in each sample, which resulted in 9-116 counted individuals of different development stages (including females with and without egg-sacs) of *Microsetella norvegica*. Depth-integrated abundances reported here are typically based on > 60 counted individuals (Table II). The number of eggs per egg-sac was counted from 10 egg-sacs per sample, and the egg size was measured on one occasion. Total length was measured on 10 individuals of each naupliar and copepodite stage (precision of 6 μm). All samples were analyzed in the Plankton sorting and identification center in Szczecin (www.nmfri.gdynia.pl), using the identification key of Hirakawa (Hirakawa, 1974).

The egg production was calculated using the egg-ratio method, by multiplying the average number of eggs per clutch with the depth-integrated number of egg-sacs, divided by the depth-integrated number of females and the temperature-specific development time of eggs. As no measurements exist on the embryonic development time of arctic or sub-arctic *Microsetella*, we estimated the development time using functions from McLaren *et al.* (McLaren *et al.*, 1969) and Nielsen *et al.* (Nielsen *et al.*, 2002), averaging the obtained development times for each sampling date. Weight-specific egg production was calculated by using the egg carbon content of 0.018 ± 0.002 $\mu\text{g C egg}^{-1}$, based on the egg diameter of 46 ± 6 μm and the carbon content of *Microsetella norvegica* eggs as in Uye *et al.* (Uye *et al.*, 2002), corrected for size. Carbon weight of females was calculated according to the length: carbon regression of Uye *et al.* (Uye *et al.*, 2002), as other available length: carbon regressions (Satapoomin 1999; Arendt *et al.*, 2012) did not include eggs or nauplii stages.

The mortality of eggs and females was calculated using the vertical life table approach as presented in Hirst and Ward (Hirst and Ward, 2008). To get the mortality of females we assumed that 5th copepodites stage had a sex ratio of 1:1. To estimate the development times of NI and CV we used the average of the temperature-dependent development times from McLaren et al. (McLaren *et al.*, 1989) and Lee et al. (Lee *et al.*, 2003). The average post-embryonic development times of NI and CV were estimated based on the proportional length of each stage according to Uye et al. (Uye *et al.*, 2002).

The maximum egg production varied from ca. 1 egg f⁻¹ d⁻¹ at station 2 to > 5 eggs f⁻¹ d⁻¹ at stations 4 and 5, corresponding to weight-specific egg production rates from 13 to 46% body weight d⁻¹, respectively (Fig. 1). The high egg production was due to a high number of egg-sacs rather than an exceptionally large clutch size. We observed several occasions when the number of egg-sacs in the samples exceeded that of females. During the reproductive season (end April-mid July; Fig. 1), in 32% of the sampling times egg-sac to female ratios of > 1 were observed, with ratios up to 4.5 (Fig. 1, Table II). In field studies from temperate or northern locations, the spawning percentage of egg-carrying copepods rarely exceeds 30-40% (e.g., Böttger-Schnack and Schnack, 2005), for instance *Microsetella norvegica* in **Sea of Japan** had a spawning percent of ca. 40% during the peak reproduction (Uye *et al.*, 2002). Assuming that inter-clutch duration is 25% of the embryonic development time (Corkett and McLaren, 1978), and female mortality is low (Fig. 1) resulting in a relatively large proportion of post-spawning females, a realistic maximum spawning percentage of *M. norvegica* at our sampling location could resemble 39%, as shown by Ohman et al. (Ohman *et al.*, 1996). Instead we regularly observed spawning percentage close to or above 100% (Fig. 1, Table II).

First, we wanted to examine any possible sources of error in sampling or data analysis which could have led us to observe too high numbers of egg sacs compared to females. These could include 1) an overestimation of the number of egg-sacs, 2) an underestimation of the number of females, 3) a differential sampling efficiency of egg-sacs and females or 4) different vertical or horizontal transport of egg-sacs and females. Neither of the first two alternatives can explain the discrepancy as 1) all eggs (both free and carried egg-sacs) were frequently needed to explain the number of first naupliar stages, implying at least some hatching from the discarded egg-sacs, and 2) even the sum of copepodites, males and females could not always balance the female: egg-sac ratios (Table II). Differential capture efficiency of stages is also highly unlikely as the 50 µm net captures early naupliar stages which have smaller dimensions than adults, and as the swimming velocity of *M. norvegica* at < 1 mm s⁻¹ (Koski *et al.*, 2005) would not provide an efficient escape from the net. Also, the vertical distribution of females with and without egg-sacs and free egg-sacs were typically similar (Koski *et al.*, unpublished data), making different transport of eggs and females unlikely.

The egg mortality was typically high, on average $0.48 \pm 0.32 \text{ d}^{-1}$ at all stations and dates and > 50 times higher than female mortality ($0.007 \pm 0.01 \text{ d}^{-1}$; Fig. 1). The different mortality of eggs and females further argues that not all eggs were carried. The mortality of eggs at 0.5 d^{-1} approaches the lower end of the estimated mortality rates of freely spawned eggs which vary from ca. 0.4 up to $> 6 \text{ d}^{-1}$ (Petersen and Kimmerer, 1994; Ohman and Hirche, 2001), and the large difference in egg and female mortality is similar to that observed for broadcast spawning copepods. In contrast, carried eggs should have similar mortality than females, around 0.05 d^{-1} (Ohman and Wood, 1996). It thus appears that 1) the ratio of egg-sacs to females in our study area was substantially higher than what could be expected for a sac-spawning copepod, 2) we could not identify a methodological error which could have led to biased egg-sac: female ratios and 3) differential mortality of females and eggs suggested that not all egg-sacs were attached to females.

We suggest that the number of egg-sacs per female exceeding one can be explained by shedding of the egg-sacs before they hatch. Although it is generally assumed that sac-spawning copepods carry their egg-sacs until hatching, there are a few observations of the opposite. Recently Devreker et al. (Devreker *et al.*, 2012) observed that the calanoid copepod *Eurytemora affinis* released their egg-sacs before hatching, and sometimes also produced a new egg-sac before the first one had hatched. Similarly, copepod species adapted to life on surfaces, such as many harpacticoids, do not necessary follow the broadcast-spawning vs. sac-spawning strategies, but can attach their unhatched eggs on diverse types of surfaces (Ingólfsson and Ólafsson, 1997). Shedding the egg-sacs before they hatch is thus not unheard of as a strategy in copepods, and *Microsetella norvegica*, with its dependency on aggregates (Ohtsuka *et al.*, 1993; Koski *et al.*, 2005), could well be expected to use such alternative strategies.

Shedding eggs prior to hatching might enable *Microsetella norvegica* to overcome the limitation imposed to reproduction by low temperature. This would mean that *M. norvegica* can at times produce as many as ~ 4.5 egg masses at the time it takes for one to hatch, which may help to achieve the high abundances seen in arctic areas. We suggest that rather than a physiological adaptation of metabolic rates, the high abundances could be achieved by a behavioral adaptation of reproduction, resulting in shedding of unhatched egg-sacs at conditions which otherwise (for instance, due to food concentration) are favorable for egg production. A flexible reproductive behavior could be particularly advantageous for a cosmopolitan species like *M. norvegica*, facilitating the adaptations to local temperature and predation regimes, and could explain part of this species global success.

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Legends

Fig. 1. Seasonal variation in clutch size (eggs egg-sac⁻¹; mean \pm SD), egg-sac to female ratio, **estimated** weight-specific egg production ($\mu\text{g C } (\mu\text{g C})^{-1} \text{ d}^{-1}$) and **estimated** specific mortality of eggs (solid circles) and females (open circles; d⁻¹) at a) station 2, b) station 4, c) station 5 and d) station 6. The solid line indicates egg-sac to female ratio of 1, corresponding to 100% of the population spawning. The clutch size was significantly higher in May and early June at stations 5 and 6 than in other times and stations (2-way ANOVA, $F_{30,1115} = 5.1$; $p < 0.001$; Tukey HSD; $p < 0.05$). The mortality of females was significantly lower than egg mortality (Mann Whitney; $t_3 = 522$; $p < 0.001$). Note different scales of egg and female mortalities.

Table I. Sampling stations, their coordinates and maximum depths (m), sampling depths and the gear used. Station 2 was located close to the mouth of the fjord branch, station 4 in the middle of the fjord, station 5 on the slope leading up to a shallow inner creek at the end of the fjord, and station 6 at the end of the fjord. All stations were sampled 13-15 times between March 24th and August 5th; every third cruise on station 4 included sampling at dawn, dusk, day and night (at 6:00, 12:00, 18:00 and 00:00).

Table II. Depth-integrated abundances of NI and attached and total eggs (10^6 m^{-2} ; assuming an average clutch size according to the station and date, and the depth down to the last sampling depth), the ratios of egg-sacs to females, egg-sacs to all copepodites and adults (CI-VI) and eggs to NI, as well as the number of females and egg-sacs counted for each data point. To compare egg and NI concentrations, egg abundance was divided by 2.8 ± 0.1 , which was the average ratio of egg to NI development time over all stations and sampling times. Only stations and sampling dates where the number of egg-sacs exceeded that of females are included; these consisted of 12 out of 37 sampling times during the spawning season. Spawning season was defined to stretch from 22.4. to 18.7 when $\geq 10\%$ of the females had egg-sacs (see Fig. 1).

Table I. Sampling stations, their coordinates and maximum depths, sampling depths and the gear used.

Station	Position	Max. depth	Sampling depth	Gear
2	64° 26 N, 50° 39 W	194	0-100; ^a 0-50, 50-100, 100-150	CTD, Multinet, WP2
4	64° 25 N, 50° 22 W	251	^b 0-50, 50-100, 100-150, 150-200, 200-235	CTD, Multinet
5	64° 25 N, 50° 18 W	125	0-75; ^c 0-50, 50-100	CTD, Multinet, WP2
6	64° 26 N, 50° 15 W	85	0-50; ^d 0-10, 10-20, 20-30, 30-40, 40-50	CTD, WP2, Multinet

^a Sampling in 3 depth strata on 24.3., 22.4., 18.5., 17.6. and 6.7.

^b Sampling on 18.6. at 25-m intervals (10 depth strata)

^c Sampling in 2 depth strata on 22.4., 18.5., 17.6. and 6.7.

^d Sampling in 5 depth strata on 10.5., 24.5. and 3.6.

Table II. Depth-integrated abundances of NI and attached and total eggs, the ratios of egg-sacs to females, egg-sacs to all copepodites and adults and eggs to NI, as well as the number of females and egg-sacs counted for each data point.

Station and time	Abundance (# x 10 ⁶ m ⁻²)			Ratio				Count
	NI	Attached eggs	Total eggs	Egg-sac: F	Egg-sac: CI-VI	Attached eggs: NI	Total eggs: NI	F / egg-sac
Station 2								
18.5.	0.02	0.74	4.10	1.2	0.6	14.1	78	282 / 136
29.6.	0.08	0.10	0.30	1.1	0.2	0.4	1.3	11 / 8
Station 4								
10.5.	0.02	2.87	9.1	1.3	0.4	53	168	322 / 132
18.5.	0.15	0.67	3.9	1.2	0.6	1.5	9.0	358 / 187
24.5.	1.06	1.96	40.4	4.5	2.4	0.7	13.5	438 / 167
18.6.	0.18	0.32	19.2	3.5	2.3	0.6	39	127 / 71
7.7	0.09	0.34	3.8	1.2	0.1	1.3	14.1	708 / 134
Station 5								
18.5.	0.92	2.01	3.1	1.5	1.0	0.8	3.6	159 / 104
24.5.	1.10	1.66	3.9	1.1	0.8	0.5	3.0	75 / 64
29.6.	0.04	0.23	1.12	4.1	1.1	2.1	85	20 / 10
Station 6								
24.5.	2.67	11.6	70.3	1.4	1.2	1.5	9.4	239 / 220
7.7.	0	0.09	3.4	1.9	1.2	-	-	103 / 12

