NOTE

Hatching rate of the egg-carrying estuarine copepod

*Eurytemora affinis*

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ABSTRACT. The egg-carrying copepod *Eurytemora affinis* is often a characteristic and dominant component in the carbon cycles of freshwater influenced marine ecosystems in the Northern Hemisphere. In egg-carrying copepods the specific egg production rate in the field can be calculated from the egg hatching rate at in situ temperatures, the egg/female ratio and the carbon content of the eggs and females. To determine the temperature dependent egg hatching time (HT) of 2 populations of *E. affinis*, hatching experiments were conducted at 6 different temperatures (T). Hatching times ranged from 14.1 to 1.6 d at 5 and 22°C, respectively. Results from the 2 populations were pooled and fitted to a power function: HT = 187T^{-1.54} (r^2 = 0.97; p < 0.001). There is a large range in the hatching times versus temperature reported in the literature, and our results reveal longer hatching times than previously reported. To overcome this pronounced variation in hatching times, we present a significant relationship (r^2 = 0.87; p < 0.001) based on all available data: HT = 36.87T^{-1.04}.

KEY WORDS: *Eurytemora affinis* · Egg production · Hatching rate

Copepods are considered to be some of the most important grazers in pelagic ecosystems. A key species in freshwater influenced marine ecosystems is the egg-carrying calanoid copepod *Eurytemora affinis*. It is widely distributed in the Northern Hemisphere and often dominates copepod biomass in estuaries (Jeffries 1962, Heinle & Flemer 1975), salt marshes, lagoons (Castonguay & Fitzgerald 1990) and brackish lakes (Jeppesen et al. 1994, Irvine et al. 1995). *Eurytemora affinis* is an omnivorous suspension feeder, capable of exploiting a large particle spectra. The diet may consist of algae, protozoa, bacteria, and detritus within the size range of 1 to 60 μm (Heinle et al. 1977, Heerkloss 1979, Gyllenberg 1980, Barthel 1983, Boak & Goulder 1983). Since small-sized particles and detritus are often found in high concentrations in estuaries, *E. affinis* potentially plays an important role in trophic dynamics, making these carbon sources available for higher trophic levels.

The consumption rate and production of copepod populations can be estimated from measurements of the weight-specific egg production rate and the standing stock (Kiorboe et al. 1985a, b). This method assumes equal weight-specific growth rates in all stages and a close correlation between ingestion and egg production (Kiorboe et al. 1985a, b, Berggreen et al. 1988, Fryd et al. 1991). Egg production rates of free-spawning copepod species are determined by incubation of adult females under in situ conditions as described by Kiorboe et al. (1985a, b). However, this method cannot be implemented directly to egg-carrying copepods, such as *Eurytemora*, because the dense egg sacs makes it impossible to count eggs on live females. Instead the population-specific egg production rate (SEP, d⁻¹) in the field can be calculated from the egg hatching rate (HR, d⁻¹) at in situ temperature, the ratio of eggs to females (eggs/Q) and the carbon content of the egg (eggC) and females (C/Q) respectively:

\[
SEP = \frac{\text{eggs/Q}}{\text{HR(eggC/QC)}}
\]

Egg hatching rate can be calculated if the egg hatching time at the in situ temperature is known. Here we define hatching time as the time from extrusion of eggs into the egg sac until eggs have hatched. A comprehensive study of the literature revealed several previous studies dealing with embryonic development in *E. affinis*, but only 4 papers report data that can be interpreted as hatching times according to this definition (see Table 1). There is a pronounced variation in the hatching times reported, but it is not clear if the differences are due only to natural variation among populations or if they are also attributable to the variation in terms and methods used.

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The aim of this study was to determine the temperature dependent egg hatching rate of *Eurytemora affinis* by using the same method in 2 different populations. We compare our findings with earlier studies of egg development of *E. affinis* and also provide an overview of the different methods and terms used. Finally we establish a general equation relating hatching time and temperature based on all available data from the literature. This equation provides a simple method of estimating the productivity in the field of this abundant copepod species.

**Materials and methods.**

**Study sites:** Sampling and experiments were carried out during 2 cruises in 1995. The Hylsfjord, situated on the southwestern coast of Norway (Fig. 1), was visited from 5 to 13 July with RV 'Håkon Mosby' (University of Bergen). The Hylsfjord is a deep fjord (max. depth 510 m) separated from the ocean by a 110 m deep sill. It is strongly influenced by freshwater discharge from a hydroelectric power plant. A more detailed description of the fjord is given in Kaartvedt & Svendsen (1995). Another cruise, to the Randers Fjord, Denmark, was carried out with RV 'Gunnar Thorson' (Danish National Environmental Protection Agency) from 5 to 9 September. Additional samplings in the Randers Fjord were carried out on 22 May 1996 from a small ship provided by Århus County. The Randers Fjord is a shallow estuary (max. depth 6 m) which connects the Gudena river with the Kattegat (Fig. 1). For a more detailed description of the estuary see Nielsen et al. (1993).

**Hatching experiments:** Copepods were collected from surface waters by horizontal net hauls using a WP-2 net (mesh size 200 μm) with a large non-filtering cod-end. The samples were diluted in surface water and kept in a large insulated plastic container (100 l). Shortly after, subsamples were placed in small petri dishes and brought to the laboratory. Here females carrying egg sacs were sorted out with a pipette, under a dissecting microscope, and transferred to 600 ml polycarbonate bottles (3 to 6 Q bottle−1) containing 45 μm screened surface water. Care was taken to maintain the animals at *in situ* temperature, because heating would have influenced the egg hatching time. Acclimation corresponding to experimental temperatures was not carried out prior to experiments.

The hatching experiments were conducted in temperature controlled incubators at 6 different temperatures: 5 and 22°C (Randers Fjord, September 1995), 12 and 18°C (Hylsfjord), and 8 and 15°C (Randers Fjord, May 1996). At the beginning of each experiment (t₀) and every 6, 12 or 24 h, dependent on incubation temperature, the contents of each of 3 replicate bottles from each experiment were concentrated on 45 μm sieves and rinsed into small petri dishes, where they were checked to see if females were alive and healthy. Animals were preserved by adding a few drops of Lugol's solution to each petri dish, and eggs and nauplii were counted under a dissecting microscope (x25).

To avoid the source of error due to the possible production of new eggs during experiments, the hatching percentage was calculated in relation to the initial number of eggs Q−1 by:

\[
\text{Hatching percentage} = \left( \frac{\text{no. of nauplii}}{\text{no. of } Q} \right) \cdot 100
\]

The initial number of eggs Q−1 was calculated as the mean number of eggs Q−1 at t₀ in all experiments (6 x 3 = 18 bottles). The hatching rate (HR) was calculated in each of the 6 experiment, from the slope of the linear regression of hatching percentage versus time. Hatching time (HT), defined as the time required to reach 100% of hatching, was also calculated from the linear regression.

There is some confusion in the literature concerning the systematics of the genus *Eurytemora*. According to Busch & Brenning (1992), the species *E. hirundoides* should be regarded only as a variety of the very variable species *E. affinis*. Consequently no distinction between the 2 species is made in the following, and only the name *E. affinis* is used.

**Results.** Both fjords were characterised by a thin plume of outgoing low salinity water separated by a steep pycnocline from more saline bottom water (see...
Table 1. Eurytemora. Previous studies on embryonic development time

<table>
<thead>
<tr>
<th>Expt</th>
<th>Temp. range (°C)</th>
<th>Equation</th>
<th>Data on hatching time</th>
<th>Term used</th>
<th>Study site</th>
<th>Source</th>
<th>Symbol used in Figs. 4 &amp; 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>7–18</td>
<td>$D_c = 34.6T^{-1.20}$</td>
<td>$r^2 = 0.69, n = 3$</td>
<td>‘Development time of egg sacs’</td>
<td>Northern Baltic Sea, Finland</td>
<td>Vuorinen (1982)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>10–25</td>
<td>$D_c = 150T^{-1.01}$</td>
<td>$r^2 = 0.98, n = 4$</td>
<td>‘Egg development time’</td>
<td>Gironde estuary, France</td>
<td>Poli &amp; Castel (1983)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>5.5–25</td>
<td>$D = 195T^{-1.65}$</td>
<td>$r^2 = 0.99, n = 5$</td>
<td>‘Development time of eggs’</td>
<td>Patuxent River estuary, USA</td>
<td>Heinle &amp; Flemer (1975)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>9–11.8</td>
<td>$V = 1640(T + 10.4)^{-2.05}$</td>
<td>$r^2 = 0.98, n = 3$</td>
<td>‘Development time to hatching of eggs’</td>
<td>Halifax, Nova Scotia, Canada</td>
<td>McLaren et al. (1969)</td>
<td></td>
</tr>
<tr>
<td>Lab</td>
<td>2–14</td>
<td>$D = 18.49T^{-0.73}$</td>
<td>$r^2 = 0.97, n = 4$</td>
<td>‘Eggs incubation time’</td>
<td>Westerschelde estuary, The Netherlands</td>
<td>Escaravage &amp; Soetaert (1993)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>2.5–25</td>
<td>$ln D = 2.722 - 0.2612(ln T)^{2}$</td>
<td>$r^2 = 1.00, n = 6$</td>
<td>‘Egg-duration time’</td>
<td>Tjeukemeer, The Netherlands</td>
<td>Vijverberg (1980)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>4–20</td>
<td>$D = 268.8(T + 3.4)^{-1.68}$</td>
<td>$r^2 = 0.98, n = 18$</td>
<td>‘Development time of eggs’</td>
<td>Lake Ohnuma, Japan</td>
<td>Ban &amp; Minoda (1991)</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>5–22</td>
<td>$HT = 107T^{-1.54}$</td>
<td>$r^2 = 0.97, n = 6$</td>
<td>‘Hatching time’</td>
<td>Present study</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>Mean all data points</td>
<td>0–25</td>
<td>$HT = 36.8T^{-1.84}$</td>
<td>$r^2 = 0.87, n = 37$</td>
<td>‘Hatching time’</td>
<td>All applicable data compiled</td>
<td>Present study</td>
<td></td>
</tr>
</tbody>
</table>

*Reported data points fitted to power function $D = bT^c$; †data from Vuorinen (1982), Poli & Castel (1983) and Heinle & Flemer (1975) excluded.

Discussion. The variation among hatching times presented in previous studies may be attributed to different methods used and to differences in the populations of Eurytemora affinis studied. The results presented here are in agreement with previous studies and suggest that hatching times are influenced by temperature. The temperature coefficient of hatching time is negative, indicating that hatching occurs more slowly at higher temperatures. The relationship between hatching time and temperature is best described by a power function. The data from the present study were fitted to a power function of the form $HT = bT^c$, where $HT$ is the hatching time in hours, $T$ is the temperature in °C, and $b$ and $c$ are constants. The values for $b$ and $c$ were estimated to be 1.86 and 0.87, respectively, with a high correlation coefficient ($r^2 = 0.97, n = 37, p < 0.001$).
regression coefficient. This suggests that variation among populations might not always be of crucial importance in the study of egg hatching times. Nevertheless, we suggest that animals used in experiments should preferably be collected in the field, because there is selection for fast growth and development in cultures (Fenchel 1982, Tiselius et al. 1995). Escaravage & Soetaert (1993) used laboratory-reared copepods, which might have biased their results towards shorter hatching times.

To minimize acclimation effects the temperature in experiments should be close to that of the collection habitat or culture. In the present study, the change in temperature from in situ to experimental temperature is comparable to what the copepods would experience if the pycnocline were crossed (see Table 2). Vuorinen (1987) found that reproducing *Eurytemora* females in the archipelago sea of southwest Finland exhibit a distinct diurnal migration pattern, and thereby experience changes in temperature of at least 8°C. In the study of Ban & Minoda (1993) some egg sacs were incubated at 15°C throughout the season, neglecting in situ temperature. Nevertheless, they found no seasonal variations in the hatching times. This result questions the importance of acclimation in egg hatching experiments.

Hatching time can be assessed using 2 general approaches: direct observations on females with eggs or by an indirect regression method. The results of McLaren et al. (1969) and Vijverberg (1980) are based on direct observations of individual ovigerous females, while Escaravage & Soetaert (1993) made their direct observations on paired (1♂ + 1♀) copepods. Escaravage & Soetaert (1993) define hatching as the moment when at least 50% of the eggs have hatched. Unless hatching is synchronized, this may explain why they

Table 2. Temperature in experiments and temperature, salinity, chlorophyll a (chl a), and biomass of *Eurytemora affinis* at the study sites (temperature and salinity values are given for above/below the pycnocline)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Temp. in experiments (°C)</th>
<th>Temp. study site (°C)</th>
<th>Salinity [%]</th>
<th>Max. chl a (μg l⁻¹)</th>
<th>E. affinis biomass (% of copepod biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hylsfjord, Norway</td>
<td>12 and 18</td>
<td>16.4/7.1</td>
<td>6.2/34.6</td>
<td>3.61</td>
<td>100</td>
</tr>
<tr>
<td>Randers Fjord, Denmark</td>
<td>5 and 22</td>
<td>16.3/15.6</td>
<td>1.2/8.2</td>
<td>36.2</td>
<td>20–70</td>
</tr>
<tr>
<td>Sep 1995</td>
<td>8 and 15</td>
<td>10.8/9.4</td>
<td>1.5/15.3</td>
<td>17.0</td>
<td>20–70</td>
</tr>
</tbody>
</table>
found shorter hatching times compared to this study where a 100% hatching criterion is used. In the indirect regression method the ratio or percentage of hatched eggs or egg sacs is plotted against incubation time and hatching rate is calculated as the slope of the linear regression. This method was used in the present study and by Ban & Minoda (1991), who incubated single egg sacs dissected from anesthetized females. Their results are supported by a large number of replicates, but the mortality of egg sacs was high, probably due to the small incubation volume (2 ml or 0.3 ml) and because the water was not renewed. This could cause oxygen deficiency and maybe also lead to bacterial or fungal infection of eggs. The advantage of this method, though, is that it eliminates the risk of cannibalism. This phenomenon is well known from incubation experiments with several free-spawning species (Kiorboe et al. 1985b), but since eggs of *Eurytemora affinis* are carried by the female, cannibalism can only be on the newly hatched nauplii. Even though we expect the newly hatched nauplii to be well above the upper size limit of adult *E. affinis* prey, we have no exact information about this and cannibalism cannot be neglected as a source of error in our study. Since we calculated the hatching time on the basis of the number of nauplii, the effect of cannibalism would be longer hatching times.

The observation frequency in hatching experiments is crucial, because new egg sacs might be produced during experiments. The appearance of new eggs is dependent on the duration of the period from hatching of one brood to the extrusion into sacs of the next brood. Vijverberg (1980) found that this was temperature dependent, ranging from 1.4 d at 5°C to 0.5 d at 25°C for *Eurytemora affinis*. This means that the time interval between successive observations should not be more than 12 h and preferably shorter, maybe 6 h. If intervals are longer, it becomes difficult to identify new egg sacs. In the present study the production of new eggs will not affect the calculated percentage of hatched eggs, since this is based on the initial number of eggs. In copepods that carry paired egg sacs, newly produced egg sacs can be identified if 1 of the 2 egg sacs is amputated prior to incubation.

It is difficult to make any conclusions about the relative importance of natural intraspecific variation to egg hatching times in *Eurytemora affinis* compared to the variation caused by the difference in methods used in previous studies. Therefore we have established the new general equation based on all available data. This equation can be used as a tool when site-specific egg hatching times of *E. affinis* are not available.

The egg production rate of copepod populations can be measured directly by incubation of fertilised females (Kiorboe et al. 1985a, b) or indirectly by combining data of the egg to female ratio of the population with egg hatching rates at *in situ* temperature (Edmondson 1965). The incubation method is well implemented in free-spawning species, while the use of this method in egg carriers is restricted to the species where the initial number of eggs in each egg sac is readily counted on females prior to incubation (e.g. *Oithona plumpfera*; Paffenhofer 1993). In species such as *Eurytemora affinis* and *Oncaea mediterranea* (Paffenhofer 1993) where egg sacs are very dense or the number of eggs is very high, it is impossible to count the eggs on live females, and the method cannot be implemented directly. Hansen & Christoffersen (1995) measured the egg production in the egg-carrying freshwater species.
Cyclops strenuus and Eudiaptomus graciloides both with dense egg sacs. Prior to experiments several egg sacs were mounted on a microscope slide and the number of eggs counted. After incubation, the same procedure was followed and the egg production was calculated. This method does not take into account the possible hatching of eggs during experiments. If hatching of eggs take place, the egg production will be underestimated. To avoid this problem the nauplii produced should also be counted. An alternative is to incubate non-ovigerous females (or remove egg sacs before incubation) and continuously remove the egg sacs produced after having narcotised females with carbonated water. This was done by Hansen & Santer (1995), but the method induced high female mortality. Also the time between broods could be artificially altered (reduced) by the removal of egg sacs leading to a biased estimate of egg production.

The hatching rate method can be applied to both egg-carrying and free-spawning copepods, though the egg production in the free-spawning species could be severely underestimated, because of the very high mortality of free eggs (Liang et al. 1994, Peterson & Kimmerer 1994, Liang & Uye 1996a, b). In egg-carrying species the egg mortality follows the mortality of females and is much lower (Ohman 1986, Liang & Uye 1997), but here the use of the hatching rate method to estimate productivity assumes the same distribution of all adult females and no selective predation on the egg-bearing ones. This is problematic since the ratio of eggs to females measured in the field can be biased because of different diurnal migration behaviour in ovigerous and non-ovigerous females (Vuorinen 1997)). Depth integrated hauls should overcome this bias.

The method applied to determine hatching time presented here can readily be implemented to other egg-carrying species. It requires only a minimum handling of copepods, samples can be fixed and counting of nauplii and eggs can take place whenever time is available. The equation presented based on all available hatching times from this study and from the literature can readily be applied to other populations of Eurytemora affinis. Finally the hatching rate method to estimate copepod productivity can also easily be applied to fixed biomass samples.

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