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The effect of temperature on the rates of development of Eurytemora hirundoides (Nordqvist) in laboratory culture

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The development times of egg sacs, nauplii and copepodids of *Eurytemora hirundoides* during laboratory cultivation are presented. Egg sac development is a function of temperature in the range 7 to 18°C according to the least squares regression equation: $D_e = 34.6T^{-1.296}$ ($r^2 = 0.69$), the duration of the nauplius stage is also a function of temperature, in the range 7 to 20°C: $D_n = 278T^{-1.289}$ ($r^2 = 0.85$) and copepodid development is a function of temperatures from 12 to 8°C: $D_c = 272T^{-1.249}$ ($r^2 = 0.69$).

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1. Introduction

"In the study of biological productivity trophic level dynamics progress depends acutely on increasing our present capabilities for copepod cultivation. We must know more about their environmental and nutritional requirements, as well as about their rates and efficiencies of growth and reproduction" (Kinne 1977).

Very little information about the development rates of copepods in the Baltic Sea that could be used in production calculations has hitherto been published. Generation frequencies (generations per month) have been published for different zooplankton groups (Schnese 1975). The duration of the naupliar and copepodid stages of *Eurytemora* sp. has been estimated by Hernroth (Wulff et al. 1977). The data are based on laboratory cultivations, but the methods of cultivation are not described. Ciszewski & Witek (1977) have published information about the development times of older stages of *Acartia bifilosa* (Giesbr.) and *Pseudocalanus elongatus* Boeck. These data are also obtained from laboratory cultivations. The animals have been fed with naturally varying algal food taken from the sea. The significance of varying food conditions has not been taken into account. Some papers (Lindqvist 1961, Wulff et al. 1977, Eerola 1979) present developmental data obtained from field observations. The variance in local environmental conditions makes it difficult to draw general conclusions from these data. In gathering data for zooplankton productivity studies it is necessary to monitor environmental factors other than merely temperature, because the effect of temperature may be masked by other factors, such as varying food conditions (see Bottrell et al. 1976 and Kankaala & Wulff 1981). Temperature is, however, the fundamental regulator of productivity. Its effect should be studied primarily by keeping other factors constant. The fact that few cultivation experiments have been done in the Baltic area may be because of the timeconsuming and laborious nature of the cultivation methods.

The copepod species studied were *Eurytemora hirundoides, Acartia bifilosa* and *Centropages hamatus* (Lilljeborg). The species are later referred to as *Eurytemora, Acartia* and *Centropages*. Together they form 60—70 % of the total zooplankton biomass in the Archipelago Sea (Northern Baltic) (Vuorinen 1981). Copepodid and nauplius stages of *Eurytemora* and *Acartia* are also the dominating food items in the diet of larval fish in the littoral zone (Vuorinen & Rajasilta 1980).

2. Material and methods

The cultivation studies were carried out in June-September in 1978 and 1979, using 500 ml Erlenmayer flasks that were kept in a water bath and in shaded light (12 h/d in 1978 and 24 h/d in 1979). The temperature of the water bath was regulated thermostatically (Lauda termotemp, accuracy ± 0.5°C). The temperatures studied were 15 and 20°C in 1978 and 7, 12 and 18°C in 1979. The flasks were closed with aluminium foil in order to prevent contamination. A small aperture was made in the foil, through which feeding was carried out. Commercial algal mixtures were used as food (in 1978 Hobby mikrozell Aufzuchtsfütter, manufactured by Dohse Aquaristik Bonn and in 1979 Elite brine shrimp feed, manufactured by Derhams Products). They consisted mainly of one species of single cell *Chlorococcales* alga (*Oocystis* type, diameter about 5 μm). The initial concentration of algae in the culture flasks was about $1 \times 10^4 - 5 \times 10^4$ cells/ml. During the experiment food was added to each flask weekly.

Natural sea water was used as culture medium (Salinity-$^0/_{00}$ 5.5 ± 0.1, O_2 11.3 ± 0.4 mg/l, pH 8.3 ± 0.1). The temperature of the sea water in the sampling area varies between 10 and 20°C during the summer months. Water was taken from the same place as the plankton samples and filtered with a 25 μm mesh plankton net in order to remove alien plankton material. The plankton samples were collected with a 200 μm mesh plankton net from the vicinity of the Archipelago Research Institute (60° 14′ N, 21° 58′ E). They were then immediately taken to the laboratory, where single adult copepod females were selected and placed in small glass bowls. The animals were then fed and kept at room temperature until the nauplii hatched.

Eurytemora females carry egg sacs and the nauplii hatch periodically. Ten to 20 nauplii usually hatch from one egg sac and this was considered an adequate number for culture flask. *Acartia* and *Centropages* deposit their eggs free in the water and the nauplii hatch continuously. Several females can be placed together in a glass bowl in order to obtain an adequate number of nauplii hatched on the same day. The hatching time of the nauplii was taken as the beginning of the nauplius stage. The nauplii were then transferred to the culture flasks.

The flasks were inspected once a day in 1978 and twice a day in 1979 (except during the weekends) by lifting the flasks from the water bath and lighting them from behind, the animals then being clearly seen. The temperature was recorded at the same time. During the inspection the contents of the flasks were stirred in order to ensure a uniform food concentration. The weekly addition of food and stirring resulted in the water always being somewhat cloudy. Either the sedimented material was removed from the bottom of the flasks or the culture water was changed in cases when excessive growth of bacteria or fungi was observed.

The transition from the naupliar to the copepodid stage was easy to observe in the flasks, being when the appearance and mode of swimming of the larvae changed. The appearance of the egg sacs (*Eurytemora*) or the appearance of the second generation nauplii (*Acartia*) can be taken as the end of the copepodid stage and the beginning of the adult stage, although the copepodid stage has of course ended somewhat earlier. The duration of the copepodid stage can be calculated by subtracting the time for egg development from the time from the beginning of the copepodid stage to the hatching of the eggs. The development time of the *Eurytemora* egg sacs (= the time between successive egg sacs) was studied by placing single females, whose offspring had just hatched, into culture flasks and observing the time needed for the production of a new clutch. Observations of the development times of the egg sacs were made from single *Eurytemora* females, and the durations of the naupliar stages were estimated from stock cultures of 10 to 20 individuals hatched on the same day. The durations of the copepodid development were estimated from the same stock cultures starting from when they had reached the copepodid stage. Because of mortality during the experiments, the number of individuals was considerably lower at the end than at the beginning of the experiment. No attempts were made to assess quantitatively the mortality during the experiments. The change in the stage was recorded when over 50 per cent of the individuals in one culture flask had changed stage.

3. Results

According to the criteria of Paffenhörer & Harris (1979), culturing was successful with *Eurytemora* and rearing with *Acartia*. Both species reproduced and the nauplii developed into adults under the cultivation conditions. *Eurytemora* produced a second generation of nauplii. The cultivation experiments with *Centropages* failed; the adults did not survive more than four days in the culture.

The development times of various stages of *Eurytemora* are presented in Fig. 1. The curves in Fig. 1 were fitted by eye. In drawing the curves the values of copepodid development at 9 °C presented by Hernroth (Wulff et al. 1977) were used, because the experiment at 7 °C was terminated when the copepodids were 25 days old. No egg sacs had appeared in the culture during this time. The *Acartia* results were obtained from only 3 egg and nauplius cultivations. The development times of *Acartia* eggs were 5, 5 and 7 days and the development times of the nauplii were 6, 7 and 9 days at 20 °C.

Least squares regression equations (log-log transformation) were calculated between the temperature and the development times of *Eurytemora*. The development time of the egg sacs in the temperature range 7 to 18 °C is:

$$D_e = 34.6T^{-1.296} \quad (r^2 = 0.69).$$

The duration of the nauplius stage in the temperature range 7 to 20 °C is:

$$D_n = 278T^{-1.289} \quad (r^2 = 0.85).$$

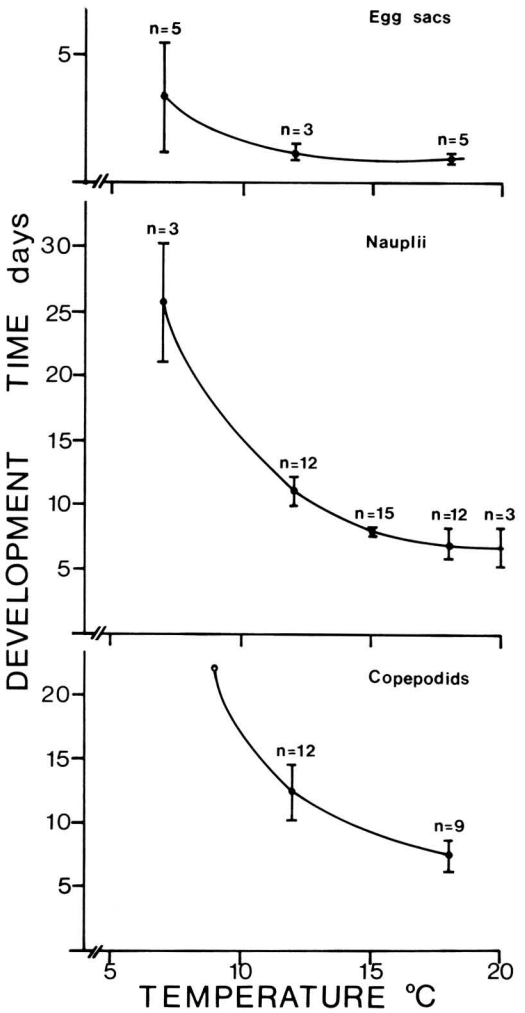

Fig. 1. The development times of egg sacs, nauplii and copepodids of *Eurytemora hirundoides* as a function of temperature ($\pm SD$). Solid dots = according to this study, open dot = according to Hernroth (Wulff et al. 1977). The curves are fitted by the eye.

The duration of the copepodid stage in the temperature range 12 to 8 °C is:

$$D_c = 272T^{-1.249} \quad (r^2 = 0.69).$$

4. Discussion

4.1. Eurytemora

The method is suitable for the cultivation of *Eurytemora*. The relative ease of culturing the mainly estuarine *Eurytemora* species compared with the more pelagic species was also observed by Katona & Moodie (1969). The results are in agreement with the development times of *Eurytemora* sp. presented from other areas (Tables 1 and 2). The methods used by Katona (1970), Heinle & Flemer (1975) and Corkett & McLaren (1970) are comparable with the methods in this study; Hernroth (Wulff et al. 1977) and Wulff et al. (1977) do not report their methods. Both *Eurytemora affinis* (Poppe) and *E. hirundoides* are distributed throughout the Baltic Sea and crossings between the two species have been recorded (Pesta 1927). This justifies the comparison of developmental times between the species.

The variations in development were compared using *SD* in Fig. 1, and coefficients of variation (Snedecor & Cochran 1967). The variation in egg sac development ($P < 0.05$) was larger at 7 °C than at 18 °C, but there were no differences between the variations in naupliar development at 7 °C and 20 °C or in copepodid development at 12 °C and 18 °C. There was no difference between

Table 1. The generation times (days) of *Eurytemora hirundoides* and *E. affinis* at different temperatures.

	10°C	15°C	20°C
E. hirundoides			
Archipelago Sea, Northern Baltic, Finland (present study)	27	18.5	15.5
E. affinis			
Hamble River, Southampton, England (Katona 1970)	28	17	12
Woods Hole, Massachussets, U.S.A. (Katona 1970)	33	20	15
Patuxent River, Maryland, U.S.A. (Heinle & Flemer 1975)	30	20	15

Table 2. The development time (days) of eggs (D_e) and nauplii (D_n) of *Eurytemora* species at 10 and 9°C.

	°C	D_e	D_n
E. hirundoides			
Archipelago Sea, Northern Baltic, Finland (present study)	10	1.8	14.3
E. affinis			
Halifax, England (Corkett & McLaren 1970)	10	3.4	10.8
Patuxent River, Maryland, U.S.A. (Heinle & Flemer 1975)	10	4.1	12
E. spp.			
Luleå, Bothnian Bay, Sweden (Wulff et al. 1977)	9	—	14
Askö, Baltic proper, Sweden (Hernroth in Wulff et al. 1977)	9	—	14

the variations in egg sac and copepodid development at 12 °C and 18 °C or in egg sac and nauplius development at 7 °C. Although, in the cases of nauplii and copepodids, the coefficients of variation do not support the observation that decreasing temperature increases the variation in the development time of eggs, this seems, however, to be the tendency when Fig. 1 is considered without statistics.

It is not known over how large an area the presented development rates are applicable when used as the basis for production calculations. The cultivation experiments should therefore be carried out using samples from various parts of the distribution area of the species in question, and should also be carried out in different seasons. The presented experiments were mostly carried out during the natural reproduction period (July-August) of the copepods in the study area. In September it was difficult to find ovigerous *Eurytemora* females, although the temperature of the sea water was in the same range as in June-July when ovigerous females were abundant. The number of eggs/sac was also lower in autumn than in summer. The reasons for this may be poor food quality or level or changed photoperiodic conditions (Checkley 1980, Marcus 1980). The results of this study should therefore be checked in samples cultivated under more natural conditions in order to judge their reliability and applicability to production studies.

4.2. Acartia and Centropages

For the cultivation of *Acartia* and *Centropages*, the method must be modified. The food in this experiment may have been unsuitable, because a mainly uni-algal diet is not natural for the copepods studied, and this may have caused the difficulties in cultivation. According to Paffenhöfer & Harris (1979): "Most investigators have used a mixture of foods, but there is good evidence that some species can be cultured with low mortality on an unialgal diet". The greater difficulties in *Acartia* and *Centropages* than in *Eurytemora* cultivation may also have been caused by starvation. *Eurytemora* is not as "planktonic" a species as *Acartia* and *Centropages*. It was frequently seen to be attached to the walls of the culture flasks and to take food from the bottom of the hatching bowls. It is possible that the food level was too low in the culture water and *Eurytemora* was therefore forced to take food from the sedimented material on the bottom. The other species may not have been able to feed on the bottom and starved, at least they were not observed to attach themselves to the walls of the culture flasks. Food levels have usually been somewhat higher in cultivations of this kind (see Corkett 1970, Corkett & McLaren 1970, Katona 1970, Corkett & Zillioux 1975 and Heinle & Flemer 1975). The food levels used in this study are however much higher than the natural phytoplankton densities in the sampling area. The starvation was perhaps caused by the difference in feeding habits rather than by an inadequate food level. The water of the culture flasks was not changed unless bacterial or fungal growth was observed. The reason for not obtaining a second generation of *Acartia* may have been the fact that eggs were released onto the bottom of the flasks. The unsuitable conditions among detritus, faeces and bacteria on the bottom may have caused the poor hatching success. On the other hand, detritus-bacteria aggregates serve, in addition to phytoplankton, as food for copepods (Hillbricht-Ilkowska 1977, Gyllenberg 1980). The cultivation experiment should therefore be made with different food types and quantities in order to ensure the best nourishment and to study the effect of varying food conditions on the development.

4.3. Evaluation of the method

When compared with the methods presented in Kinne (1977) and Paffenhöfer & Harris (1979) this method has the following advantages. The use of standard laboratory apparatus in the cultivation makes the construction of complex cultivation equipments, as presented in Kinne (1977), unnecessary. The time needed for the maintenance of the cultivation equipment is reduced because of the simplicity of the construction. The construction is simpler than the numerous methods presented in Paffenhöfer & Harris (1979) for the following reasons. Aeration, circulation and sterilization of the water are unnecessary. Changing of the water, cleaning of the flasks and transfer or handling of the animals during the cultivation are minimized. The use of a commercial algal mixture as food means that the bother of supporting another culture at the same time can be dispensed with. The shape of the flasks minimizes the risk of nauplii being trapped by the surface tension. The small size of the flasks makes inspection easy. Small flask size and stagnant, unchanged water and high food levels may, on the other hand, be adverse factors. The results should

therefore be checked and the method developed further by varying the flask size, the interval between changes and food levels.

The fact that the temperature may have an influence on other factors affecting productivity, e.g. size (see Lock & McLaren 1970) and reproductive capacity (see Katona & Moodie 1969), should also be taken into account. These should be measured at the same time as the data concerning development time are recorded. One way of measuring the reproductive capacity is to measure the hatching percentage. In this study the eggs of *Eurytemora* hatched well. Although the hatching percentage was not calculated, it was found to be almost 100 according to inspections made with a dissecting microscope when the animals were in the hatching bowl. In the hatching process, the eggs in an egg sac hatched within one to five minutes of each other. Paffenhöfer & Harris (1979) have noted that nauplii hatch directly from the egg sac of healthy *Eurytemora*. In this study nauplii were seen to hatch successfully from the egg sac that had been released on to the bottom of the hatching bowl. Hatching directly from an egg sac still attached to the female was recorded, too. Mortality also indicates the suitability of conditions in the culture, and should be measured. The mortality of nauplii in this study was high if they were trapped by surface tension in the hatching bowl.

In the culture flasks the surface to volume ratio was reduced and the mortality decreased. It increased again towards the older stages and lower temperatures. The method should be modified and tested further by varying the size of the flasks and (or) the number of individuals/flask. These factors may affect the development because the competition foor food may cause the animals stress, and the presence of the males may cause the females stress. Copulation occurred easily in the cultures and copulation attempts were recorded even in the copepodid stages. A maximum of about ten spermatophores were recorded attached to one female, making swimming difficult.

In general the results of such laboratory cultivations with restricted diet and cultivation volume are not directly applicable to production calculations because the natural development times may vary according to local environmental conditions. On the other hand, if cultivation is done under natural conditions it is necessary to measure the environmental variables, and results should be reported only in relation to these. Even so, they may still not be applicable to all areas. Furthermore these experiments would be very laborious because e.g. food level and quality would have to be monitored in pace with food consumption.

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