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Submitted: 10 October 1974
Accepted: 8 April 1975

The relationship between temperature and the development of life stages of the marine copepod *Acartia clausi* Giesbr.[1]

Abstract—The durations of life stages of the marine copepod *Acartia clausi* relative to egg development time are consistently maintained when these animals are cultured with excess food at 10, 15, and 20°C. Seasonal acclimation effects, which have been shown to affect the egg development of *A. clausi*, are carried through an entire generation. Generation time or the development time of any postembryonic stage can be calculated from life history data at one temperature and the relationship between egg development at other temperatures regardless of the effect of acclimation.

Corkett and McLaren (1970) proposed that under optimal food conditions calanoid copepods molt at intervals which maintain a constant relative relationship to the duration of the egg stage regardless of temperature. If true, this would allow calculation of development time of any stage at any temperature from life history data at one temperature and egg development times throughout the copepod's temperature range. The general applicability of this relationship was challenged by Geiling and Campbell (1972), who observed that the freshwater calanoid copepod *Diaptomus* did not follow a simple developmental pattern with temperature and that the relationship between stages did not remain constant. Further, Munro (1974) found a broad temperature-independent plateau in the development of some stages of the freshwater cyclopoid copepod, *Cyclops vicinus*. Both of these papers are conspicuously detailed with respect to the development of many life stages, involving observations of numerous animals at four different temperatures. In contrast, Corkett and McLaren (1970) studied three species (*Pseudocalanus minutus*, *Eurytemora hirundoides*, and *Temora longicornis*) but only in the most superficial way (2 stages/species —eggs and CI, based on the observations of 3 (range 1–6) individual copepodids at each temperature).

I have re-examined the proposal of Corkett and McLaren (1970) for 6–10 life stages of the marine copepod *Acartia clausi* reared at three temperatures. Temperature acclimation affects the egg development of *A. clausi* by causing the eggs from winter-acclimated females (10°C) to develop significantly faster under summer conditions (20°C) than eggs from summer-acclimated animals (Landry 1975); the effect of acclimation on the development of advanced life stages of this copepod is also investigated.

I gratefully acknowledge the assistance of B. Frost in preparing and reviewing the manuscript. This study was carried out at the University of Washington Friday Harbor Laboratories. I thank the director and

[1] Contribution No. 845 from the Department of Oceanography, University of Washington. This study was supported by NSF grant DES 74-22640 to B. W. Frost.

staff for the use of the facilities and for their help.

Acartia clausi was collected from Jakle's Lagoon, San Juan Island,Washington (*see* Hardy 1973). Adult females were sorted into beakers containing a dense mixture of phytoplankton cells, about 500,000 ml^{-1} of *Isochrysis galbana* (4 μ), *Dunaliella tertiolecta* (8–10 μ), and *Peridinium trochoideum* (10–20 μ), and the beakers were placed in constant temperature incubators. After at least 1 day under experimental conditions the females were separated from the eggs which they had produced and placed in fresh vessels with the same food concentration. At 12-h intervals the eggs were separated from the females and about 1,000–2,000 placed in each of 4–7 replicate 500-ml beakers with 200 ml of filtered (glass fiber, Gelman type A) seawater. Replicate beakers from two to four 12-h intervals were incubated at each experimental temperature (10, 15, and 20°C $\pm$ 0.1°C SD), shaded from direct light to avoid phototaxic concentrating effects, under a daily cycle of 16-h light and 8-h darkness.

When *A. clausi* is reared in unstirred beakers, best results (i.e. fastest development and highest survival: 90–95%) are obtained when only flagellated cells are offered as food and when the copepods are raised in relatively high densities so that grazing intensity slightly outstrips the reproductive potential of the phytoplankton. This procedure prevents phytoplankton senescence and large accumulations of settled phytoplankton (nonflagellates) on the bottom of the beaker, so that the copepods maintain relatively clean beakers and consequently require less handling. Naupliar stages NIII–NVI seem to be most sensitive to handling: even the most careful transfer of these stages from one beaker to another gave unpredictable survival. Zillioux and Wilson (1966) and Katona and Moodie (1969) have also observed the importance of a mixed diet, maintaining clean culture vessels, and minimizing handling when culturing marine copepods. However, contrary to their observations for other species, not reported in detail, the development and survival of *A. clausi* at the food concentrations used seem not to be adversely affected by high copepod density or the size of the culture vessel, provided that somewhat of a balance is maintained between grazing pressure and phytoplankton growth. Details of the scheme used in increasing culture volume, increasing phytoplankton density, and decreasing copepod numbers within beakers were adapted so that this balance would be maintained as the copepods developed.

Phytoplankton was first added to the experimental beakers just before the copepods molted to the first feeding stage (NII). The initial concentration of 100,000–150,000 cells ml^{-1} of the three phytoplankters was increased stepwise until a maximum of 500,000 cells ml^{-1} was reached at copepodid stages CII–CIII; Corkett and McLaren (1970) found that food concentrations in excess of 150,000 cells ml^{-1} of *I. galbana* allowed maximal development rates for *Pseudocalanus* nauplii. The initial volume of 200 ml was increased daily by the addition of food and filtered seawater until the 500-ml beaker was filled (usually stage NIV–CI), whereupon about 100 ml was replaced daily with new food and filtered seawater. At the CI or CII stage the copepodids were transferred with a widebore pipette to clean 1-liter beakers, of which about 100 ml was replaced daily with new media. As fecal material builds up rather rapidly for the advanced copepodids, another transfer was usually necessary before the animals reached the adult stage.

Replicate beakers were periodically (4–8-h intervals) sampled by gently stirring the water and drawing up a fraction of the volume with a pipette so that 50–200 animals were captured. This method of capture has proved to be unbiased; when a beaker was repeatedly sampled at a given time, the stage frequency estimates did not change with successive samples. Within experimental beakers, only a narrow range of stages was represented at any time so that all of the individuals tend to be similarly distributed and have similar escape capabilities. Biased sampling results are

Table 1. Median development time (T, h) of the life stages of Acartia clausi *at three experimental temperatures. The relative development (R) of any stage is the ratio of its development time at the given temperature to its development time at 20°C (fall group). Confidence limits (95%) are given for egg hatching times (i.e. when 50% of the eggs have hatched to NI).*

Stage	20°C Fall T	20°C Spring T	20°C Spring R	15°C Summer T	15°C Summer R	10°C Spring T	10°C Spring R
N1	31.2(±0.36)	29.3(±0.52)	0.939	45.8(±0.68)	1.468	72.0(±0.52)	2.308
N2	47.0						
N3	82.6					184.6	2.235
N4	107.4					239.0	2.225
N5	130.5					295.8	2.267
N6	155.3	145.3	0.936	217.6	1.401	348.9	2.247
CI	180.4	171.4	0.950	260.9	1.446	409.4	2.269
CII	204.8	196.0	0.957	299.0	1.460	475.4	2.321
CIII	230.7	218.9	0.953	339.0	1.469		
CIVm	254.0	242.1	0.953	363.6	1.431		
CVm	278.7	263.0	0.944	402.6	1.445		
male	312.0	290.0	0.929	485.1	1.554		
CIVf	260.0	239.6	0.922	376.8	1.449		
CVf	287.0	269.2	0.938	443.1	1.544		
female	319.0	300.9	0.942				

expected and obtained when the above method is used for beakers containing both young nauplii and advanced copepodids.

Stage frequencies of preserved samples from the replicate beakers were determined by microscopic examination, and the percent frequency of the population beyond each molt calculated. Median development time of a specified stage is defined as the time when 50% of the animals in a culture have passed into that stage, calculated from linear regressions of percent frequency of a stage against time, fit by the least squares method.

For unknown reasons mortality in a few replicate beakers was high (over 50%). High mortality is always accompanied by slower development than in beakers with low mortality. Beakers in which high mortality was observed were excluded from the calculations, since the aim of the study was to establish minimum development times for the given experimental conditions.

Development times for the stages of four experimental groups of copepods are presented in Table 1. At 20°C, copepods collected when the water was cold (12°C, spring) produced offspring that developed faster at every stage (including eggs) than the offspring from copepods collected during the fall (18°C). Rate enhancement of cold-acclimated animals at 20°C is like that for the development time of eggs of *A. clausi* (Landry 1975). Development time for the other two groups (at 10 and 15°C) increased as expected with decreasing temperature.

Relative development times for three experimental conditions (10, 15, and 20°C spring) were calculated with respect to the 20°C fall group and are presented in Table 1. Confidence intervals which take into account variance in both the numerator and denominator of each ratio can be calculated by operating on the original data after logarithmic transformation; this is done for the ratios of egg development (Table 2). The ratios for postembryonic stages, however, are subject to experimental errors relating to the condition of food and the accumulation of metabolic wastes, factors which do not affect egg development. Re-

Table 2. Ratio (R) of development time relative to development at 20°C (fall group) for the egg and combined postembryonic stages of Acartia clausi *at three experimental temperatures. The 95% confidence limit (CL) for each mean is given.*

	Egg R	Egg CL	Postembryonic stages R	Postembryonic stages CL
20°C Spring	0.939	0.927–0.951	0.942	0.934–0.950
15°C	1.468	1.451–1.486	1.467	1.429–1.505
10°C	2.308	2.285–2.331	2.261	2.227–2.295

sults from several replicate beakers (with potentially different conditions) were used to calculate the development times of the various stages. As mentioned above, very bad replicates, i.e. those with low survivorship, were easily recognized and discarded. However, there is no way of knowing if the development of some stages presented here was calculated from beakers in which the animals were unhealthy and their development only slightly depressed. An error of several hours is relatively undetectable in a 300–400-h experiment, yet could introduce significant variability in the calculation of ratios, which is not amenable to conventional statistical analysis. For each experimental group, then, a mean ratio of development time and 95% confidence limits for the mean were calculated from the combined ratios of all life stages excluding the egg (Table 2).

At each experimental temperature, there is no significant difference between relative egg development and the relative development of other life stages. This result confirms and complements that of Corkett and McLaren (1970): given excess food, the life stages of the marine copepod *A. clausi* maintain a constant development time relative to each other and to the egg stage. Given a constant relationship between stages derived from life history data at one temperature, the development time of any life stage at other temperature conditions (i.e. experimental temperature and previous temperature acclimation) can be calculated from factors relating egg development time at the experimental conditions to development under the conditions where the full life history is known. These factors need not be corrected for temperature acclimation since acclimation effects seem to be carried through an entire generation. However, conditions allowing maximum development rates for all stages must be met.

It is important to note that evidence contradictory to the results of Corkett and McLaren (1970) and those reported here has only been offered for freshwater copepods. It is tempting to speculate that development in *A. clausi* is controlled by a series of biochemical reactions which is entirely rate regulated by temperature effects, provided that all substrates are available in excess (i.e. optimal food). The extra metabolic demand of osmoregulation in freshwater copepods could be related to temperature by a competing function so that the combined effects of two systems would be to distort predictable developmental results. The effect of temperature on the development of additional species should be studied to determine if the differences observed for marine and freshwater copepods are real differences or simply the result of choosing nonrepresentative experimental species.

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Submitted: 22 January 1975
Accepted: 16 May 1975