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Title: The Effects of the Temperature Acclimation of Parental Generations and Incubation Temperature on Lability of Egg Hatching Time in the Copepod Acartia tonsa Dana

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Dr. Jefferson J. Gonor

Experimental results indicate that both parental acclimation temperature and egg incubation temperature have significant effects on the egg hatching time of Acartia tonsa between the temperatures of 15°C and 25°C. The effects of parental acclimation temperature and egg incubation temperature are additive if the long-term parental acclimation temperature is constant. A. tonsa eggs are labile to temperature during development and after they are laid up to the time of hatching. Egg hatching times acclimate to changes in parental culture temperatures from 20° C and 25°C within 86 hours and to temperature changes from 15°C within 8 days.

Hatching times of eggs from field collected A. tonsa demonstrate that acclimation of hatching times occurs in the field and responds to time periods of less than one generation.

Results from feeding studies using ^{14}C labeled algae indicate that eggs are labeled within 24 hours and that the ^{14}C activity is reduced to a base level 48 hours after substitution of unlabeled food.

The lability of A. tonsa eggs to temperature and the acclimation of egg hatching times to temperature changes suggests that any

predictions of egg hatching times for this species should be accompanied by a statement of the environmental conditions under which the values are determined and any population parameters based on birth rates for this species should be calculated carefully from empirically derived data using acclimated animals.

The Effects of the Temperature Acclimation of Parental Generations and Incubation Temperature on Lability of Egg Hatching Time in the Copepod *Acartia tonsa* Dana

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Typed by Nancy Brown for Patricia Ann Tester

To B. L. T.

for encouraging me to finish this

so other things could begin.

To P. E. G.

for your patience

and for accepting the importance

of this work because it was

important to me.

Nothing in the world can
take the place of persistence.
Talent will not; nothing is more common
than unsuccessful men with talent.
Genius will not; unrewarded
genius is almost a proverb.
Education will not; the world
is full of educated derelicts.
Persistence and determination
alone are omnipotent.

C. Coolidge

"The interaction between organisms and their environment is an old but very important problem for biologists. Organisms respond to environmental changes in different ways according to the time during which the environmental changes persist and according to the magnitude of the stress. In living organisms those alterations which favor survival in a changed environment are said to be adaptive. Similar adaptive variations of organisms may be genetically determined or they may be environmentally induced . . . "

C. Ladd Prosser

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I. INTRODUCTION

Statement of the Problem

Of all factors in the marine environment, temperature has the most pervasive effects on the functions and distribution patterns of organisms (Somero and Hochachka 1976). These effects are the most investigated responses of marine animals. Because of the influence of temperature on rates of growth and development, such effects have received considerable attention in relation to population dynamics and productivity of zooplankton. Many methods proposed for calculating secondary production of zooplankton require estimates of egg hatching times at various temperatures (Edmondson 1960; Edmondson and Winberg 1971; Snell 1978). Egg hatching rates of several common marine copepods have been studied (McLaren 1965, 1966; Corkett 1972; Landry 1975a; Palmer and Coull 1980). There is a considerable literature on the effects of temperature on egg hatching rates of freshwater copepods as well (Munro 1974; Smyly 1974; Bottrell 1975; Cooley and Minns 1978). Generally, these studies show that subitaneous egg hatching rate or embryonic duration in copepods is strongly temperature dependent and is relatively insensitive to other common environmental factors (Elster 1954; McLaren, Walker and Corkett 1968; Uye and Fleminger 1976).

Determining egg hatching times as a function of temperature over the entire environmental temperature range of a species is a lengthy and tedious task. Therefore, workers have sought to demonstrate the

value of certain equations to predict development times under different conditions for the same species and for closely related species. McLaren (1963,1966) advocated the use of Bêlehradék's (1935) equation. This function has been widely used to describe the effects of temperature on egg hatching rates for insects (Humpesch and Elliott 1980), and freshwater and marine copepods (McLaren 1966; Smyly 1974; Cooley and Minns 1978; Palmer and Coull 1980).

Other workers have not agreed that Bêlehrádek's equation gives the best fit for development time as a function of temperature. Bottrell (1975) gave an excellent treatment of rate-temperature functions and argued against the validity of using a single line to describe the relationship between egg hatching time and temperature for a number of species. However, even Bottrell conceded that the use of a mean slope, common intercept or a single line may be necessary for short term, general surveys of entire ecosystems.

To appreciate the complexities and the magnitudes of thermal effects on the physiology and acclimation responses of egg hatching times details of the thermal history of the parents and egg incubation temperature are required. Factors influencing the shapes of the rate-temperature curves must be carefully considered since any error will be multiplicative rather than additive in production calculations. This is especially important when a particularly abundant species is being considered. Bullock (1955) warned that it is mandatory to measure rate-temperature curves of poikilotherms using animals that have been completely acclimated to the temperature at which the rate of the response is being measured. Hall (1964) realizing the importance of this in his work with Daphnia, suggested that any reference to the rate of increase

of a population should be accompanied by a statement of the environmental conditions under which the value was determined. The thermal history of an animal may change both the position and the slope or form of the temperature response curve. Edmondson (1960), working on the hatching of rotifer eggs, found a marked difference between the temperature duration curves for acclimated and non-acclimated animals.

Acclimation to temperature occurs in poikilotherms, and often quickly. Levins (1969) found most of the thermal acclimation of Drosophilia took place in the first 12 hours. For the crayfish Astacus pallipes, acclimation to temperature is complete in about two days (Bowler 1963). Acclimation to increased temperature occurs quite rapidly (less than 24 hours) in the calanoid copepod Eurytemora affinis (Bradley 1978), which also acclimates to decreased temperature but less rapidly. Palmer and Coull (1980) suggested that the harpacticoid copepod Microarthridion littorale acclimated during the two-day period over which temperatures were adjusted prior to the start of the experiments.

Landry (1975a) reported that egg hatching rates for the marine calanoid copepod Acartia clausi are affected by the temperature history of the female parent. He reported that the hatching rate of A. clausi eggs spawned by a winter population was significantly faster than the hatching rate of eggs from the summer population when incubation temperatures were above 19°C. Landry also suggested that adaptation of winter acclimated animals to summer conditions is a slow process requiring more than one generation at 20°C. Further, Landry (1975b) found that the duration of larval stages of A. clausi relative to egg development time was consistently maintained when the animals were

cultured with excess food at 10°C, 15°C and 20°C. He concluded the seasonal acclimation effects were carried through the F_1 and the F_2 generations as well.

Several subsequent observations complicate the line of evidence proposed by Landry (1975a, b). Uye and Fleminger (1976) and Uye (1980) failed to confirm Landry's (1975a) results and concluded that eggs produced by A. clausi and A. steueri have similar physiological properties throughout the year. Miller, Johnson and Heinle (1977) found the duration of larval stages to be consistent (isochronal) under conditions of excess food and constant temperature for Acartia, independent of egg hatching time. Yet, it is known that environmental conditions during development can irreversibly modify labile physiological processes (Kinne 1965). Non-genetic adaptation of crustaceans to low or high temperatures is well documented. Acclimation to reduced temperatures tends to shift the lower lethal limit downward, and acclimation to increased temperatures tends to shift the upper limit upward (Kinne 1965). Such variations in lethal limits are often maintained for days or weeks after removal of the stress. A. tonsa and A. clausi exhibit a tolerance to higher temperature with acclimation to increased temperatures (González 1974). Increased temperature during the development of Eurytemora affinis led to increased temperature tolerance in the adult stage (Bradley 1978). Apparently changes affecting temperature tolerance can occur during development and may be only partially reversible in the adult stage.

Acartia tonsa Dana (1853) is a widely distributed and abundant pelagic calanoid copepod. It inhabits tropical, sub-tropical and warm temperate estuaries and coastal zones worldwide. Along the Atlantic

coast of America, A. tonsa is recorded as far north as the Miramichi River estuary in New Brunswick (Bousefield 1955) and has been collected from the Bahía Fosforescente, Puerto Rico (González 1974). A. tonsa is abundant in the Gulf of Mexico (Turner 1981) and is a prominent, year-round resident of Lake Pontchartrain, Lousiana (Darnell 1962). On the European coast A. tonsa is known from the Gulf of Finland (Smirnov 1935) to the Canal de Caen (Remy 1927) and the British coastal waters off Southampton (Conover 1957). Esterly (1928) recorded A. tonsa from the California coast and this species is also known in the Indo-Pacific (Steuer 1915) and Australia (Wilson 1932).

As a coastal species, A. tonsa is perennial in protected waters of the West Atlantic from northern New Jersey southward (McAlice 1981). In the Navesink River estuary, a tributary of New York Bight, it comprised 71% of the copepods sampled over a 13-month period (Kantz 1978). Heinle (1966) found it to be the most abundant copepod seven months of the year in the Patuxent River estuary in Chesapeake Bay, where densities up to 100,000 copepods m^{-3} were found during the summer months. Potential production rates calculated by Durbin and Durbin (1981) for A. tonsa were as high as 22.9 mg C m^{-3} and biomass doubling times were generally less than one day in the summer. Woodmansee (1958) found A. tonsa to be the dominant copepod in Biscayne Bay, Florida. This species is both eurythermal (-1°C to 30°C) (González 1974) and euryhaline (5 to 35 $^{o}/_{oo}$ salinity) (Lance, 1962, 1963, 1964; Darnell 1962). A. tonsa produces both subitaneous and resting eggs (Zillioux and González 1972). This study did not consider resting eggs. For a review of resting eggs in marine copepods see Grice and Marcus (1981).

Objectives

Laboratory experiments were performed to determine the temperature lability of subitaneous egg hatching times of Acartia tonsa Dana under controlled conditions by testing the effects of temperature and temperature changes on eggs before and after they are laid. Parent cultures were temperature acclimated for at least three generations to test the effects of long-term temperature acclimation and for less than one generation to test the effects of short-term temperature acclimation on egg hatching times. The effects of incubation temperatures on egg hatching times were also considered.

The specific objectives were as follows:

1. Experimentally demonstrate the effects of long-term and short-term parental acclimation temperature and egg incubation temperature on the hatching time of A. tonsa eggs.
2. Determine the length of time required for a temperature change of the parent culture to affect egg hatching time.
3. Compare the egg hatching times in the temperature change experiments with predicted egg hatching times.
4. Demonstrate the temperature acclimation of egg hatching times found in the laboratory experiments also occurs in the field.
5. Determine the development time of A. tonsa eggs.

II. METHODS

Plankton Sampling and Culture Maintenance

Vertical plankton tows were made within one meter of the surface using a 1/2 meter plankton net with 333 μm Nitex mesh. Samples were taken on outgoing tides from Pivers Island Channel, the channel between the Newport River estuary and the Beaufort Inlet, Carteret County, North Carolina, and from the Newport River at its junction with the Intra-coastal Waterway. For field water temperature studies, A. tonsa individuals were sorted from the mixed plankton as quickly as possible to minimize temperature change, and held in one gallon glass jars in running seawater at ambient field temperature. The salinity of these cultures was adjusted to 9-13 °/oo and a continuous low level of illumination ($0.5\text{-}2.2 \times 10^{14}$ quanta sec^{-1} cm^{-2}) was provided. Animals destined for laboratory acclimation studies were allowed to come to either 15°C, 20°C or 25°C overnight and then were sorted into 10 to 16 liter battery jars or one gallon glass jars containing filtered seawater diluted to 9-13 °/oo salinity and maintained at the above temperatures. Cultures were held in either Partlow environmental chambers at the Duke University Marine Laboratory, in a Partlow walk-in constant temperature box at the National Marine Fisheries Service Laboratory, or in Gilson respirometer water baths at either 15°C, 20°C or 25°C as experiments required. The Partlow equipment maintained temperature to ±0.5°C and the Gilson respirometer water baths controlled water temperature to ±0.1°C. All egg incubation was done in water baths. Temperatures were recorded continuously (Partlow) or daily during the experiments. Throughout the entire study, temperatures were adjusted and recorded

using the same thermometer which was calibrated at the start of the experiments using an NBS calibrated thermometer.

All copepod cultures were aerated gently and held under continuous low illumination ($0.5\text{-}2.2\times10^{14}$ quanta sec^{-1} cm^{-2}). Cultures at 15°C were fed every third day, those at 20°C every two days, and cultures at 25°C were fed half rations each day. Food concentrations were not strictly maintained but rations were approximately 2×10^{6} cells ml^{-1} of Pseudoisochrysis paradoxa (strain VA-12) and Thalassiosira weissflogii (strain VA-59), both obtained from the Virginia Institute of Marine Science. These microalgae were cultured in half-strength medium (Guillard and Ryther 1962) at 20°C in 20 °/oo salinity.

Pivers Island Channel Water Temperatures and Experimental Temperature Range

For the seven year period between 1975 and 1981 the highest mean weekly water temperature in Pivers Island Channel was recorded in August of 1980 at 29.3°C and the seven year mean weekly high was 28.7° C±0.39°C (±95% CI). The lowest mean weekly temperature was 2.8°C in January of 1977 and the seven year mean was 5.2°C±2.3°C (±95% CI) (unpublished data from William Hettler, National Marine Fisheries Service, Beaufort, North Carolina). Throughout the unusually cold winters of 1976-1977 and 1977-1978 (lowest mean weekly temperatures of 2.8°C and 3.5°C respectively) A. tonsa were not present in Pivers Island Channel. During the mild winters of 1980-1981 and 1981-1982 adult A. tonsa were collected from Pivers Island Channel where they were quite abundant until late January and early February. As water temperatures approach 25°C usually in late May to mid-June, A. tonsa

abundance declines from an April high and it is not abundant again until the mean weekly temperatures drop below 25°C in late September to early October. Similar patterns of abundance have been noted for A. tonsa in other estuaries (Kantz 1978; Durbin and Durbin 1981). Although the mean annual water temperature range in Pivers Island Channel is near 23-24°C, a temperature range of only 10°C (from 15°C to 25°C) was used in these experiments. Zillioux and González (1972) reported that for A. tonsa, production of resting eggs starts when temperature falls below 14.5°C. The upper experimental temperature of 25°C was the highest temperature at which cultures could be maintained in the laboratory and is probably near the upper thermal limit of this species in the Newport River. González (1974) cultured A. tonsa from the coast of Florida for several generations at 27°C but he found it necessary to use antibiotics to discourage bacterial growth. In this study no antibiotics were used to rear A. tonsa at 25°C although it was necessary to sterilize the laboratory glassware and culture vessels frequently to avoid high mortality.

Egg Laying Periodicity

Notwithstanding the controversy and discussion about the various equations used to describe the shape of the egg hatching rate-temperature curve, comparatively little attention has been given to the assumptions made to arrive at such curves. Time to 50% or 100% hatch, as predicted by linear regression or extrapolation of the curve to the time axis is the standard measure of egg hatching time (Edmondson 1960; Burgis 1970; Landry 1975a). A linear relationship is assumed between number of eggs laid and time at a given temperature and supposes no

egg laying or hatching periodicity. In copepods which retain eggs in egg sacs until hatching, eggs are laid over a relatively short period and the assumption of the linearity probably holds. For many pelagic calanoids, including Acartia, which broadcast eggs one at a time as they are produced, egg laying periodicity is a potential problem. Parrish and Wilson (1978), Landry (1975a) and Uye (1980) all indicated that Acartia produces eggs continuously. The light levels or conditions were not stated in Landry's paper. Parrish and Wilson used continuous light and Uye did not control the light in his experiments. Because of the potential importance of knowing of any periodicity in egg laying by A. tonsa, day-night periodicity was examined.

Adult A. tonsa were conditioned for three days to a 12:12 L:D cycle to 2.2×10^{14} quanta sec^{-1} cm^{-2} at 20°C and then females were placed in each of three 500 ml beakers at the beginning of a 12 hour light or dark cycle. After 12 hours the females and their eggs were counted. The experiments continued for six 12-hour periods. Controls for these experiments were set up in a similar manner except that the copepods had been conditioned under constant low light levels (2.2×10^{14} quanta sec^{-1} cm^{-2}) and were tested under these conditions. Although the difference in the number of eggs produced in the light, dark or constant illumination was not statistically different, generally more eggs were produced by females held in darkness. Because of this, all cultures used to produce eggs for hatching experiments were kept under constant low light ($0.5\text{-}2.2 \times 10^{14}$ quanta sec^{-1} cm^{-2}). A uniform egg laying period (ELP) was used when possible and eggs were taken at the same time of the day, usually between noon and 16:00 h.

The Data, Their Treatment, the Experimental Design and Data Analyses

Egg hatching in A. tonsa is similar to that described for Calanus (Marshall and Orr 1972) except it is quicker in Acartia, taking approximately 5-10 seconds after the first movement of the nauplius. If the egg membrane is not ruptured within about 30 seconds after the chorion is split, the nauplius usually does not manage to free itself from the egg membrane and hatching is not completed. At the onset of hatching, observations were made to determine the number of eggs hatched each 15, 30 or 60 minutes as the experiment progressed. The data were recorded as the percentage of the total number of eggs in the experiment which hatched vs. time. The level of the response used was time to 50% hatch. The arcsine transformation (Sokal and Rohlf 1969) and the probit transformation (Finney 1962) were used on the percentage data. A probit analysis program, 298028F from Texas Instruments, Inc. for the TI-59 hand calculator, was used to compute probit regressions.

The data on percent hatch were regressed against time, and time to 50% hatch was determined for each experiment. Egg laying period (ELP) was generally about four hours and it was sometimes necessary to prolong the ELP beyond that to have enough eggs for the experiments. Because of a difference in ELP for some experiments, time to 50% hatch - 1/2 ELP was taken as the mean time to hatch and used as the response variable throughout this study.

Landry's (1975a) data on egg hatching times for A. clausi suggested that the temperature history of the parental and possibly the grandparental generation affected hatching rates of the F_1 generation. It was therefore essential that the temperature history of the stock cultures used in this study be known and maintained constant for

at least three generations prior to the start of the experiments. The experimental design for testing the effects of parental acclimation temperature and egg incubation temperature on time to hatch was a 3 x 3 matrix wherein eggs from parent cultures, long-term acclimated to either 15°C, 20°C or 25°C, were incubated at each of these three temperatures.

In the short-term temperature acclimation studies, cultures were held in the laboratory at a constant temperature (either 15°C, 20°C or 25°C) for less than one generation, usually for four to seven days, but up to 13 days at 15°C before the experiments started. The percent hatch in the 15°C/15°C experiments was greater than 85%. This indicates that four days at 15°C was enough time for females isolated from the field, when temperatures were less than 15°C, to commence producing hatching rather than resting eggs. The experimental design was the same as that for the long-term acclimated cultures.

The method of Grainger (1959), modified by Kahn (1965) and Keen and Parker (1979), was used to calculate the expected times of hatching under conditions of changing temperature. This method integrates the area under the rate-development curve and is used to predict correct embryonic durations and to detect apparent acceleration or retardation of development.

The lability of eggs to temperature was examined by comparing the hatching times of eggs laid over different periods. The eggs were taken from parent cultures acclimated to 15°C. The ELP varied from 8-27 hours at 15°C, and eggs were incubated at 20°C.

Hatching times for eggs of A. tonsa taken directly from the field were also determined. Animals were sampled eight times from 7 April

to 27 June, 1979 when field water temperatures ranged from 16.7°C to 27.8°C. Eggs from these samples were incubated at 20°C.

To test the effects of changing the temperature of the parent cultures on the time for the eggs from that culture to hatch, A. tonsa were acclimated for one generation before the temperature was changed by either 5°C or 10°C. Eggs were taken at time = t_x, t_{x+1}, t_{x+2}, t_{x+3}, etc. after the temperature change, and incubated at the raised or lowered temperature. Hatching times were determined as in the previous experiments. In one of these experiments the parental cultures had been at 15°C for three generations before the temperature was raised to 20°C. Hatching times of eggs incubated at 20°C from this culture were determined in separate experiments. When the hatching time of these eggs equalled the hatching time of the 20°C/20°C long-term temperature acclimation experiments, eggs were incubated at 15°C (the original temperature of the parental cultures) and at 25°C. This experiment was a test of the ability of eggs laid under continuing temperature changes to acclimate to temperature.

The mechanics of the experimental techniques were as follows: at time zero adults were screened using a 571 µm mesh sieve, placed in fresh culture medium, fed, and left to lay eggs for the specified ELP. Adults were screened from the eggs which were caught on a 64 µm screen. Eggs were sorted into 0.25 ml chambers made by fixing a 1 cm (ID) rubber "O" ring to the bottom of 5 1/2 cm petri dishes with hot paraffin. Six to eight "O" ring chambers were used per petri dish and approximately 10 eggs were placed inside each. Close fitting clear plastic lids prevented evaporation. When enough eggs were available, each experiment used at least 100 eggs and three replicates per treatment.

The petri dishes were floated on the surface of the water baths during the incubation period. Observations were made through the lids, using a Wild M-7 microscope with a fiber optics light source when it was available. The total number of eggs hatched in each experiment was usually 85% or more. Even though some of the temperature combinations were less than optimal for hatching success, no experiment was used in which fewer than 50% of the eggs were observed to hatch.

Data from these experiments were analyzed using a Tektronix 4051 analysis of variance program for a two-way analysis of variance (ANOVA) for unbalanced data. The ANOVA was performed in one of two ways. The method of fitting constants (Steele and Torrie 1960) was used for the long-term temperature acclimation data. If there is a significant interaction term, tests of the main effects in the method of fitting constants is not valid and the method of weighted squares of means was used (Steele and Torrie 1960). A multiple regression was also run on the long term data.

Egg Development Time

If parental acclimation temperature is a factor in the hatching time of A. tonsa eggs it becomes important to know the time required for an oocyte to develop into an egg. Is the yolk labile to temperature and over what time does the yolk accumulate? Marshall and Orr (1972) reported that occasionally stage V Calanus females were found with a large, well-developed ovary and they thought that some stage V females could be already ripe when they molted to stage VI. Algae were labeled with ^{14}C and fed to immature copepods (stage C_I) throughout their development to adults (stage C_{VI}). In another experiment

labeled algae were also fed to adults. If eggs accumulate yolk over a long period the eggs of the copepods fed labeled algae during their entire development from C_I to C_{VI} would be expected to show a higher level of activity than the eggs of adults fed labeled algae after maturity.

The methods used for the ^{14}C experiment were modified from Copping and Lorenzen (1980). Two-liter cultures of unicellular Thalassiosira weissflogii ($5x10^3$ cells ml^{-1}) were adjusted to pH 8 with NaOH, innoculated with 50 µci of $NaH^{14}CO_3$ (Amersham, specific activity 652 µci mg^{-1}) and cultured under constant illumination at 20°C. After approximately four days the uniformly labeled algal cultures, ready to serve as food, were centrifuged at 5,000 rpm in a Sorvall centrifuge for three minutes to concentrate the algae. The supernatant was decanted and the algae resuspended in sea water of 15 °/oo salinity before being used as food for the copepods.

Eggs were sampled either after the copepodites reached maturity or from 3.5-96 hours after the adult A. tonsa were given labeled algae as food. Unlabeled algae were then substituted for labeled algae and eggs were again taken 24-48 hours later. All ELPs were two hours and all samples were done in triplicate. Eggs from these experiments were screened from the cultures, suspended in 12 °/oo salinity and transferred through three rinses to avoid contamination by fecal pellets. Samples of the eggs were then filtered onto one cm Millipore filters (5 µm pore size) and washed twice with 0.5 N HCl to remove ^{14}C remaining on the filter as inorganic carbonate. The filter was then quickly rinsed with distilled water to remove the acid and placed on blotter paper, and the eggs were enumerated. Both the filter and eggs

were placed in a glass scintillation vial with 25 ml of organic counting scintillant (OCS 196322 Amersham). After 48 hours, the samples were counted in a Beckman liquid scintillation counter for their entire ^{14}C pulse height spectrum at 2% error. As a control, two hour old unlabeled eggs were allowed to incubate for one hour in medium from the ^{14}C copepod culture to determine if newly laid eggs would take up ^{14}C directly from the medium. These eggs were then treated as were the experimental eggs. This ^{14}C blank and a standard blank of unlabeled eggs were run in triplicate.

III. RESULTS

The variable, 50% hatch - 1/2 ELP, was used untransformed. Neither of the two transformations, arcsine or probit, improved estimates of the variance and the variance of the untransformed data was not a function of the mean. If, as in the 15°C/20°C experiments, the ELP varied widely, the time to 50% hatch - 1/2 ELP gave a considerably better estimate of the true hatching time and a smaller standard deviation than did the other methods (Table 1).

Effects of Long-Term Temperature Acclimation of Parent Cultures

The results from 66 experiments using 12,605 eggs from long-term temperature acclimated parental cultures indicated an inverse relationship between both parental acclimation temperature and egg incubation temperature on mean hatching time for temperatures between 15°C and 25°C (Table 2). Increasing the long-term acclimation temperature of the parental cultures decreased the hatching time, except for those eggs from 25°C parental cultures which were incubated at 15°C. Lowering the incubation temperature by 10°C reduced hatching of eggs produced at 25°C. In the four experiments attempted at this combination of parental acclimation and incubation temperatures, no more than 10% of the eggs hatched and the nauplii produced were not active. A 10°C temperature decrease from 25°C to 15°C, was lethal for eggs while the 10°C increase from 15°C to 25°C was not. The 95% confidence intervals of the means of hatching times in the 20°C/20°C and 25°C/20°C experiments overlapped.

In the analysis of variance using the method of fitting constants, egg incubation temperature had a highly significant ($p<0.001$) effect

Table 1. Comparison of the means and standard deviations for four methods of calculating time to hatch (hours) for Acartia tonsa eggs. I. Time to 50% hatch predicted by linear regression, II. Time to 50% hatch - 1/2 ELP, III. Time to 50% hatch as predicted by linear regression of arcsine transformed data, and IV. Time to 50% hatch as predicted by probit regression.

Experimental conditions[1]	I. $\bar{X}$	I. s	II. $\bar{X}$	II. s	III. $\bar{X}$	III. s	IV. $\bar{X}$	IV. s
20°C/20°C	21.98	1.89	19.87	1.89	22.26	1.85	22.57	2.10
N=15 n=2881								
20°C/15°C	19.15	1.64	17.08	1.58	19.40	1.59	19.11	1.42
N=5 n=1365								
15°C/20°C	29.06	6.46	24.26	2.01	29.06	6.42		
N=5 n=574								
25°C/25°C	15.07	2.11	12.55	1.34	15.22	2.03		
N=4 n=815								

[1] Experimental conditions are parental acclimation temperature/egg incubation temperature
N = number of experiments
n = number of observations

Table 2. Time in hours to 50% hatch - 1/2 egg laying period as a function of the long-term temperature acclimation of the parent cultures and egg incubation temperature for eggs of Acartia tonsa. Parent cultures were acclimated to a constant temperature (either 15°C, 20°C or 25°C) for at least three generations before the experiments started. 95% CI are given.

EGG INCUBATION TEMPERATURE	PARENTAL ACCLIMATION TEMPERATURE: 15°C	20°C	25°C
15°C	40.1±3.9 N=6 n=940	29.3 N=1 n=247	No Hatch N=4 n=453
20°C	24.4±1.8 N=7 n=874	20.8±1.1 N=21 n=3732	18.8±1.7 N=6 n=1157
25°C	18.2 N=1 n=157	16.4±2.2 N=13 n=3282	12.5±1.3 N=7 n=1745

on time to hatch (Table 3). When adjusted for the sum of squares of the main effect of egg incubation temperature, the effect of long-term parental acclimation temperature on time to hatch was also highly significant ($p<0.001$). Since the interaction term in the ANOVA is not significant ($p<0.05$), the egg incubation temperatures and long-term thermal history of the parent cultures do not interact. Between the temperatures of 15°C and 25°C hatching times can be predicted on the basis of the additive effects of egg incubation and long-term parental culture temperatures except for the 25°C/15°C combination. The regression analysis equation is as follows:

$Y = 71.2 +$ egg incubation temperature $(-0.868) +$ long-term acclimation temperature (-1.55) $\quad r^2=0.79.$

Since the interaction term was not significant the tests of the main effects are valid. In a plot of these data (Fig. 1) slope reflects the effects of long-term parental acclimation temperatures. The heights of the curves reflect the effects of egg incubation temperature on mean time to hatch. The lower the acclimation temperature the slower the hatching time at all incubation temperatures tested. There is translation of the rate-development curve with no significant change in slope.

Effects of Short-Term Temperature Acclimation of Parental Cultures

Data from 43 experiments representing 3,502 observations of mean egg hatching times in short-term acclimation experiments are summarized in Table 4. In these experiments parental cultures were exposed to a constant temperature (either 15°C, 20°C or 25°C) for less than one generation (4 to 7 days at 25°C and 20°C and up to 13 days at

Table 3. Two-way analysis of variance (unbalanced data) of time in hours to 50% hatch - 1/2 egg laying period as a function of the long-term acclimation of the parent cultures and egg incubation temperature for eggs of Acartia tonsa. Parent cultures were acclimated to a constant temperature (either 15°C, 20°C or 25°C) for at least three generations before the experiments started. The method of fitting constants (ANOVA) is used for making inferences about the main effects since there is one cell with no observation.

Preliminary Analysis of Variance

Source	Degrees of freedom	Sum of squares	Mean squares	F	Significance level
Total	61	3527.49			
Main	4	3092.52	773.13	109.81	0.001 ***
Interaction	3	54.75	18.25	2.59	0.001 ***
Error	54	380.20	7.04		
	Method of Fitting Constants - Analysis of Variance				
Total	61	3527.48			
Egg incubation temp.	2	2850.64	1425.32	202.44	0.001 ***
Parental acclimation temperature adjusted by egg incubation temperature	2	241.89	120.94	17.18	0.001 ***
Parental acclimation temperature	2	1810.31	905.15	128.56	0.001 ***
Egg incubation temp. adjusted by parental acclimation temp.	2	1282.22	641.11	91.06	0.001 ***
Interaction	3	54.75	18.25	2.59	0.062
Error	54	380.20	7.04		

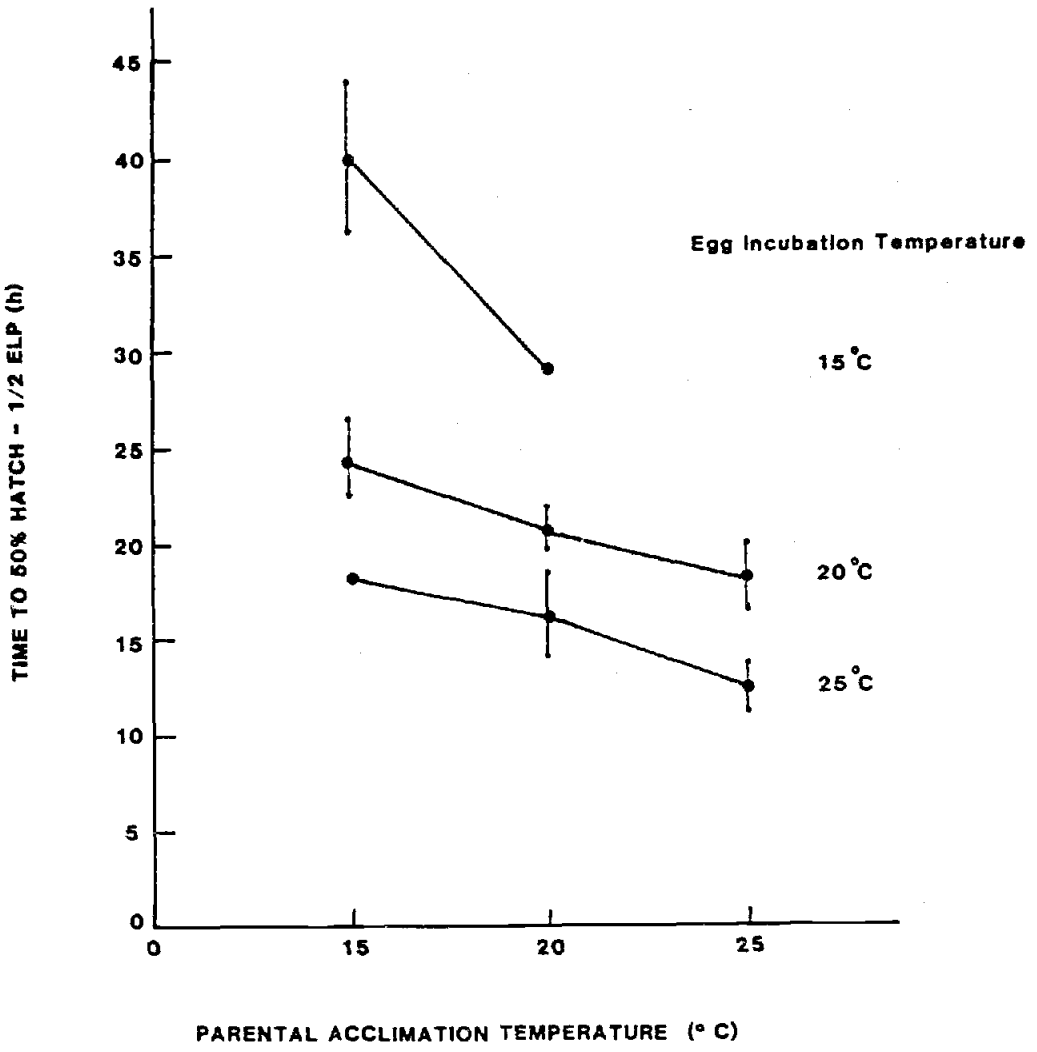

Figure 1. The effect of parental acclimation temperatures and egg incubation temperatures on time to 50% hatch - 1/2 egg laying period (ELP) for the eggs of Acartia tonsa. Acclimation of parent cultures was at a constant temperature (either 15°C, 20°C or 25°C) for at least three generations before the start of the egg laying period. 95% CI are given.

Table 4. Time in hours to 50% hatch - 1/2 egg laying period as a function of the short-term temperature acclimation of the parent cultures and egg incubation temperature for eggs of Acartia tonsa. Parent cultures were acclimated to a constant temperature (either 15°C, 20°C or 25°C) for less than one generation before the start of the experiments. 95% CI are given.

EGG INCUBATION TEMPERATURE	PARENTAL CULTURE TEMPERATURE 15°C	20°C	25°C
15°C	38.4±2.5 N = 6 n = 534	43.2±7.9 N=3 n=222	58.8 N=1 n=74
20°C	23.4±2.5 N=11 n=952	23.9±1.4 N=6 n=580	20.8±1.8 N=7 n=482
25°C	20.8±2.1 N=3 n=257	15.9±3.9 N=3 n=230	17.9±1.0 N=3 n=171

15°C) before the start of the egg laying period. The data from these experiments do not show as clear a pattern as do the data from the long-term temperature acclimation experiments (Fig. 2). The means of five of the six cells in the 20°C and 25°C egg incubation temperature rows are within the 95% CI of the adjacent cells. Only the means of the 20°C/20°C and the 20°C/25°C cells differed significantly. Of the nine different treatments in each of the two data sets (long and short-term) five cells have observations of the time to 50% hatch 1/2 ELP which are within the 95% CI of the corresponding observations (Tables 2 and 4). The 95% CI of the mean hatching times of eggs from 15°C acclimated parent cultures at all three egg incubation temperatures overlap in the two data sets. This is also true for the 20°C/25°C and the 25°C/20°C treatments.

In only one of the seven short-term acclimation experiments at 25°C/15°C did eggs have a hatching success of more than 50% and these eggs exposed to 25°C during their development in the ovary are adversely affected when the temperature is lowered 10°C (to 15°C). Also, eggs from 20°C short-term acclimated parents incubated at 15°C took longer to hatch than did their counterparts in the long-term acclimation study. These adults were held for four days at 20°C after being isolated from the field where the mean weekly temperature was 23°C. Lowering the parental temperature from the 23°C field temperature for four days to 20°C and subsequently incubating the eggs 5°C lower, at 15°C, produced much the same, disproportionate response as the 20°C/15°C short-term acclimation experiments. Acclimation occurs at different rates and acclimation to lower temperatures is usually slower than acclimation to higher temperatures (Prosser 1973). This

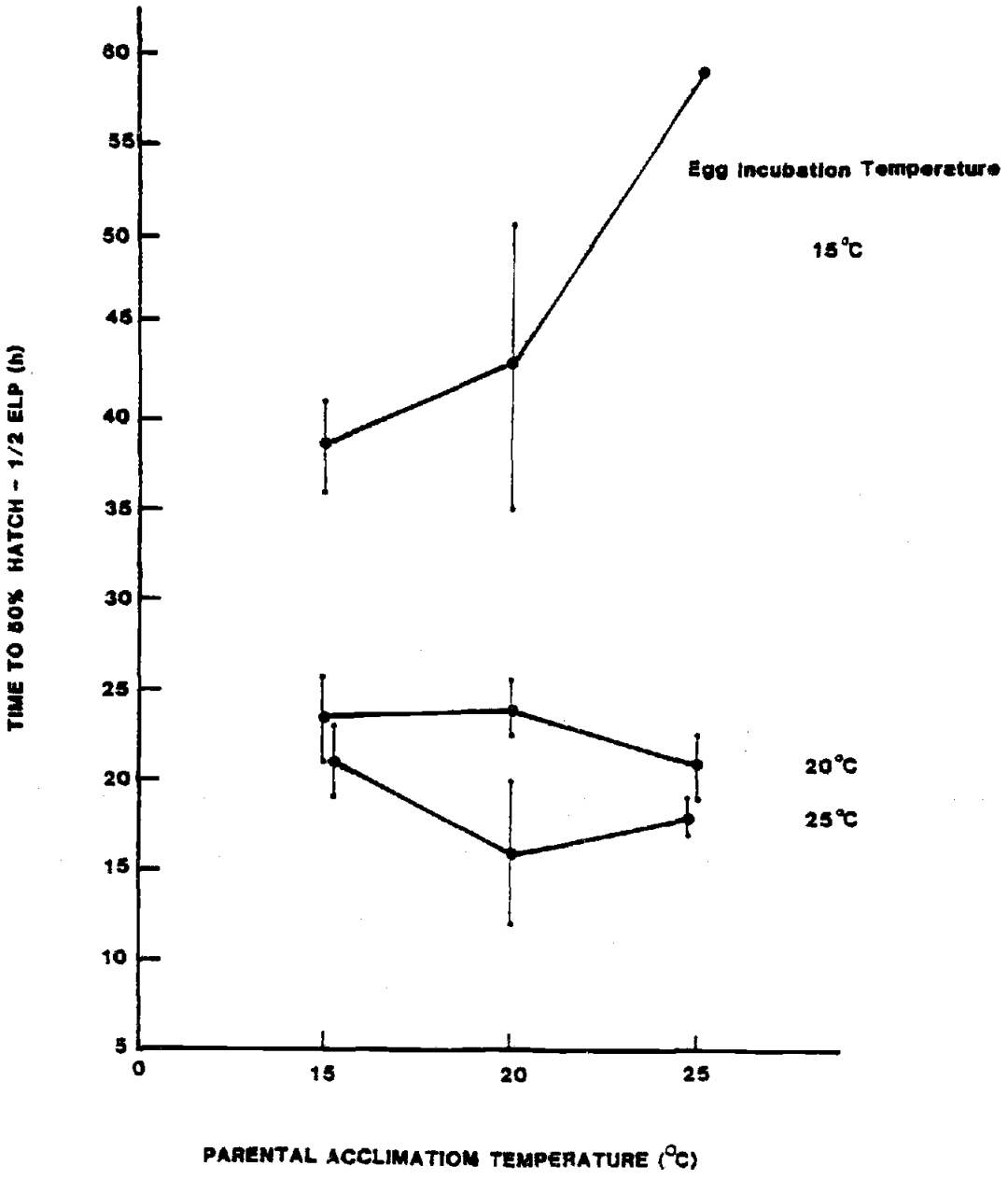

Figure 2. The effect of short-term parental acclimation temperatures and egg incubation temperatures on time to 50% hatch - 1/2 egg laying period (ELP) for the eggs of Acartia tonsa. Acclimation of parent cultures was at a constant temperature (either 15°C, 20°C or 25°C) for less than one generation before the start of the experiments. 95% CI are given.

response to lowered temperatures is essentially the same for the field isolated and the laboratory acclimated A. tonsa.

In the ANOVA using the method of weighted squares of means, both the short-term parental acclimation temperatures and egg incubation temperatures have highly significant ($p<0.001$) effects on mean egg hatching time (Table 5). Since the interaction term is also highly significant ($p<0.001$), the effects of short-term parental acclimation temperature and egg incubation temperature are not independent and are not additive.

Predicted Egg Hatching Times

Grainger (1959) suggested that changes of temperature could have two different effects on egg hatching times. First, the rate of subsequent development may be affected after temperature change. This "after effect" can be detected by keeping eggs at a constant temperature after they have been exposed to another temperature and comparing hatching time with control eggs which had been maintained all the time at this same constant temperature (Tables 2 and 4). An "after effect", the consequence of parental acclimation, is evident from these data and will be considered in the next series of experiments. Secondly, hatching time may be affected during the time that the temperature is changed. This "immediate effect" can be detected in the following manner. The amounts of development which would be expected to take place at each experimental temperature, using the results of constant temperature experiments as a basis for the calculations, are summed and the time to hatch is calculated (Grainger 1959). An "immediate effect" of temperature change is not evident in either the long-term or short-term mean hatching times (Table 6). The predicted mean egg

Table 5. Two-way analysis of variance (unbalanced data) of the time in hours to 50% hatch - 1/2 egg laying period as a function of the short-term acclimation of the parent cultures and egg incubation temperature for eggs of Acartia tonsa. Parent cultures were acclimated to a constant temperature (either 15°C, 20°C or 25°C) for less than one generation before the experiments started. The weighted squares of means method of analysis of variance was used for inferences about the main effects because the data were unbalanced.

Preliminary Analysis of Variance

Source	Degrees of freedom	Sum of squares	Mean squares	F	Significance level
Total	42	3980.61			
Main	4	3320.77	830.19	123.89	0.001 ***
Interaction	4	432.00	108.00	16.12	0.001 ***
Error	34	227.84	6.70		
	Weighted Squares of Means - Analysis of Variance				
Egg incubation temperature	2	3370.67	1685.34	251.50	0.001 ***
Parental culture temperature	2	117.64	58.82	8.78	0.001 ***
Interaction	4	194.95	48.74	7.27	0.001 ***
Error	34	227.84	6.70		

Table 6. Actual and predicted time to mean hatch (50% hatch - 1/2 ELP) for the long-term and short-term temperature acclimated parent cultures.

Experimental conditions[2]	N/n[3]	Long-term temperature acclimated parent cultures		N/n	Short-term temperature acclimated parent cultures	
		Actual time (h)	Predicted time (h)		Actual time (h)	Predicted time (h)
15°C/25°C	1/157	18.2	17.6	3/257	20.8±2.1	18.4
15°C/20°C	7/874	24.4±1.8	25.1	11/952	23.4±2.5	23.2
20°C/15°C	1/247	29.32	34.5	3/222	43.2±7.9	37.5
20°C/25°C	13/3282	16.4±2.2	14.21	3/230	15.9±3.9	13.7
25°C/15°C	4/453	No hatch	-	1/74	58.7	33.9
25°C/20°C	6/1157	18.8±1.7	17.75	7/482	20.8±1.8	18.4

[2]Experimental conditions are parental acclimation temperature/egg incubation temperature

[3]N= number of experiments n= number of eggs

hatching times and the actual egg hatching times are in close agreement except for the 25°C/15°C case.

Effect of Temperature Change on Egg Hatching Time

Further experiments investigated the lability of the egg to temperature influence during ovarian development. The time required for a 5°C to 10°C temperature change of an acclimated parental culture to alter the hatching time of the egg was determined. The time required for hatching of eggs from cultures under new temperature conditions to equal the hatching times of eggs from long-term acclimated parents was determined by experiments in which the temperatures of parental cultures reared for at least one generation in the laboratory at either 15°C, 20°C or 25°C were changed by 5°C or 10°C to new maintenance temperatures. Eggs were taken periodically from these cultures and incubated at the new temperature. All combinations of acclimation temperatures and temperature changes were attempted. These data are observations from different experiments but each combination has at least one experiment where eggs were sampled in triplicate from the same parent culture at time = t_x, t_{x+1}, t_{x+2}, t_{x+3}, etc. sequentially through the experiment.

15°C → 25°C and 20°C → 25°C. The temperature of parental cultures held for at least one generation at 15°C and at 20°C was raised to 25°C and eggs from these cultures were incubated at 25°C (Fig. 3). The mean hatching times of eggs taken only 22 and 24 hours after the temperature was raised to 25°C were within the 95% CI of the mean hatching times of eggs from parental cultures acclimated at 25°C for long periods.

15°C → 20°C and 25°C → 20°C. Eggs from parental cultures acclimated for at least one generation at 15°C and 25°C before being changed

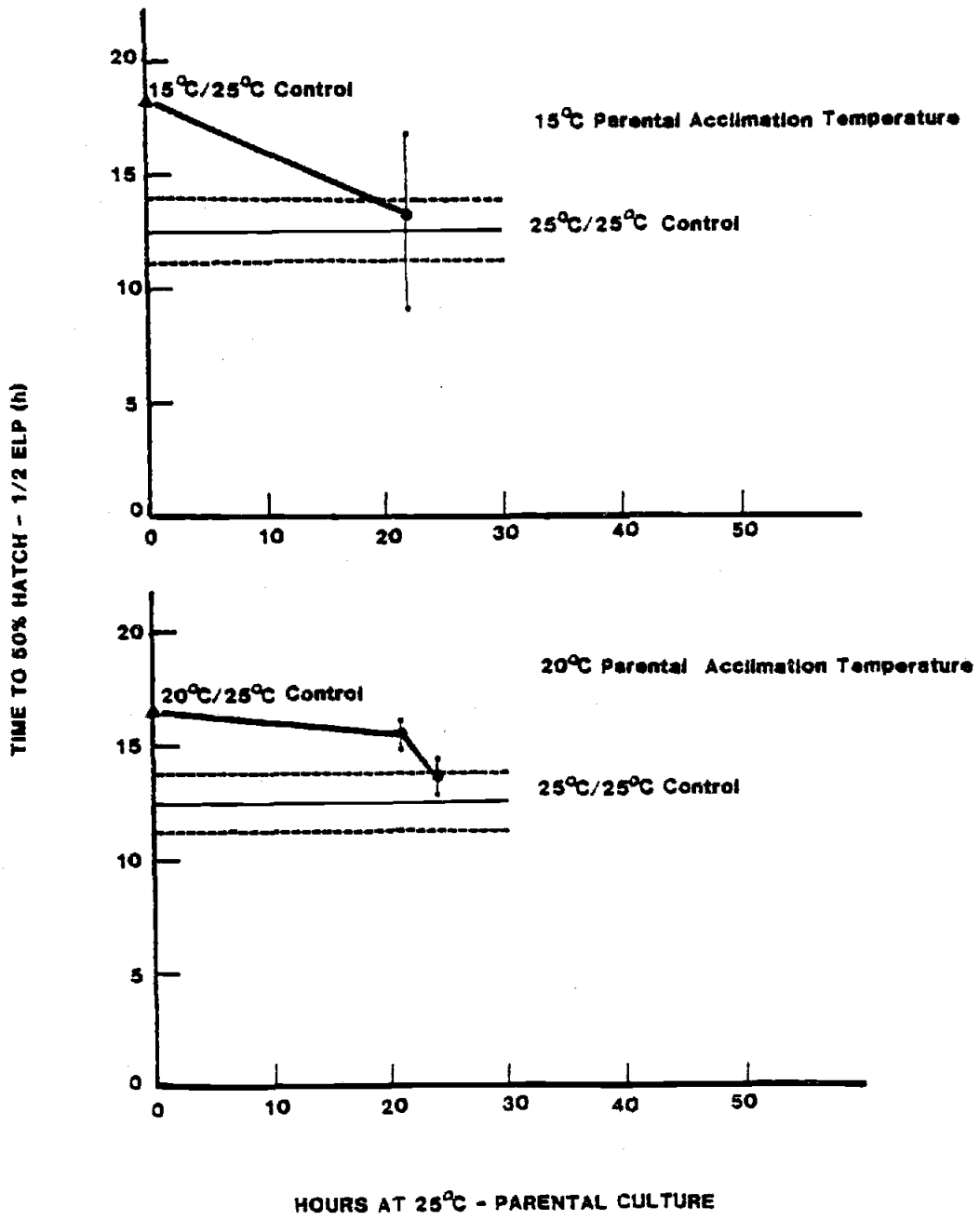

Figure 3. Effect of exposure to 25°C on time to hatch of Acartia tonsa eggs from parental cultures acclimated for at least one generation to 15°C or 20°C. Eggs were incubated at 25°C. Control is the time to 50% hatch - 1/2 egg laying period from the 25°C/25°C long-term acclimated parental cultures (12.5±1.3 h). 95% CI indicated by broken lines.

to 20°C were taken 21 to 86 hours after the 5°C temperature change (Fig. 4) and incubated at 20°C. The mean hatching times of eggs from the 15°C acclimated parental culture was 23.2 hours after the culture had been at 20°C for 86 hours. After 86 hours at 20°C the eggs from the 25°C acclimated parental culture had mean hatching times of 22.4±1.7 hours. These hatching times were within the 95% CI of the mean hatching time of the controls.

20°C → 15°C. The temperature of parental cultures held for at least one generation at 20°C was lowered 5°C and eggs laid by this culture thereafter were incubated at 15°C (Fig. 5). The mean hatching time of these eggs laid after 22 hours at the new temperature was 41.8±3.6 hours and did not differ from the 15°C/15°C long-term acclimated experiments (40.1±3.9 hours). In experiments where 25°C cultures were changed to 15°C a few eggs were laid but did not hatch.

To summarize these results (Fig. 6), exposure of A. tonsa (reared at 25°C) to lower temperatures resulted in eggs which did not hatch at all for the change to 15°C, or an acclimation response of 86 hours for the change to 20°C. The cultures reared at 20°C for at least one generation appeared to acclimate quickly when the temperature was changed 5°C higher or lower. Within 24 to 48 hours of the temperature change the mean egg hatching times were equal to the mean hatching times of the long-term temperature acclimated cultures. For the culture acclimated to 15°C, a temperature rise to 25°C resulted in the acclimation of hatching times within only 22 hours. But, the rise from 15°C to 20°C required 86 hours for mean hatching time to acclimate. The effects of lowering the temperature of cultures exposed to 25°C has already been observed in the long-term and short-term acclimation

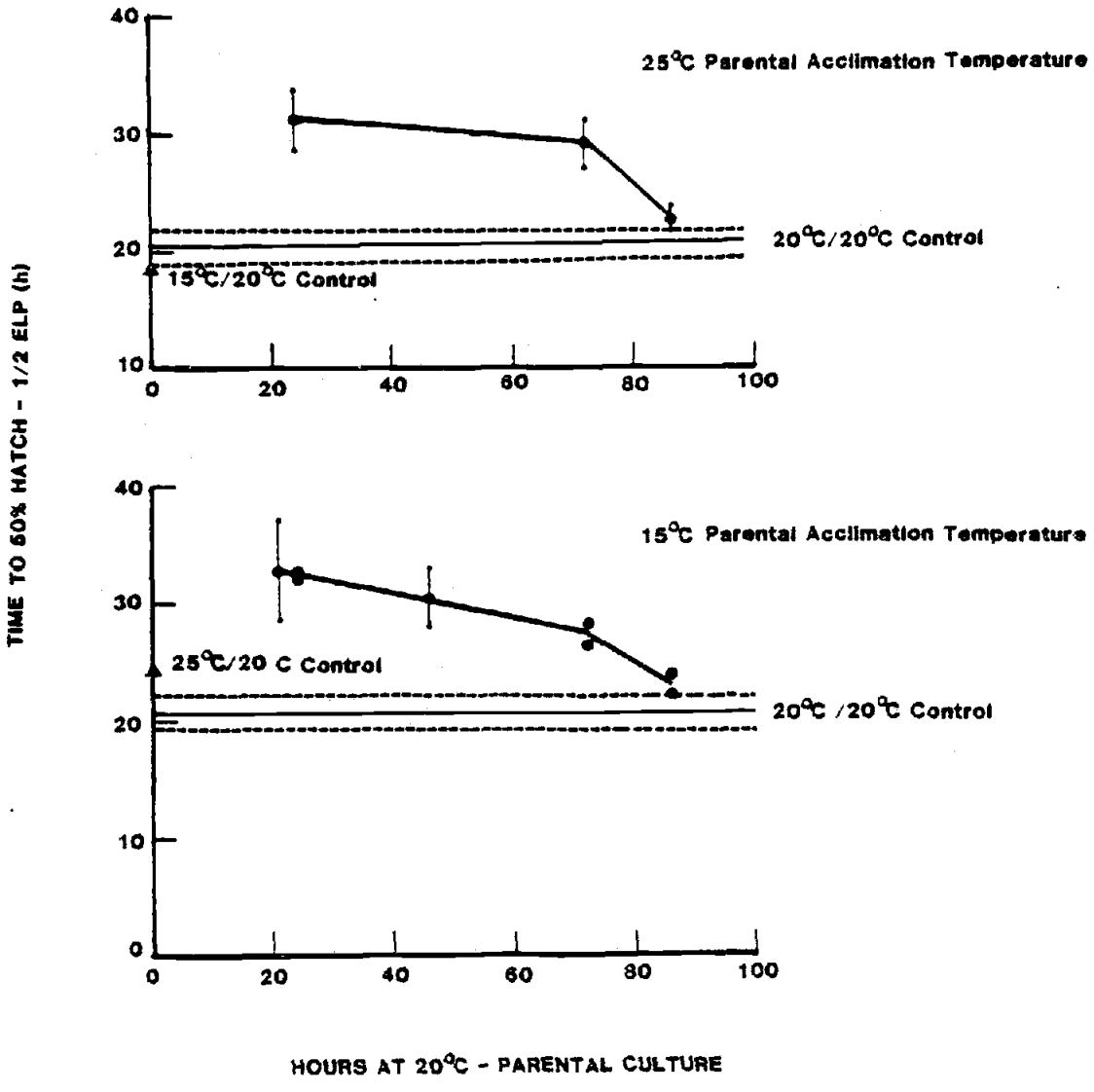

Figure 4. Effect of exposure to 20°C on time to hatch of Acartia tonsa eggs from parental cultures acclimated for at least one generation to 15°C or 25°C. Eggs were incubated at 20°C. Control is the time to 50% hatch - 1/2 egg laying period from the 20°C/20°C long-term acclimated parental cultures (20.8±1.1 h). 95% CI indicated by broken lines.

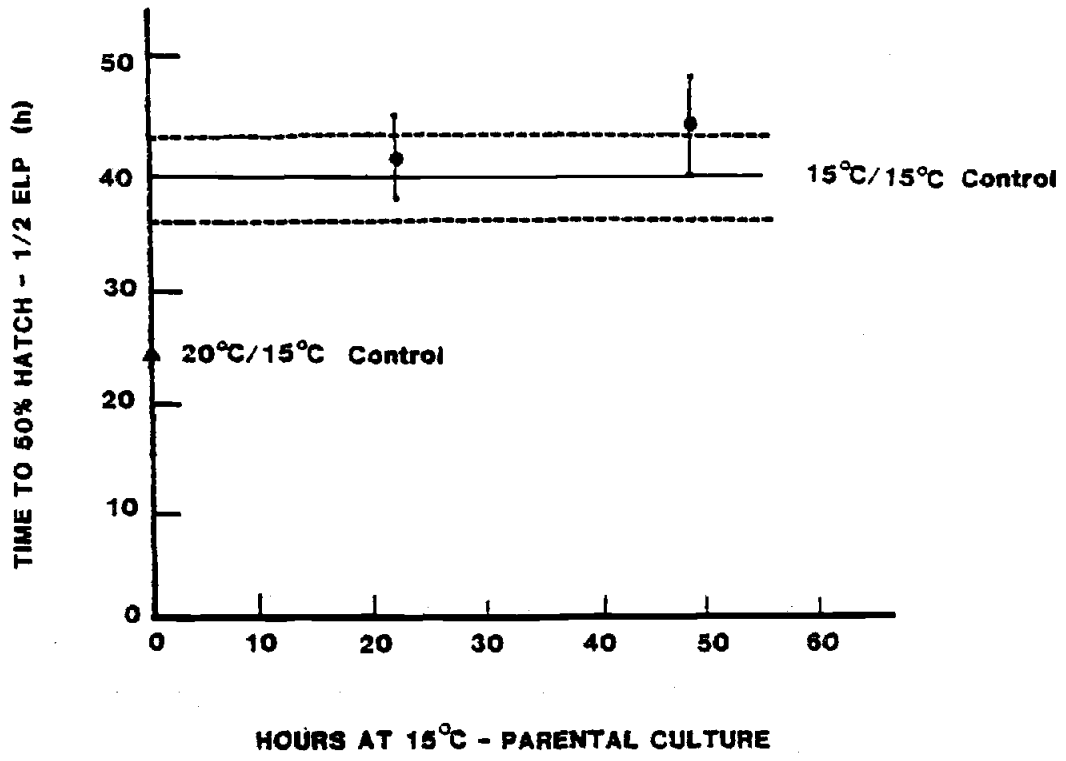

Figure 5. Effect of exposure to 15°C on time to hatch of Acartia tonsa eggs from parental cultures acclimated for at least one generation to 20°C. Eggs were incubated at 15°C. Control is the time to 50% hatch - 1/2 egg laying period from the 15°C/15°C long-term acclimated parental cultures (40.1±3.9 h). 95% CI indicated by broken lines.

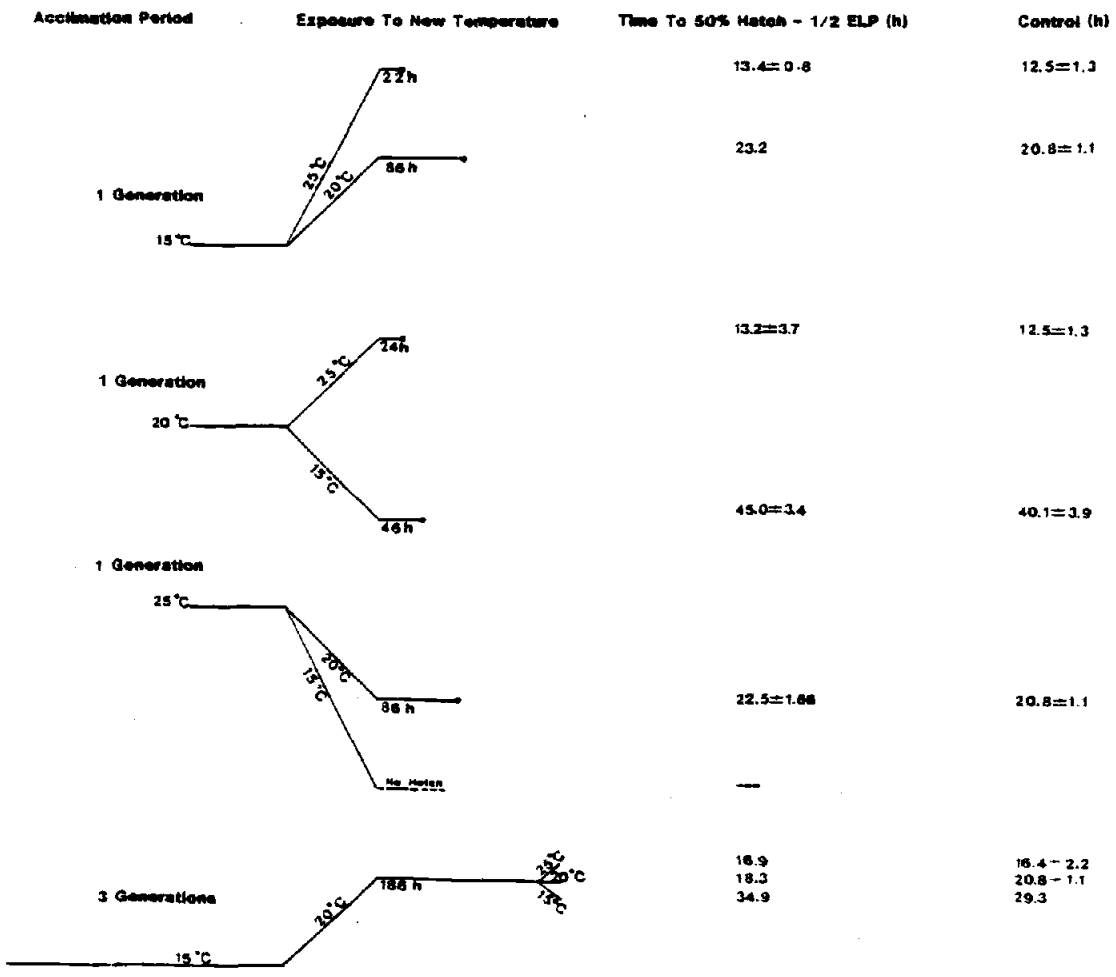

Figure 6. Time in hours for hatching times of Acartia tonsa from parental cultures which experienced a temperature change to equal the hatching rates of fully acclimated cultures. This figure combines the data from Figures 3, 4, 5 and 7.

experiments, but the slow acclimation of the 15°C acclimated culture to 20°C was unexpected.

One set of data was available from parental cultures held for three generations at 15°C before the culture temperature was raised to 20°C (Fig. 6). After eight days at 20°C the hatching time of eggs taken from this culture and incubated at 20°C (Fig. 7) equaled those of the 20°C/20°C long-term experiments. Some of the eggs from this culture were incubated at 15°C and at 25°C after the culture had been at 20°C for 8 days. The time to hatch for the eggs incubated at 15°C was 34.9 hours, and is close to the hatching time of 29.3 hours for the 20°C/25°C long-term temperature acclimated experiment (Fig. 6). Eggs incubated at 25°C hatched in 16.9 hours, close to the time of the control. Eggs from the 20°C/25°C long-term temperature acclimated experiments had a mean hatching time of 16.2±2.2 hours. The eggs demonstrate continued lability with respect to temperature changes. These eggs had essentially the same response or hatching time as eggs from a 20°C long-term acclimated culture and demonstrate that even three generations of constant temperature acclimation at 15°C has not affected the ability for eggs of parent cultures to acclimate to new temperatures and continue to exhibit lability to temperature.

The Effect of Mean Weekly Water Temperatures on Egg Hatching Time

To test the effect of field water temperature change on time to hatch in *A. tonsa* eggs, plankton was collected eight times from 7 April to 25 July, 1979. Adult individuals of *A. tonsa* from this plankton were held at ambient temperature and the egg laying period started immediately. For these samples, time to hatch for eggs incubated at

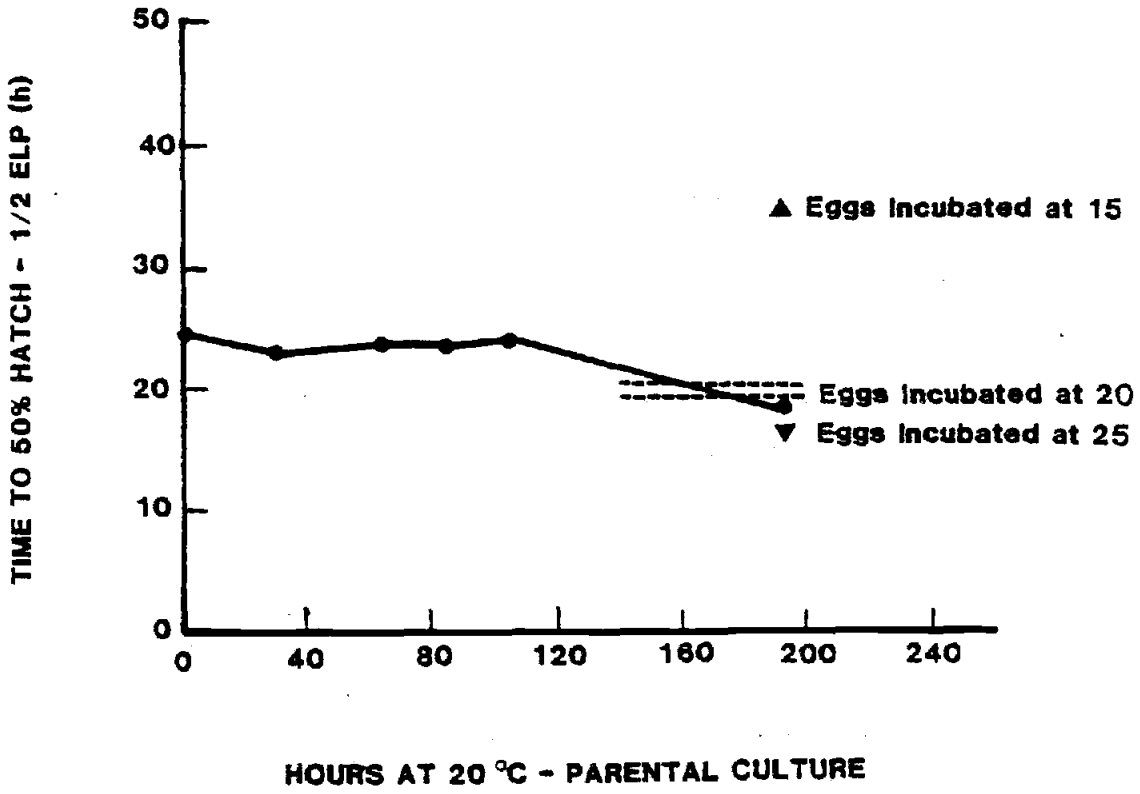

Figure 7. The effect of exposure to 20°C on the time to hatching of Acartia tonsa eggs from parental cultures acclimated for over three generations to 15°C. Eggs were incubated at 20°C except where noted on the final observation. Broken lines indicate the 95% CI around the time to 50% hatch - 1/2 egg laying period for eggs from long-term temperature acclimated parent cultures at 20°C and incubated at 20°C (20.8±1.1 h).

20°C ranged from 34.2 to 14.6 hours. Time to 50% hatch - 1/2 ELP = -1.84 (°C) +64.34, with r^2 = 0.87. The time to mean hatch predicted by this equation is within the 95% CI of the means of 15°C/15°C and the 20°C/20°C long-term acclimation experiments (Fig. 8).

The effect of ELP on time to hatch and the importance of a uniform and short ELP is demonstrated in Table 7. In preliminary long-term experiments with parental cultures at 15°C and incubation temperature at 20°C, the ELP varied widely. This prolonged ELP and consequent exposure to the 15°C parental culture temperature for a longer period caused the slower hatching times for those eggs with a longer ELP. This experiment demonstrates that eggs are temperature labile virtually up to the time of hatching. The eggs produced at 15°C with a 27 hour ELP hatched immediately after being exposed to 20°C. At 15°C the relationship between time to mean hatch and ELP is:

Time to 50% hatch - 1/2 ELP = -0.25 ELP (at 15°C) + 20.64 r^2=0.78.

Egg Development Time

At this point it became important to know the period required for *A. tonsa* eggs to develop in the ovary and therefore the time they might be influenced by the temperature of the female parent. The uptake of ^{14}C from labeled food was rapid (Fig. 9); egg samples taken 3.5 hours after the feeding of labeled algae showed activity above the baseline. After 24 hours the activity level of the eggs was relatively constant in both the eggs produced by adults fed labeled algae and the first eggs laid by the copepods fed labeled algae since the C_I stage. The loss of the activity of the eggs was also rapid; within 48 hours after females had been given only unlabeled food, the activity level had dropped from an average of 410 cpm egg^{-1} to less than 100 cpm egg^{-1}.

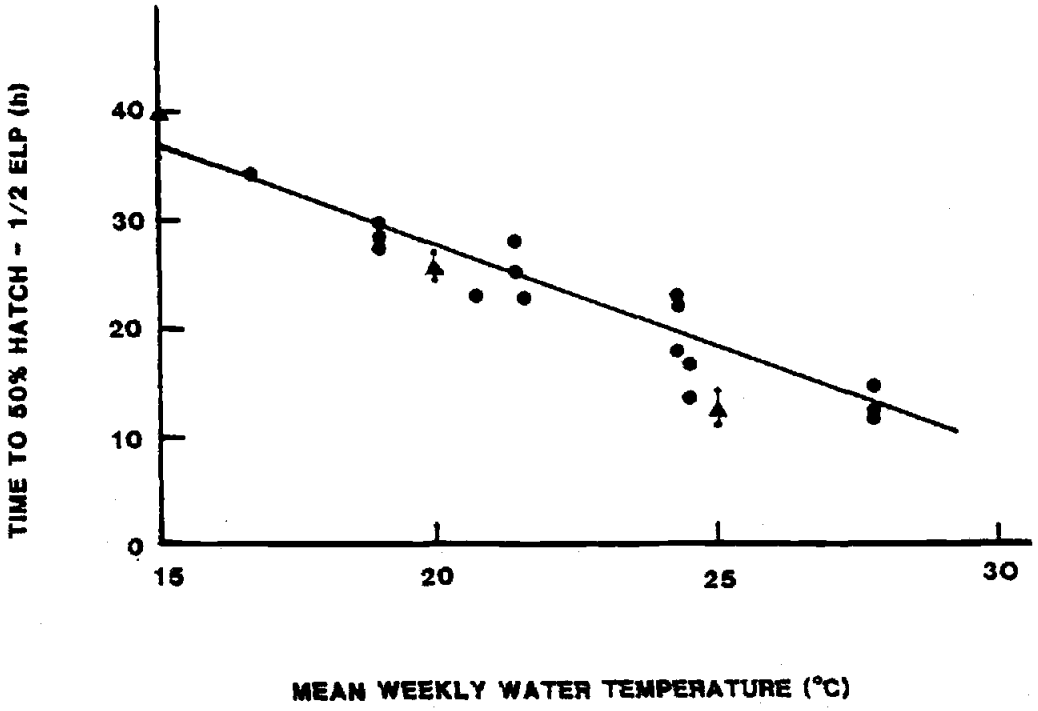

Figure 8. Time to 50% hatch - 1/2 ELP for Acartia tonsa eggs from field isolated parents. Experiments started immediately after adults were taken from the field. All egg incubation was at 20°C. Triangles represent data from the long-term acclimated experiments which served as the control for this experiment.

Table 7. The effect of different egg laying periods on time to hatch in *Acartia tonsa* eggs from 15°C long-term acclimated parental cultures. Incubation temperature was 20°C.

Long-term acclimation temperature / Egg incubation temperature	Egg laying period (h)	Time to 50% hatch - 1/2 ELP (h)
15°C/20°C	8	21.4
15°C/20°C	13	24.9
15°C/20°C	13	24.4
15°C/20°C	17	26.3
15°C/20°C	27	27.1

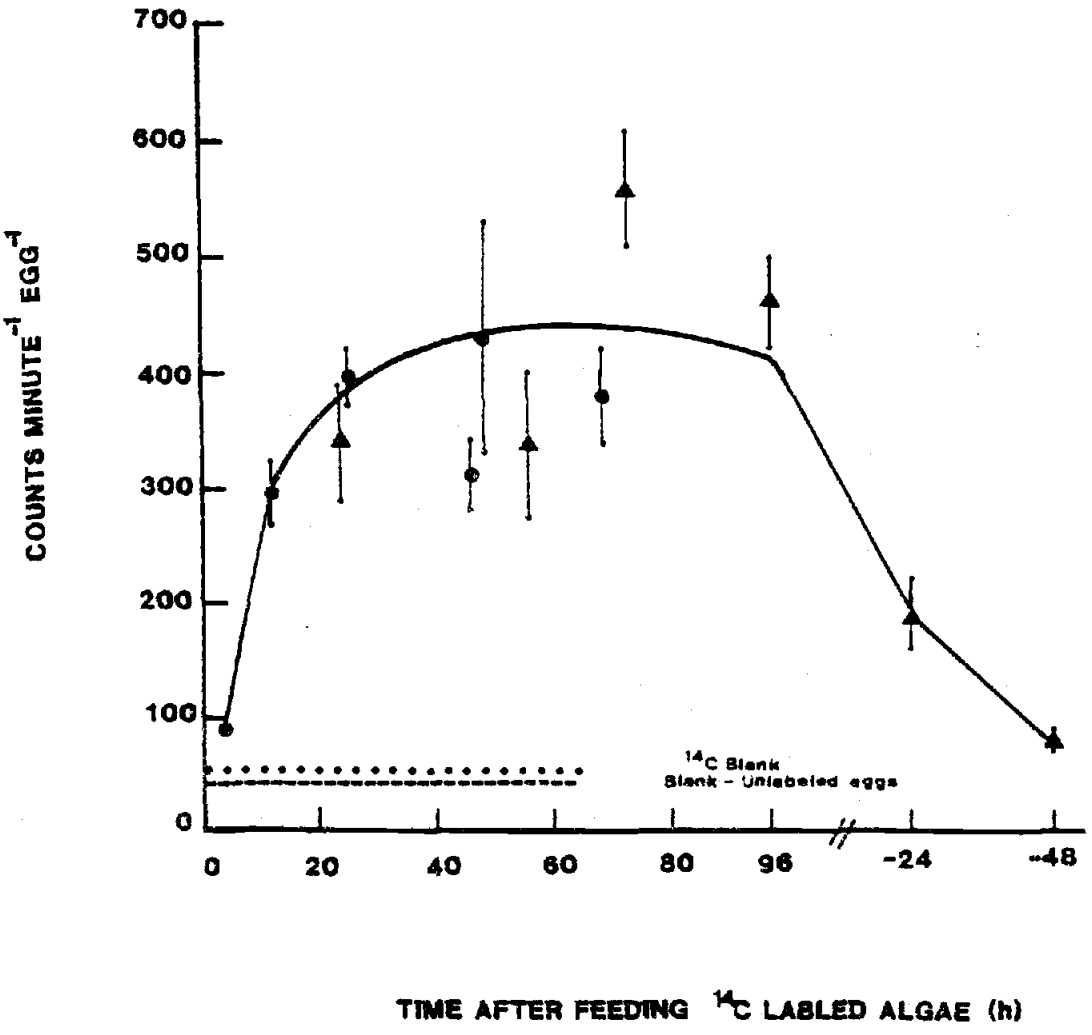

Figure 9. Activity of eggs from Acartia tonsa after being fed ^{14}C labeled algae. Adults were exposed to the labeled algae for 96 hours after which unlabeled algae was substituted for the labeled algae and the activity of the eggs was monitored for another 48 hours. Standard deviations are shown. Triangles represent data from Acartia tonsa fed on labeled algae since stage C_I. Closed circles represent data from adults fed labeled algae.

IV. DISCUSSION

The element of time plays a critical role in determining the magnitude of stress imposed by temperature change and the acclimation responses of an organism. The eggs of A. tonsa are temperature labile both during oogenesis and after being laid. The "after effect" of Grainger (1959) is evident in the mean hatching times for eggs for both the long and short-term temperature acclimation experiments. For eggs produced between 15°C and 25°C the long-term parental acclimation temperature and egg incubation temperature have significant effects on hatching time, and these effects are additive.

Hatching times of eggs from short-term temperature acclimated parents are affected significantly by both parental culture temperatures and egg incubation temperatures. The interaction term in the ANOVA for the short-term temperature acclimation data is significant but does not, of course, take into account the thermal history of the parents prior to the start of the experiments. Copepods used in these experiments were collected when field water temperatures varied from about 7°C to 28.3°C and were held in the laboratory at constant temperatures for only a short period before the experiments started. No allowance was made for field water temperature, rate of change to the experimental temperature or age of parental female. Despite this, in five of nine temperature combinations the mean egg hatching times in the short-term acclimation experiments were within the 95% CI of the mean egg hatching times of the long-term acclimation experiments. The unexpectedly long hatching time of the 25°C/15°C experiment also contributed to the interaction term in the ANOVA of the short-term acclimation experiments. The only hatching time in either of the long

or short-term data to differ significantly from the predicted hatching times was for the 25°C/15°C experiments. No observations were made at this temperature combination in the long-term data and the three remaining cells in the long and short-term data sets which differ may do so for reasons not accounted for in this experimental design. From these data the egg hatching responses in both the short and long-term temperature acclimation experiments are nearly identical.

Lowering the temperature by 10°C from 25°C to 15°C had a greater effect on mean time to hatch than did raising the temperature 10°C from 15°C to 25°C. Munro (1974) and others have found that the duration of all stages of copepod development decreases with increasing temperature but that the extent of this effect diminished as the temperature increased.

The "enhancement effect" described by Landry (1975a) due to cold acclimation of A. clausi females is not evident in these experiments with A. tonsa. Landry stated that A. clausi eggs from cold acclimated parents incubated at an elevated temperature, not only hatched faster than eggs incubated at the parental acclimation temperature but also hatched faster than eggs from females acclimated to the elevated temperature, when incubated at that temperature. In the experiments with A. tonsa the eggs from 15°C acclimated parents, when incubated at 20°C, did not hatch faster (24.4±1.8 hours) than the eggs from 20°C acclimated parents (20.8±1.1 hours), when incubated at 20°C. Enhancement of hatching times by cold exposure of adults was also not found by Johnson (1967) for Tortanus discaudatus, Hart and McLaren (1978) for Pseudocalanus sp., nor Uye (1980) for A. clausi.

The copepods in Landry's experiments were held for "at least one

day under experimental conditions before the egg samples were taken" (Landry 1975a). He did not consider that this temperature experience could affect egg hatching times or the possibility that females could acclimate to experimental temperatures within a day or two. Consequently, his observations may have resulted from the short-term acclimation of the parents. That even 24 hours of acclimation may change rate-temperature functions was not widely appreciated in copepod development studies until Munro's (1974) work. In contrast with previously published work on copepod development, the ovigerous females used by Munro were taken from a water storage reservoir at or close to the experimental temperature.

The "immediate effect" of Grainger (1959) is not evident in the long- or short-term temperature acclimation data. The predicted and actual mean egg hatching times for both these data sets are not significantly different (except the 25°C/15°C experiment). This means that there is no effect of the temperature change itself on mean hatching time. The series of experiments designed to test the effect of changing the temperature of the parent cultures on time for eggs to hatch is therefore a valid one.

The time required for mean egg hatching times to adjust to an increase in parental culture temperature is faster than the time required for the same temperature decrease, but both are accomplished within 22 to 86 hours. In a similar experiment, Sweeney and Schnack (1977) found that egg development in the aquatic insect, Segara allernata, adjusted very quickly to diel changes in temperature. Eggs from the long-term (three generations) acclimated at 15°C parent culture took 186 hours to acclimate to 20°C. But eggs from this reacclimated

culture (20°C) were incubated at 15°C, 20°C and 25°C with hatching times not unlike those from the long-term acclimation experiments which serve as controls for this experiment. Nevertheless, the acclimation time for cultures held for three generations at 15°C was certainly less than one generation and less than the reproductive lifetime of an adult female A. tonsa. Also, the prediction of mean egg hatching times by the mean weekly field temperature (r^2=0.87) indicated that periods of days rather than generations are effective in changing the egg hatching times in field populations.

The ^{14}C experiments and other data were intended to determine the time required for an oocyte to develop into an egg. By using these data and other lines of evidence, correspondence between egg development time and the time required for the egg hatching times to adjust to a temperature change of the parent culture presumably could be established. Hilton (1931) recorded the most rapid period of yolk formation in Calanus finmarchicus was at the stage of half-grown oocytes. Eggs from the females fed labeled algae from C_I stage through C_{VI} stage and eggs from females fed labeled algae for only 24 hours at 20°C appeared uniformly labeled. Either A. tonsa eggs are produced rapidly at 20°C, or the exchange between the carbon pool in the rest of the body and the ovary is rapid, or both. Copping and Lorenzen (1980) found the specific activity of C. pacificus equaled that of the ^{14}C labeled phytoplankton 48 hours after first exposure to it. Their experiments were conducted at 15°C. In Diaptomus leptopus eggs are produced in clutches, and when the gametic cycle was determined at 18°C, the gravid cycle (dark oviducts) was 3.4 days and a non-gravid phase (clear oviducts) was 0.9 days (Watras and Haney 1980). Estimates of the daily production of A. tonsa

eggs vary. Parrish and Wilson (1978) found 25.8 eggs female^{-1} day^{-1} at 18°C. Corkett and Zilloiux (1975) recorded 15.6 eggs female^{-1} day^{-1} at 10.5°C and 18.4 eggs female^{-1} day^{-1} at 20.2°C. At 20°C, 28 eggs female^{-1} day^{-1} were reported by Tester and Costlow (1981). The quick response of A. tonsa egg production to food conditions led Parrish (personal communication, letter dated 24 January 1979) to conclude that production of eggs was continuous under conditions of sufficient food and eggs were laid as they were formed. Dagg's (1977) work with A. tonsa suggests that this species can withstand only short periods without food and its metabolic behavior will closely track any changes in the food environment. Parrish and Wilson (1978) found that A. tonsa had an abrupt reduction in the numbers of eggs laid on day one without food. The production was only 50% of the mean production the preceding day. On the second day without food the production dropped to 10% and on the third day production was about 5% of that preceding starvation. When feeding resumed the egg production assumed pre-starvation levels by the third or fourth day. In a similar study using A. clausi, Uye (1980) found egg laying to respond in the same manner as in A. tonsa with respect to food availability.

Predictions of A. tonsa egg hatching rates or population parameters based on birth rates should be calculated carefully from empirically derived data for the local population in question. Otherwise, local acclimatization of the population and seasonal acclimation to temperature could affect the results. That regional climatic and geographic factors operate to cause and maintain differences in temperature responses for the same species is certainly a possibility. Johnson (1980) concluded tidal flushing removed a maximum of 3.4 to 8.7%

of the A. californiensis population from upper Yaquina Bay, Oregon. His model assumed passive behavior of A. californiensis. Show's (1979) model of A. tonsa in a tidal lagoon predicts that losses from the lagoon would be minimized to the extent that a local population is maintained inside the lagoon which is distinct from the population found immediately outside.

Moreira, Jillett, Vernberg and Weinrich (1982) have found "striking" differences between New Zealand and Brazilian populations of Euterpina acutifrons and they imply the existance of different physiological races in this species. Cooley and Minns (1978) analyzed egg hatching data for several freshwater copepods and found eggs of the same species in different environments will likely have different responses to temperature. Zillioux and González (1972) found less evidence for dormancy in A. tonsa eggs from Biscayne Bay than eggs from Narragansett Bay, and Uye and Fleminger (1976) suggested that eggs of A. tonsa in Southern California had a weak ability for dormancy.

Levins (1969) suggests that the greater the individual flexibility, the smaller the genetic differences required to meet a given degree of geographic and temporal long-term variation in the environment. Slobodkin (1968) and Slobodkin and Rapoport (1974) maintain that behavioral and physiological mechanisms preclude changes in gene frequency unless the disturbance is too great to be dealt with behaviorally or physiologically. It is axiomatic that a species is adapted to its environment but in the case of a highly successful species such as A. tonsa it is not only adapted to its environment but also highly physiologically adaptable as well. This species is a dominant in many latitudes and in variable habitats. It is known to be euryhaline,

eurythermal and, from this work, A. tonsa has been shown to be temperature labile in its reproduction as well. Egg hatching is influenced by the temperature of the parent cultures as well as by the temperature of incubation. A. tonsa can therefore take advantage of the immediate temperature fluctuations in its environment. This species is capable of adjusting its reproductive output and rate with temperature; that is, the lability to temperature of the eggs allows the species to benefit from any advantage of higher temperatures, either during oogenesis or during egg incubation.

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