Development of Neritic Copepods *Acartia clausi* and *A. steueri*

I. Some Environmental Factors Affecting Egg Development and the Nature of Resting Eggs[1],[2]

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Abstract

Effects of temperature, salinity-temperature combinations, illumination and the presence of bottom mud on the egg development of neritic copepods *Acartia clausi* and *A. steueri* were investigated. Since eggs of both species obtained from various months behaved similarly in haching at various temperatures and in survival during storage within the mud, the eggs of these copepods have the same physiological properties throughout the year (i.e. subitaneous eggs). Resting eggs accumulated within the natural sea-bottom mud are also subitaneous ones, which are merely inhibited from hatching by unfavorable environmental conditions (e.g. low temperature, low oxygen concentration, darkness). The time-release effect of hatching from resting eggs may act to enhance the maintenance of endemic copepod populations in highly variable environments.

Eggs of most species of the calanoid copepods are laid freely into the water. Generally, these eggs are slightly denser than the seawater and sink slowly through water column till hatching. During the sinking, their development is under control of the environment of the water column. In shallow waters, however, since the eggs are most likely to sink to the sea-bottom before hatching, their development is largely to be influenced by the bottom condition. Many studies (MCLAREN 1966, MCLAREN et al. 1969, CORKETT 1972, KASAHARA et al. 1975a, LANDRY 1975a, b, UYE & FLEMINGER 1976, UYE et al. 1979) have demonstrated that the development of copepod eggs is affected by a variety of environmental factors.

In the present study, the effects of temperature, salinity and temperature combinations, illumination and the presence of mud on the development of newly spawned *A. clausi* and *A. steueri* eggs were investigated at first. Then, the effects of temperature upon hatching of resting eggs of these two species isolated from the natural bottom sediments were also investigated. By these experiments, temperature dependent egg hatching could be compared between eggs recently spawned and those isolated from the mud.

Materials and Methods

Zooplankton was collected monthly from July, 1976 to February, 1977 by vertical or oblique tows with a 0.5 m net (0.32 mm mesh aperture) at the innermost area of Onagawa Bay,

northeast mainland of Japan. Live specimens contained in a thermoflask were brought within 2 h of transport to the laboratory in the Faculty of Agriculture, Tohoku University, Sendai. Females of *A. clausi* and *A. steueri* were isolated from the samples immediately after arrival to the laboratory. About 100-200 females were cultured in Pyrex beakers containing approximately 900 ml of Millipore (HA type: 0.45 μm) filtered seawater mixed with *Dunaliella tertiolecta* and *Thalassiosira decipiens* (ca. 1×10^6 cells $\cdot$ ml^{-1}) at temperatures equivalent to those at 5 m depth of the sampling station. Eggs produced by these females within first 4 h were used except for the experiments to study the effect of mud on egg hatching. In the latter experiments, the eggs were 12-24 h old. In addition to these newly spawned eggs in the laboratory, eggs of both species existing in dormant state within the bottom mud collected by a Ekman-Berge grab sampler were also isolated and used for the experiments. Methods used in the following experiments are described below.

Temperature: Each 20 of newly spawned and resting eggs of each species was introduced into a Pyrex test tube (1.8 cm diameter, 18 cm deep) containing approximately 15 ml of filtered seawater. Three replicates were prepared for each of 9 different temperatures ranging between 2.5 and 29.6°C (±0.2°C). Hatching to nauplii was checked at 2-6 h intervals for the first 3 days and 8-24 h intervals thereafter. The eggs were transferred into freshly filtered seawater at intervals of 2-5 days.

Salinity and Temperature Combinations: Salinity of the natural inshore Onagawa Bay water was concentrated to approximately double its original level (ca. 32‰) by mild evaporation and then the water was Millipore filtered. A series of various salinities was prepared by dilution of either the concentrated or the natural seawater with distilled water. Twenty eggs of *A. clausi* were introduced into each test tube containing ca. 20 ml of these water, shielded on its top with Parafilm. Three series of experiments were run, i.e. at 8.3, 14.5 and 22.2°C for 10, 7 and 7 days, respectively. *A. steueri* eggs were incubated at 14.5 and 24.3°C for 7 days. Hatching was monitored at 4-12 h intervals.

Illumination: Test tubes containing filtered seawater and 20 eggs were shielded by wrapping with 2 layers of aluminum foil. They were incubated at 18.0°C with 2 unshielded controls under the continuous light of ca. 500 lx.

Bottom Mud: Fifty newly spawned eggs were introduced into small test tubes (1.5 cm diameter, 4 cm deep) filled with filtered seawater and left until settling on the bottom. After this, silty bottom mud, that had been taken at the sampling station on 9 June, 1976 and preserved at −10°C, was gently added to cover the eggs with a layer of 5-7 mm thick. They were stored at 5 and 20°C. Periodically those eggs appearing to be viable in these vessels were transferred to freshly filtered seawater and incubated at 18.0°C for 7 days to check their survival.

Results

Effect of Temperature

The hatching success and time required for the hatching of 50 % of the viable eggs at various

temperatures are shown in Fig. 1a for *A. clausi* and Fig. 1b for *A. steueri*. The temperature range in which eggs could hatch was between 2.5 and 27.7°C for *A. clausi* and 7.2 and 29.6°C for *A. steueri*, respectively. Hatching success of both species was generally higher than 80 %
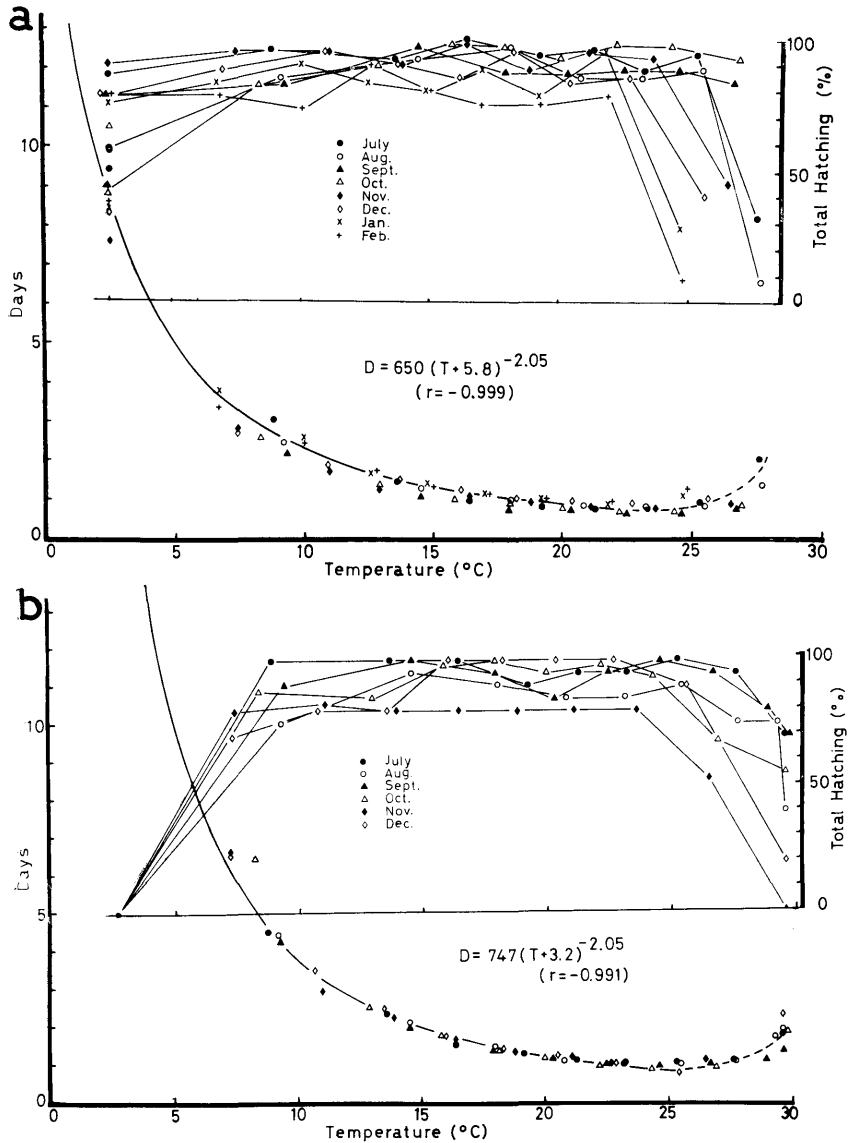

Fig. 1a, b. Effect of temperature on hatching success and development time of eggs of
(a) *Acartia clausi* spawned in each month between July, 1976 and February, 1977,
and (b) *A. steueri* spawned between July and December, 1976.

within the optimal temperature range. Some effects of seasonal temperature acclimatization was noted. Hatching percentage at the lowest temperature of *A. clausi* eggs which were spawned in warmer months (e.g. August and October) was lower than those spawned in

colder months (e.g. November to February), and vice versa. Similarly, eggs of *A. steueri* spawned in warm months hatched at higher success at the highest incubation temperature of 29.6°C.

The development time to hatching was dependent on temperature. The development time was shortened with increasing temperature, then it was slightly prolonged at temperatures above 22.7°C for *A. clausi* and above 24.6°C for *A. steueri*. Unlikely to hatching success, in hatching time, there was no apparent difference between warmer and colder months. The
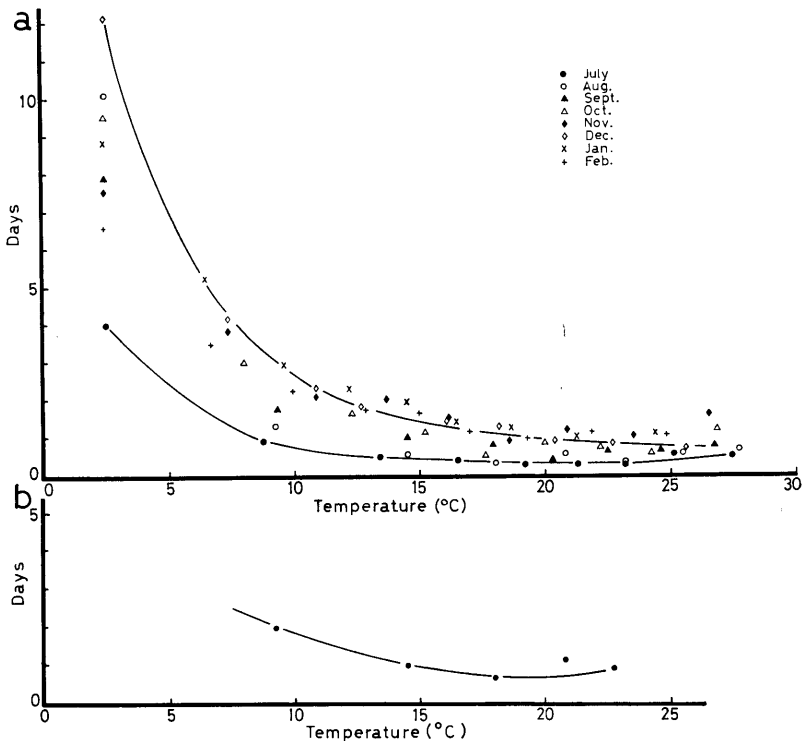

Fig. 2a, b. Effect of temperature on development time of eggs which were isolated from the natural sea-bottom mud for (a) *Acartia clausi*, and (b) *A. steueri*. Freehand curves are simply drawn for taking plots in July and December, 1976 for *A. clausi* and in August, 1976 for *A. steueri*.

Bĕlehrádek equation, which is commonly used to describe the relationship between the development time and the temperature, is applied to the present species. This equation has the form:

$$D = a(T - \alpha)^b$$

where D is the development time in days to hatching, T is temperature in Celsius, and a, b, and α are fitted constants. MCLAREN et al. (1969) demonstrated b was approximately -2.05 for many species of copepod, a was related to egg diameter, and α or so-called "biological zero" could be an index of temperature adaptation of the given species. A total of 55 sets of data for *A. clausi* and 37 sets for *A. steueri*, which were obtained within natural tempera-

ture range are available here. These data yielded the equation $D=650(T+5.8)^{-2.05}$ for *A. clausi* and $D=747(T+3.2)^{-2.05}$ for *A. steueri*. The curves obtained fit the data very well; the correlation coefficients of the logarithmic regression exceed 0.99 for both species. The value of "biological zero" of *A. steueri* $(-3.2°C)$ is higher than that of *A. clausi* $(-5.8°C)$ by 2.6°C, indicating the former is adapted to warmer environment than the latter.

Effect of temperature on the hatching of eggs isolated from the bottom muds was also investigated. The relationship between the time for 50% hatching of the viable eggs and the temperature is shown in Fig. 2 for *A. clausi* eggs recovered from the muds sampled monthly between July, 1976 and February, 1977, and *A. steueri* eggs in August, 1976. Since the eggs hatched sporadically over several days at any given temperature, it seems to be meaningless to derive a fitted equation from the data. Nevertheless, it may be obviously revealed that most eggs sorted from the muds can hatch in approximately the same time as eggs newly produced in the laboratory.

Eggs which failed to hatch during 7 days at higher temperatures above 25°C were transferred to 18.0°C to check their viability. About half could develop into nauplii in *A. clausi* during an additional 7 days, while very few eggs of *A. steueri* could hatch.
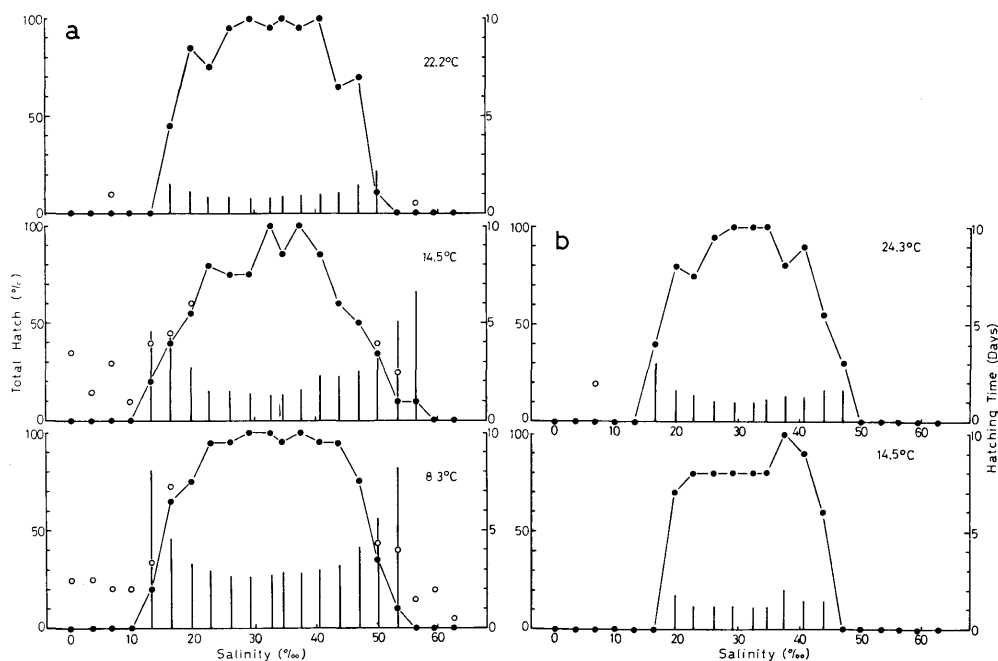

Fig. 3a, b. Effect of salinity-temperature combinations on hatching success (closed circles) and development time (vertical lines) of eggs of (a) *Acartia clausi*, and (b) *A. steueri*. Open circles denote hatching success in 33‰ seawater after various periods of incubation (see text) at given salinities.

Effect of Salinity and Temperature Combinations

Eggs of *A. clausi* and *A. steueri* hatched over a wide range of salinity. Temperature had

no effect on the salinity range where hatching occurred; the ranges between 19.5 and 46.9‰ S·
and between 19.5 and 43.7‰S always permitted over 50 % hatching success of *A. clausi* and
A. steueri eggs, respectively (Fig. 3).   Hatching time (50 % grouped eggs) varied with salinity
levels but was shortest at salinity close to that of normal Onagawa Bay water.

After initial incubation at various salinities, unhatched eggs were transferred to optimal
condition to induce hatching.   Some additional hatchings were observed for both species
(Fig. 3).   However, those for *A. steueri* were very few, probably indicating that the egg
membrane of this species is less impermeable to change of osmotic pressure than that of *A.*
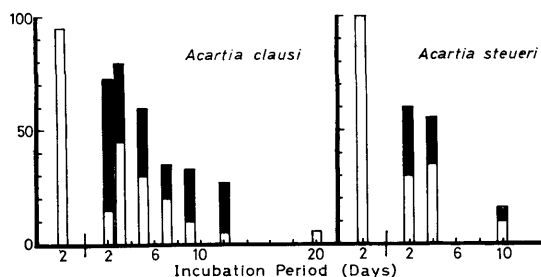*clausi*.

Fig. 4.   Egg hatching of *Acartia clausi* and *A. steueri* in continuous light and in darkness.
White column on left: total hatching success under continuous light; black column:
total hatching success upon return to light after various periods of incubation in dark-
ness; white segments of columns: hatching success in complete darkness.

Effect of Illumination

Eggs of both species hatched normally within 2 days under continuous light, whereas their
hatching was inhibited under continuous darkness (Fig. 4).   When unhatched eggs were then
incubated in light, some were observed to develop into nauplii.   However, no subsequent
hatching was observed for the eggs beyond 12 days in darkness for *A. clausi* and 10 days
for *A. steueri*.   Similar inhibition of egg development by darkness was found for *A. clausi*
from west coast of the United States (LANDRY 1975b, UYE & FLEMINGER 1976).

Effect of Bottom Mud

No nauplii were observed to hatch from the eggs while burried within silty mud.   Decompo-
sition of the eggs within the mud was found to be greater for longer of storage period.
After various storage days the eggs incubated at optimal conditions to check their viability.
For each species, there was no seasonal difference in the maximum period of egg viability in
the mud.   However, there was marked difference in survival rate between storage tempera-
tures, 5 and 20°C (Fig. 5).   Viability was sustained for maximum of 165 and 100 days at
5°C but 70 and 75 days at 20°C for *A. clausi* and *A. steueri*, respectively.   This may indicate
that *A. clausi* eggs can survive longer than *A. steueri* eggs on the sea-bottom during cold
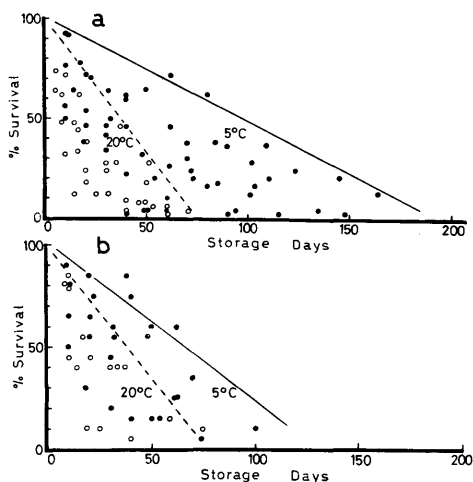season.

Fig. 5a, b. Survivability of eggs in the bottom mud at temperatures of 5 and 20°C for
(a) *Acartia clausi*, and (b) *A. steueri*.

Discussion

The present results show that among the several environmental factors investigated, salinity
may be negligible in the natural environment, since the salinity at the sampling station varied
seasonally within a narrow range around 32‰. Other factors such as temperature, illumi-
nation and the presence of mud, however, are important. Temperature in situ fluctuated
from 4.7 to 23.2°C during the investigations. Eggs of both species are able to hatch through-
out the year, although *A. steueri* eggs take more than 10 days to hatch at the lowest temper-
ature. In waters of the innermost part of Onagawa Bay, in fact, the recruitment as hatched
nauplii into the planktonic population was found to be continuous throughout the year for
these two species.

LANDRY (1975a) demonstrated that development rate of *A. clausi* eggs spawned by the
winter population was significantly faster than that of the eggs by the summer population
when incubated at temperatures above 19°C. In the present experiments, however, such an
effect of the seasonal temperature acclimatization was only observed in the hatching success
at lower or higher extremes of the thermal range tested. This fact suggests that eggs pro-
duced by *A. clausi* and *A. steueri* have similar physiological properties throughout the year.
This implies that the development rate in nature can be determined by in situ water temper-
ature which the sinking eggs encounter with. On the other hand, the sinking rate of eggs
of *A. clausi* and *A. steueri* in still water at 18°C, which was taken from Onagawa Bay and
HA Millipore filtered, was measured as 1.28 m·h⁻¹ and 2.41 m·h⁻¹, respectively. Thus,
unless strong upwelling occurs, it is likely that most eggs spawned in the surface layer will
sink to the bottom (ca. 15 m deep at the station) before hatching. Further, even at night,
since adult females of *A. clausi* have been found to aggregate near the bottom, spawned eggs
have more of a chance to contact the bottom sediment. In fact, in top layer of the sediment

at the sampling station, a large quantity of the eggs were present throughout the year. Their number, eggs of *A. clausi* were usually one order of magnitude more numerous than *A. steueri*, fluctuated seasonally, as had already been demonstrated for several calanoid copepods in the Inland Sea of Japan (KASAHARA et al. 1975b, UYE et al. 1979). Abundance of *A. clausi* eggs was greatest in summer and early fall (more than 2×10^6 eggs·m^{-2}) and least in winter and spring (ca. 0.5×10^6 eggs·m^{-2}). Hatching success of the eggs sorted from the mud was always greater than 70 %. Their hatching time tended to be influenced by temperature in a similar manner to freshly laid eggs, but it must be noted that the greater variability in hatching time was observed for the eggs sorted from the mud. The latter fact indicates that the embryonic development is not stopped at a fixed developmental stage in the mud, but that development may be arrested at some variable stages (cf. UYE et al. 1979).

Inhibition of hatching of eggs by the silty bottom sediment has been reported for many species of calanoid copepod (KASAHARA et al. 1975a, LANDRY 1978, UYE & FLEMINGER 1976, UYE et al. 1979). KASAHARA et al. (1975a), UYE & FLEMINGER (1976) and UYE et al. (1979) demonstrated that, since under very low oxygen concentration no eggs hatched the hatching inhibition by the mud was mainly caused by scarce oxygen in the sediment. Burying the eggs under the mud also creates darkness. Consequently, while inhibition of egg development by darkness itself was less complete for *A. clausi* and *A. steueri*, the effect of darkness should be important in nature where dark and anoxic conditions are created in couples in the sediment.

Mortality of the eggs in sediment is intuitively thought to be caused biologically by predation of larger benthic animals and attack of micro-organisms or chemically by toxic substances such as hydrogen sulfide. In this investigation the mortality of laboratory spawned eggs within prefrozen mud was determined at 5 and 20°C (Fig. 5). Since no large predator was present in this examination, the present results show the possible lowest mortality rate of the eggs in sediment caused only by micro-organisms and chemical substances.

UYE & KASAHARA (1978) have suggested that one of the most important strategic roles of resting eggs of neritic copepods is to maintain their potential populations endemically during unfavorable seasons. However, since adult of *A. clausi* and *A. steueri* can be present throughout the year in Onagawa Bay, the role of their resting eggs as strategy for maintenance of the population during unfavorable periods is less important. Many *Acartia* species are inhabiting estuarine-coastal waters where unexpected deterioration of environment occurs. For instance, in estuarine regions benthic resting eggs may act to maintain the endemic copepod populations against the strong flushing. In delimited water body, simultaneous hatching of the spawned eggs is possibly resulted into overcrowded population and enhance the risk of food shortage and, in turn, heavy cannibalism. Time-released hatching may ease such situations. In this sence, silty bottom sediment in shallow waters acts as a "seed-bed" of the neritic copepods by allowing temporal stay of their eggs in a dormant state (UYE & KASAHARA 1978). The existence of facultative resting eggs other than obligate diapause eggs can be advantageous in maintaining endemic copepod populations in highly variable

estuarine-coastal waters.

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