Original Article

Effects of temperature and salinity on the metamorphosis of nauplius of a planktonic shrimp Acetes intermedius Omori, 1975

YUNG-HUI CHEN AND I-MING CHEN*

Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan 804, Republic of China

ABSTRACT: The effects of temperature and salinity on the metamorphosis of nauplii into protozoa I of a planktonic shrimp Acetes intermedius were studied. Eggs or nauplii I, hatched under optimal conditions (30°C, 30 ppt), were incubated under 15 different combinations of three temperatures (20°C, 25°C, and 30°C) and five salinities (15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt) until reaching protozoa I stage. At salinities of 20–35 ppt, eggs required 28 h, 45 h and nearly 4–5 days to develop into protozoa I at 30°C, 25°C and 20°C, respectively. Nauplii metamorphosed into protozoa I at salinities of 25 ppt and 30 ppt earlier than at 20 ppt and 35 ppt at temperatures of 25°C and 30°C, whereas no regularity in time length existed among salinities at 20°C. Irrespective of hatching conditions, the rate of metamorphosis reached approximately 90–100% at temperatures of 25°C and 30°C at salinities of 20–30 ppt, but was significantly reduced at 20°C for all salinities examined. The metamorphosis rate of nauplii was generally higher at salinities of 20–30 ppt at all temperatures. Multiple linear regression analysis revealed that the temperature experienced by nauplii accounted for most of the variation in the metamorphosis rate.

KEY WORDS: Acetes, metamorphosis, nauplius, salinity, temperature.

INTRODUCTION

The planktonic shrimp Acetes intermedius Omori 1975 has long been a commercially and ecologically important species in the coastal waters off south-western Taiwan.1 Gravid shrimps occur abundantly within estuaries throughout the year.2,3 As a result, the larvae that occur during the different seasons experience seasonal changes of water temperatures from 20°C in winter to 28–30°C in summer.1 Similarly to other Acetes spp., the larvae of A. intermedius undergo four developmental stages: (i) nauplius; (ii) protozoa; (iii) mysis; and (iv) postlarvae before reaching the juvenile stage.5,6 Larvae at the naupliar stage depend on an internal energy source to survive before they metamorphose into feeding larvae;3 that is, the protozoa I stage. Abiotic factors are particularly important in the development of larvae at the non-feeding naupliar stage.

The larvae of genus Acetes occur seasonally and abundantly in estuaries,7–10 suggesting that larval development may be affected by variations in both temperature and salinity, as has been documented in other decapods. Survival rate of nauplii of the shrimp Metapenaeus bennettae are significantly higher at temperatures greater than 24°C and salinities above 30 ppt.11 The larvae of prawn Pandalus borealis, maintained under a regimen of increasing temperature from 4.2°C to 6.0°C in 60 days, grows more rapidly than those maintained at a constant temperature.12 Survival rate of the semiterrestrial grapsid crab, Armases miersii, is frequently higher at salinities of 15–25 ppt than at 35 ppt.13 Penaeus chinensis juveniles grow best in salinities ranging between 20 ppt and 30 ppt and at temperatures of 30°C.14 In general, either temperature or salinity alone can influence the growth and survival of the larvae, but the best larval growth usually occurs when both salinity and temperature are optimal.

Previous studies on the spatial distribution of larvae of Acetes spp. have focused mainly on larvae at the zoal and/or mysid stages,5,15 and not on naupliar larvae. This is because of the difficulties in
The temperature range spans that of the seasonal temperature changes which occur in the coastal waters off south-western Taiwan. Chen and Chen have indicated previously that the lowest temperature and salinity for egg hatching of *A. intermedius* is 20°C and 15 ppt, respectively. After hatching, the development of naupliar larvae was examined under an anatomical microscope at certain time intervals until they metamorphosed into protozoea I. The metamorphosis rate of nauplii was calculated as the percentage (%) of the number of protozoea I to the sum of naupliar larvae and protozoea I. The rate was determined when it did not obviously fluctuate with time. The required time length was determined accordingly. On average, 100 eggs were used in each beaker. At least three beakers were used to estimate the metamorphosis rate for each condition. Each experiment was carried out at least twice.

**Nauplius to protozoea I**

The proportion of internal energy consumed by eggs during development depends on hatching conditions. It can be considered that nauplii which hatch under different environmental conditions contain different amounts of energy for their subsequent development. In order to understand the effects of temperature and salinity on the metamorphosis of nauplii exclusively from the influences of environmental conditions on egg hatching, the nauplii I, which were newly hatched at a temperature of 30°C and a salinity of 30 ppt, were randomly introduced into the 15 different conditions described earlier. Chen and Chen have demonstrated previously that eggs of *A. intermedius* reach maximum hatching success in the shortest time at a temperature of 30°C and in salinities ranging between 25 ppt and 30 ppt. The experimental procedures and estimation of the metamorphosis rate are mentioned as described earlier.

**Statistic analysis**

The significance of temperature and salinity interactions on metamorphosis rate of nauplii was examined using two-way ANOVA. If significant, one-way ANOVA was employed to examine the significance of the differences in metamorphosis rate among different the levels of each factor (temperature/salinity) under the same levels of other factors (salinity/temperature). Duncan multiple comparisons test was used in order to sort out the metamorphosis rate into different groups where necessary. Multiple linear regression analysis was

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**Fig. 1** Map of the south-western coast of Taiwan indicating the collecting site of gravid female shrimps *Acetes intermedius* used in the experiments.

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**MATERIALS AND METHODS**

**Collections**

Gravid shrimps of *A. intermedius* Omori 1975 were collected along the bank of Kaohsiung Port, Taiwan, Republic of China (Fig. 1), using a bag net. They were introduced individually into a 0.5 L beaker to spawn at room temperature (25–27°C) at a salinity of 25 ppt (filtered seawater, 0.22 μm). Because gravid females spawn throughout the night, only those eggs that were spawned within a similar time period of 15–25 min were used. After spawning, eggs were siphoned off, pooled together and then distributed randomly into 0.5 L beakers containing 400–450 mL of filtered seawater at different salinities. The beakers were incubated in a temperature-controlled water bath.

**Egg to protozoea I**

Eggs were subjected to 15 combinations of three different temperatures (20°C, 25°C, and 30°C) and five salinities (15 ppt, 20 ppt, 25 ppt, 30 ppt, and 35 ppt). The temperature range spans that of the seasonal temperature changes which occur in the coastal waters off south-western Taiwan. Chen and Chen have indicated previously that the lowest temperature and salinity for egg hatching of *A. intermedius* is 20°C and 15 ppt, respectively. After hatching, the development of naupliar larvae was examined under an anatomical microscope at certain time intervals until they metamorphosed into protozoea I. The metamorphosis rate of nauplii was calculated as the percentage (%) of the number of protozoea I to the sum of naupliar larvae and protozoea I. The rate was determined when it did not obviously fluctuate with time. The required time length was determined accordingly. On average, 100 eggs were used in each beaker. At least three beakers were used to estimate the metamorphosis rate for each condition. Each experiment was carried out at least twice.
used to analyze the relative importance of each variable on the basis of variation of metamorphosis rate.

RESULTS

Developmental time

Nauplii metamorphosed into protozoea I at night in all conditions. At temperature 25°C and 30°C, nauplii started to metamorphose into protozoea I around 45 h and 29 h after spawning, respectively. The metamorphosis rate reached approximately 90% in 2 h and became steady thereafter. Nauplii metamorphosed approximately 2 h and 6 h earlier at 25 ppt and 30 ppt compared with at 20 ppt and 35 ppt, respectively (Fig. 2). At 20°C, eggs generally took approximately 4–5 days after spawning to develop into protozoea I irrespective of the salinity. The metamorphosis rate of nauplii fluctuated without regularity. The protozoea I usually showed weak mobility, and their spines of uropod were either absent or bent. The abnormality was especially obvious at salinities 15 ppt and 35 ppt.

Egg to protozoea I

At the temperature 30°C, the metamorphosis rate of nauplii was, on average, more than 90% when salinity was greater than 15 ppt, whereas it was higher (88–94%) at salinities of 20 ppt and 25 ppt at the temperature 25°C. At 20°C, it was greatly reduced for all salinities, whereby the higher metamorphosis rates (54–69%) occurred at salinities ranging between 20 ppt and 30 ppt (Fig. 3). The metamorphosis rate of nauplii was significantly different among temperatures \( P < 0.01 \) as well as among salinities \( P < 0.01 \). At the same temperature, the

Fig. 2 Metamorphosis rate of nauplii in different temperature (25°C and 30°C) and salinity (20 ppt, 25 ppt, 30 ppt, and 35 ppt) conditions, which changed over time. The initial times were: (a) 44 h after spawning for the 25°C treatment group; and (b) 24 h for the 30°C treatment group.
was significantly different among temperatures \((P < 0.01)\), as well as among salinities \((P < 0.01)\). The significance of the interactions between temperature and salinity \((P < 0.01)\) suggested that the variations in the metamorphosis rate were not influenced by temperature or salinity alone. At the same temperature level, the metamorphosis rate was similar among salinities at temperatures of 25°C and 30°C, but was significantly lower at salinities of 15 ppt and 35 ppt compared with salinities at a temperature of 20°C (Table 2a). At the same salinity, the metamorphosis rate was not significantly different between the temperatures 25°C and 30°C, but it was lowest at the temperature 20°C, except at 20 ppt (Table 2b).

### Table 1  
Duncan’s multiple comparisons of the metamorphosis rate (%) of nauplii in which eggs were incubated in different conditions until reaching protozoea I stage. Metamorphosis rates among salinities/temperatures were compared while maintaining the other variables (temperature/salinity) constant.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
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<tbody>
<tr>
<td>20</td>
<td></td>
<td>1.43</td>
<td>69.94</td>
<td>60.14</td>
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<tr>
<td>25</td>
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<td>63.02</td>
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<td>88.86</td>
<td>73.78</td>
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<td>30</td>
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<td>94.31</td>
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</table>

For a given temperature/salinity (row), values indicated by identical letters do not differ significantly at the level \(P = 0.05\).

### Nauplius to protozoea I

At the temperature 30°C, nearly 100% of nauplii metamorphosed successfully into protozoea I for all salinities. The metamorphosis rate ranged from 79.84% to 95.63%, in which the higher rates occurred at salinities ranging from 20 ppt to 30 ppt at 20°C and 25°C, except from 20 ppt to 35 ppt at a temperature of 30°C (Table 1a). At the same salinity, the metamorphosis rate was always lowest at temperature 20°C among the various salinities. It was similar between 25°C and 30°C at salinities ranging between 15 ppt and 25 ppt, but was significantly different between salinities 30 ppt and 35 ppt (Table 1b).

### Table 2  
For a given temperature/salinity (row), values indicated by identical letters do not differ significantly at the level \(P = 0.05\).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>20</th>
<th>25</th>
<th>30</th>
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<tbody>
<tr>
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For a given temperature/salinity (row), values indicated by identical letters do not differ significantly at the level \(P = 0.05\).

### Fig. 3  
Metamorphosis rate of nauplii (% mean ± SD) incubated at the same temperature and salinity conditions as that for eggs. (See Table 1 for detailed comparisons).

### Fig. 4  
Metamorphosis rate of nauplii (% mean ± SD), hatched at 30°C and 30 ppt, in different temperature and salinity conditions. (See Table 2 for detailed comparisons).

lowest metamorphosis rate always occurred at the salinity 15 ppt. The metamorphosis rate was generally similar among salinities ranging from 20 ppt to 30 ppt at 20°C and 25°C, except from 20 ppt to 35 ppt at a temperature of 30°C (Table 1a). At the same salinity, the metamorphosis rate was always lowest at temperature 20°C among the various salinities. It was similar between 25°C and 30°C at salinities ranging between 15 ppt and 25 ppt, but was significantly different between salinities 30 ppt and 35 ppt (Table 1b).

\[
\text{MR} = -280 + 24.19 \times MT - 0.44 \times MT^2 + 0.05 \times MT \times HT (r^2 = 0.45, F = 52.26, n = 199, P < 0.01)
\]
whereby MR is the metamorphosis rate of nauplii, HT is the hatching temperature (°C), and MT is the temperature experienced by the nauplii. The metamorphosis temperature (MT), overall, explains nearly 43% of variations in the metamorphosis rate of nauplii. Lack-of-fitness was significant \( F = 7.90, P < 0.01 \), suggesting that other factors exist which affect the metamorphosis rate of nauplii.

**DISCUSSION**

Irrespective of hatching conditions, nauplii usually have a higher metamorphosis rate at salinities ranging from 20 ppt to 30 ppt than those at 15 ppt and 35 ppt for all temperatures. In addition, abnormal individuals of protozoa I were commonly found at the salinities 15 ppt and 35 ppt at 20°C. Nauplii incubated at 25 ppt and 30 ppt metamorphosed into protozoa I earlier than those at other salinities at temperatures of 25°C and 30°C. These observations indicate that the optimal salinity range for metamorphosis of nauplii is approximately 25–30 ppt. It is suggested that nauplii might be found abundantly in the lower parts of estuaries. The use of brackish habitats for the early development of larvae has also been found for the congeneric species *Acetes erythraeus*.\(^9\)

Eggs took nearly 4–5 days to develop into protozoa I at 20°C but only 28–30 h and 44–50 h at 30°C and 25°C, respectively. Regardless of the time needed for eggs to hatch under different conditions,\(^18\) nauplii required nearly 3–4 days to metamorphose into protozoa I at 20°C, whereas they took only approximately 15–16 h at temperatures of 30°C and 25°C. The metamorphosis rate was significantly lower at the temperature 20°C compared with at 25°C and 30°C for all salinities examined, regardless of the experimental conditions in which the nauplii were hatched. These observations indicate that 25°C is close to the minimum limit of the optimal temperature range for the metamorphosis of nauplii of *A. intermedius*. The surface temperature in the coastal waters off south-western Taiwan can reach 28–29°C in summer, but might be as low as 20°C in winter.\(^4\) The results of the present study showed that the metamorphosis of *A. intermedius* nauplii is adversely affected by incubation temperatures below 25°C. It is likely that the recruitment of *A. intermedius* is reduced in winter as a result of the adverse effects on the metamorphosis of nauplii. This hypothesis is supported by Wen’s studies, which indicate that the recruitment of *A. intermedius* in the south-western coastal waters off Taiwan is weaker during winter than during summer.\(^2\)

Linear multiple regression analysis revealed that approximately 43% of variations in the metamorphosis rate of *A. intermedius* nauplii could be explained by the temperatures (MT) at which nauplii were incubated after hatching. Salinity was statistically excluded from the regression equation because it did not contribute significantly in explaining the variation of the metamorphosis rate when compared with other variables. This indicates that temperature, especially at the naupliar stages, affects the metamorphosis of nauplii more significantly than does salinity. This does not, however, totally exclude the influence of salinity on the metamorphosis of nauplii. As shown earlier, nauplii took less time in reaching a higher metamorphosis rate at the salinities 25–30 ppt compared with at other salinity levels.

The results of the present study showed that the nauplii of *A. intermedius* that hatched at optimal conditions (30°C, 30 ppt) usually had a higher metamorphosis rate than those hatched at other suboptimal conditions (Figs 3, 4). This is because the nauplii of *A. intermedius* share a common energy source with eggs, and the proportion of energy reserved for nauplii for subsequent development depends on the utilization conditions of

**Table 2** Duncan’s multiple comparisons of metamorphosis rate (%) of nauplii hatched at 30°C and 30 ppt, but incubated under different combinations of temperature and salinity conditions. Metamorphosis rates among salinities/temperatures were compared while maintaining the other variable (temperature/salinity) constant.

<table>
<thead>
<tr>
<th>(a) Temperature (°C)</th>
<th>Salinity (ppt)</th>
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<th>20</th>
<th>25</th>
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<tr>
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<table>
<thead>
<tr>
<th>(b) Salinity (ppt)</th>
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<td>69.34</td>
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<td>77.20</td>
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<td>35</td>
<td>29.55</td>
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</table>

For a given temperature/salinity (row), values indicated by identical letters do not differ significantly at the level \( P = 0.05 \).
eggs during the hatching process. The high-energy contents reserved for nauplii after hatching is likely to enhance the metamorphosis success of nauplii.

The significance of lack-of-fitness implies that there might be other factors affecting the metamorphosis rate of nauplii that were not examined in the present study. Recent studies have shown that egg quality, as a function of egg size, not only affects the hatching success of eggs but also the survival of newly hatched fish larvae before they start to feed. Fish larvae hatched from big eggs usually have a better survival rate than those hatched from small ones. This could also be the case for A. intermedius because the size of eggs varied from 168 μm to 210 μm. It is likely that the metamorphosis rate of A. intermedius nauplii is related not only to the hatching conditions of eggs but also egg quality. To our knowledge, not much information is presently available on the relationships between egg quality and the metamorphosis of non-feeding nauplii of decapods. Previous efforts have mainly concentrated on the effects of egg quality on hatching success. It would be worthwhile to examine the relationship between egg quality (e.g., energy content) and the metamorphosis of nauplii of A. intermedius, as well as of other decapods, in the future.

ACKNOWLEDGMENT

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