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# Effects of temperature and salinity on larval growth, survival and development of *Penaeus semisulcatus*

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**Abstract**

Four temperatures (22°C, 26°C, 30°C and 34°C), and the combined effects of three salinity (25, 30 and 35 ppt) and three temperature (26°C, 30°C and 34°C) levels from protozoea 1 (PZ1) to postlarvae 1 (PL1) stages of *Penaeus semisulcatus* were studied in two separate experiments. In the first experiment, the PZ1 larvae at the lowest temperature of 22°C showed the highest survival (69%) to PL1 compared to 61% at 26°C, 44% at 30°C and 12% at 34°C. However, 22°C slowed the growth and delayed the larval development by about 2–4 days. Growth rate at 30°C (0.44–0.48 mm $day^{-1}$) was double of that (0.22–0.25 mm $day^{-1}$) at 22°C. The results showed that *P. semisulcatus* is tolerant to low, rather than to high temperatures during the larval development. Hence, a water temperature level of about 30°C is optimal for the larval culture of this species.

The second experiment showed that temperature exerted a greater influence than the salinity on the growth and survival during the larval development. The range of temperature in which the larvae showed high survival and growth is relatively narrow as compared to that of salinity. At all salinity levels, survival to PL1 (69–77%) was higher at 26°C as compared to 30°C (44–73%) and 34°C (14–21%). However, daily growth rate at 30°C and 34°C was about 60% higher than at 26°C. Larval development was also 3–4 days faster at 30°C and 34°C. Based on the survival and growth results, the best salinity and temperature combination for the culture of *P. semisulcatus* was 30 ppt and 30°C. 

*Keywords:* *Penaeus semisulcatus*; Larvae; Salinity; Temperature; Growth; Survival; Development

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## 1. Introduction

Salinity and temperature are two of the most important abiotic factors affecting the growth and survival of aquatic organisms. Larval stages of most penaeid shrimp species occur in full strength seawater and at stable water temperatures. Hence, it is generally accepted that penaeid shrimps are not equipped with the capabilities of withstanding major environmental changes during their larval development. For this, research about the salinity and temperature optima for penaeid larvae has been limited to only a few studies (Preston, 1985; Kumlu and Jones, 1993; Parado-Estepa et al., 1993). Most farmers conventionally use full strength seawater and ambient water temperatures in the larval culture of penaeid shrimps (Chen, 1990; Parado-Estepa et al., 1993). Yet, it is not always true that the larvae of all penaeid shrimp species grow best at conventional rearing salinity and temperature levels (Kumlu and Jones, 1993). In addition, it is also well known that the response to these environmental parameters is species-specific and that salinity and temperature may also interact to influence growth and survival (Staples and Heales, 1991; O'Brien, 1994). It is generally agreed that temperature has a more pronounced effect on growth and survival of penaeids (Parado-Estepa, 1998).

*Penaeus semisulcatus* is an Indo-Pacific species distributed along the coast of Eastern Mediterranean and is one of the most important commercial species in this part of the world. A few commercial farms in Turkey practice its culture on a small scale. The optimal salinity for *P. semisulcatus* is 30–35 ppt (Kumlu et al., 1999). As gravid females are often captured in cooler months of the year, it is thought that this species might have adapted to cool waters encountered in sub-tropical conditions in the Eastern Mediterranean. A better understanding for the effects of temperature and salinity on the larval culture of this penaeid shrimp species is important in order to define adequate conditions for optimal production.

The purpose of the present study was to determine the growth and survival of *P. semisulcatus* at four different temperature levels (22°C, 26°C, 30°C and 34°C), and the combination of three temperatures (26°C, 30°C and 34°C) and three salinities (25, 30 and 35 ppt) during the larval development.

## 2. Materials and methods

### 2.1. Rearing procedures

This study was conducted in the Marine Research Station of the Faculty of Fisheries, University of Cukurova, Yumurtalik, Turkey. The broodstock were caught from the Eastern Mediterranean Sea at 30-m depth where salinity was 38 ppt. They were kept in a 10-ton fibreglass tank and spawned in 100-l tanks at 38 ppt. At the protozoea 1 (PZ1) stage, three batches of larvae obtained from different spawners were pooled and stocked into 2-l round bottom glass flasks at a density of 100 $l^{-1}$. They were acclimated to three salinity levels (25, 30, 35 ppt) by lowering the salinity at a rate of 5 ppt $h^{-1}$ by adding freshwater (24 h aerated well water). The larvae were then acclimated to different temperatures at a rate of 5°C $h^{-1}$. Salinity and temperature were measured with a digital

salinometer (YSI, USA). The culture water was filtered trough a sand filter and a series of cartridge filters (10, 5 and 1 μm) before being used.

The larvae were fed a mixture of *Tetraselmis chuii* (20 cells $\mu l^{-1}$), *Chaetoceros calcitrans* (50 cells $\mu l^{-1}$) and *Isochrysis galbana* (30 cells $\mu l^{-1}$) throughout the culture. These alga species were produced in batch culture system by using Walne Culture Medium. A haemocytometer was used to estimate the number of algal cells everyday in the algae culture vessels and larval flasks. Newly hatched *Artemia nauplii* were introduced into the culture flasks at a density of 5 $ml^{-1}$ when 50% of the larvae entered into the mysis 1 (M1) stage. All the larvae in each flask were counted and a random sample of 10–15 larvae were staged and measured for total length (from tip of rostrum to the end of telson) everyday throughout the culture period. Duration of larval development was determined when 50% of the larvae entered into M1 or postlarvae 1 (PL1) stages.

### 2.1.1. Experiment 1

The effects of four different temperature levels (22°C, 26°C, 30°C and 34°C) on the growth, survival and development of *P. semisulcatus* larvae at 38 ppt salinity were investigated in this experiment. The 2-l glass larval flasks were placed in four different thermostatically controlled water baths ($\pm 0.5$°C). Each temperature was conducted in two replicates.

### 2.1.2. Experiment 2

The combined effects of both temperature (26°C, 30°C and 34°C) and salinity (25, 30 and 35 ppt) on larval survival and growth were investigated from PZ1 to PL stages. The 2-l glass larval culture flasks were placed in thermostatically controlled water baths ($\pm 0.5$°C) throughout the experiment. Each combination of salinity and temperature was conducted in two replicates.

## 2.2. Statistical calculations

The growth curves of the shrimp larvae in different treatments were compared separately during the PZ1–M1 and M1–PL1 stages with the General Linear Model

Table 1
Survival, growth rate, total length and duration of development of *P. semisulcatus* larvae grown at different temperature levels (22°C, 26°C, 30°C and 34°C) from PZ1 stage to PL1 stage
Values marked with different superscripts are significantly different from each other ($P < 0.05$).

| Temperature (°C) | Survival (%) | | Growth rate (mm $day^{-1}$) | | Total length (mm ± S.D.) | | Duration (days) | |
|---|---|---|---|---|---|---|---|---|
| | M1 | PL1 | PZ1–M1 | M1–PL1 | M1 | PL1 | M1 | PL1 |
| 22 | 86.50 | 69.25 | $0.25^{b}$ | $0.22^{b}$ | $2.81 \pm 0.29^{b}$ | $5.11 \pm 0.26^{b}$ | 7 | 12 |
| 26 | 78.00 | 61.25 | $0.31^{b}$ | $0.28^{b}$ | $3.01 \pm 0.17^{a}$ | $5.22 \pm 0.28^{b}$ | 5.5–6 | 10 |
| 30 | 70.00 | 43.75 | $0.48^{a}$ | $0.44^{a}$ | $3.01 \pm 0.20^{a}$ | $5.62 \pm 0.56^{a}$ | 4 | 8 |
| 34 | 45.25 | 12.00 | $0.36^{b}$ | $0.51^{a}$ | $2.97 \pm 0.34^{a}$ | $5.15 \pm 0.39^{b}$ | 5–5.5 | 8 |

Table 2
Survival, growth rate, total length and duration of development of *P. semisulcatus* larvae grown at different salinity and temperature combinations from PZ1 stage to PL1 stage
Values marked with different superscripts are significantly different from each other at 0.01 for growth rate and at 0.05 for total length.

| Temperature (°C) | Salinity (ppt) | Survival (%) | | Growth rate (mm day$^{-1}$) | | Total length (mm ± S.D.) | | Duration (days) | |
|---|---|---|---|---|---|---|---|---|---|
| | | M1 | PL1 | PZ1–M1 | M1–PL1 | M1 | PL1 | M1 | PL1 |
| 26 | 25 | 94.00 | 77.25 | 0.336$^{c}$ | 0.343$^{e}$ | 2.58 ± 0.08$^{b}$ | 3.96 ± 0.32$^{c}$ | 5 | 10–10.5 |
| | 30 | 92.25 | 69.00 | 0.312$^{c}$ | 0.380$^{e}$ | 2.55 ± 0.07$^{b}$ | 4.07 ± 0.25$^{c}$ | 5 | 9.5–10 |
| | 35 | 93.75 | 68.80 | 0.314$^{c}$ | 0.377$^{e}$ | 2.59 ± 0.16$^{b}$ | 4.04 ± 0.26$^{c}$ | 5 | 9.5–10 |
| 30 | 25 | 89.50 | 72.80 | 0.299$^{c}$ | 0.564$^{c}$ | 2.70 ± 0.29$^{ab}$ | 5.02 ± 0.67$^{b}$ | 4.5–5 | 8 |
| | 30 | 86.50 | 70.00 | 0.353$^{b}$ | 0.655$^{a}$ | 2.90 ± 0.33$^{a}$ | 5.87 ± 0.52$^{a}$ | 4 | 7 |
| | 35 | 94.50 | 43.80 | 0.417$^{a}$ | 0.616$^{b}$ | 3.18 ± 0.34$^{a}$ | 5.68 ± 0.67$^{a}$ | 4 | 7 |
| 34 | 25 | 64.50 | 14.00 | 0.206$^{e}$ | 0.475$^{d}$ | 2.36 ± 0.30$^{b}$ | 4.55 ± 0.78$^{b}$ | 4.5–5 | 8.5–9 |
| | 30 | 65.00 | 16.30 | 0.304$^{c}$ | 0.602$^{b}$ | 2.76 ± 0.17$^{ab}$ | 5.68 ± 0.21$^{a}$ | 4 | 7 |
| | 35 | 88.75 | 21.00 | 0.253$^{d}$ | 0.663$^{a}$ | 2.51 ± 0.25$^{b}$ | 5.27 ± 0.32$^{a}$ | 4.5 − 5 | 7 |

(GLM) by using days as a covariate. Prior to this analysis, the linearity of the data was examined by residual plots from regression analysis. Growth rates (mm day$^{-1}$) were derived from the output of the analysis and are given in Tables 1 and 2. The larval total length results were compared using the two-way analysis of variance (ANOVA) and any significant difference was determined at 0.05 probability level by Scheffé's pairwise comparison test after the normality and homogeneity (Bartlett's test) of the data were checked (Sokal and Rholf, 1981) in Minitab statistical software.

## 3. Results

### *3.1. Experiment 1*

The larvae grown from PZ1 stage to PL1 stage displayed higher survival (61–69%) in low temperatures (22°C and 26°C) compared to 44% at 30°C and 12% at 34°C (Table 1). Throughout the experiment, these low water temperatures constantly gave better larval survival than the higher temperatures. At 22°C, 87% of the larvae developed into M1 stage, whereas, at 34°C, only 45% of the larvae reached this stage. At the end of the experiment, 69% of the PZ1 larvae grown at the lowest temperature level (22°C) metamorphosed into the PL1 stage in contrast to only 12% of those cultured at 34°C (Table 1).

Contrary to the survival data, the larval growth and development were inversely affected by extreme low and high temperatures (Table 1). The larvae grown at 30°C had the fastest growth rate (0.48 mm day$^{-1}$) and development (4 days) between PZ1 and M1 stages. At 30°C, the daily larval growth rate was almost double of that obtained at 22°C. Growth rate at 34°C was significantly lower than that at 30°C during the PZ stages ($P < 0.01$) but not during the M stages ($P > 0.05$). The largest larval total length (5.62

mm) was obtained at 30°C at metamorphosis ($P < 0.05$). At 30°C and 34°C, the larvae reached PL1 stage on day 8, whereas the larvae reached this stage on day 10 at 26°C and on day 12 at 22°C. Thus, with respect to larval growth at 30–34°C, the larval development was delayed by 4 days at 22°C and by 2 days at 26°C (Table 1).

### 3.2. Experiment 2

The results summarised in Table 2 show that temperature had a greater influence on survival than salinity during larval development. Survival was considerably lower at 34°C at each salinity level tested. Only between 14% and 21% of the larvae at 34°C were able to metamorphose into PL1 stage as compared to 44–73% at 30°C and 69–77% at 26°C. Among the nine combinations of salinity and temperature levels, the highest larval survival (77%) at PL1 stage was obtained at 26°C and 25 ppt (Table 2).

The greatest larval growths as TL at M1 stage (2.70–3.18 mm) were obtained at 30°C regardless of the salinity level tested and at 34°C–30 ppt (2.76 mm) (Table 2). The larval TL at M1 stage was lowest at 26°C at all the salinity levels ($P < 0.05$). At PL1 stage, the combinations of 30°C–30 ppt, 30°C–35 ppt, 34°C–30 ppt and 34°C–35 ppt gave significantly higher larval growth (ranging from 5.27 to 5.87 mm) than the other combinations ($P < 0.05$) (Table 2). The PLs had the lowest TL (3.96–4.07 mm) at 26°C at all the salinity levels ($P < 0.05$).

The fastest larval development until M1 stage occurred in 30°C–30 ppt, 30°C–35 ppt and 34°C–30 ppt. At all these salinity/temperature combinations, it took 4 days for the PZ1 larvae to develop into the M1 stage (Table 2). The influence of temperature and salinity was more evident on the larval development during the M stages. The slowest larval development (10–11 days) occurred at 26°C and 25 ppt. Regardless of the salinity level, at this temperature, the duration of larval development was around 10 days. At 30°C, at all salinity levels, larval development took about 7–8 days. At 34°C, larval development was fastest (7 days), except at 25 ppt (Table 2).

The highest and lowest larval growth rates until M1 stage were promoted by 30°C–35 ppt (0.417 mm $day^{-1}$) and 34°C–25 ppt (0.206 mm $day^{-1}$). The second best growth rate (0.353 mm $day^{-1}$) was obtained at 30°C and 30 ppt. During the M stages, the larvae grew best at 34°C–35 ppt (0.663 mm $day^{-1}$) and 30°C–30 ppt (0.655 mm $day^{-1}$) ($P < 0.01$). At all salinity levels, 26°C consistently gave the lowest growth rates (0.343–0.377 mm $day^{-1}$) between M1 and PL1 stages.

Taking into account the survival, growth, and development data, the best salinity and temperature combination for the culture of *P. semisulcatus* was 30 ppt and 30°C.

## 4. Discussion

The present study showed that temperature is an extremely important parameter as it affects larval development, growth and survival of *P. semisulcatus*. Relatively low temperature (22°C) slowed larval development and growth but did not cause high mortality. The larvae subjected to the lowest test temperature (22°C) displayed higher survival (69%) at metamorphosis in comparison to that (12%) at the highest temperature

(34°C). Yet, 22°C slowed down the growth and delayed the larval development for about 4 days. Our findings agree with those of Parado-Estepa (1998) who studied the effects of both the temperature and the salinity on early PL of *P. monodon*. High temperatures (30–34°C) significantly increased the growth rate during the PZ stages (0.36–0.48 mm $day^{-1}$) and the M stages (0.44–0.51 mm $day^{-1}$) as compared to that (0.25–0.22 mm $day^{-1}$) at 22°C. At 30–34°C, the larvae developed into PL1 stage 4 days earlier than at 22°C. O'Brien (1994) reported that the growth rate of juvenile *P. esculentus* at 30°C was 27% higher that at 20°C. They also stated that growth was arrested at 15°C. Lin and Su (1995) studied the effects of low temperature on the larvae of *P. monodon* and concluded that the larval development from nauplius 4–5 (N4–5) to PZ2 took 17 days at 22°C. In our study, the development of *P. semisulcatus* from the late nauplius to PZ2 took no longer than 3–4 days at 30°C. Our PZ1 larvae cultured at 22°C reached the PL1 stage within 12 days. These facts indicate that *P. semisulcatus* (at least our strain) is much more tolerant to 22°C than *P. monodon*.

In the first experiment, the PZ1 larvae at 26°C displayed the second highest survival (61%) but lower growth rate (0.28–0.31 mm $day^{-1}$) than the larvae at 34°C. This fact confirms the suggestion that high temperatures, to a certain point, increase the moulting frequency and larval growth but reduce the survival of penaeid shrimps (Staples and Heales, 1991; O'Brien, 1994; Parado-Estepa, 1998), possibly because less protein is being incorporated into the body tissues. Our results indicated that *P. semisulcatus* is tolerant to low (22°C) rather than high temperatures (34°C) during the larval development. Therefore, water temperature should be maintained at around 30°C for the larval culture of this shrimp species.

In our previous study, the optimal salinity for the larvae of this species ranged between 30 and 35 ppt at 28°C (Kumlu et al., 1999). We concluded that, during the early PZ stages, *P. semisulcatus* larvae obtained from the Eastern Mediterranean broodstock are tolerant to a salinity range of 25–55 ppt. The second experiment of the present study showed that temperature exerted a greater influence than salinity on the larval development of *P. semisulcatus*. The range of temperature in which the larvae showed high survival and growth is relatively narrow as compared to that of salinity. Our study showed that salinity (25, 30 and 35 ppt) combined with temperature regimes (26°C, 30°C and 30°C) had a little impact on larval survival. At all salinity levels tested, 34°C caused high mortality both during the PZ stages and M stages. At 25 and 30 ppt, the survival of larvae grown at 26°C and 30°C at M1 were quite similar. The present results indicated that the optimal salinity and temperature combination during the PZ stages was 30 ppt and 30°C. However, since a small deviation from the optimal temperature may have a pronounced effects on growth, it is thought that a slightly lower temperature than 30°C should be more adequate for the larval culture of *P. semisulcatus*. During the mysis stages, at all salinity levels, the PL1 had the lowest survival (14–21%) at 34°C. Regardless of salinity, 26°C consistently gave higher survival (69–77%) than 30°C (44–73%). However, growth rate was about 60% faster at 30°C than at 26°C (Table 2). Staples and Heales (1991) suggested that in order to determine optimum growing conditions, a compromise should be made between growth and survival data. Based on the survival and growth results, the best salinity and temperature combination for the culture of *P. semisulcatus* during the M stages was again 30 ppt and 30°C. The optimal

level of temperature for the growth and survival was 30°C when salinity was maintained between 30 and 35 ppt.

It is known that larvae of *P. monodon* are grown successfully at extremely high temperatures (32–34°C) in commercial hatcheries in Southeast Asia (Chen, 1990; Fegan, 1992). Based on the present results, *P. semisulcatus* larvae obtained from the Eastern Mediterranean broodstock should not be reared at temperatures higher than 30°C.

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