Roles of Salinity on Survival Rate, Metamorphosis Period, and Rate of Acclimatization of Dwarf Blue Shrimp, *Caridina* c.f. *babaulti* (Bouvier, 1918)

Worapong Nensri[1], Phongchate Pichitkul[1*], Idsariya Wudtisin[1] and Sommai Janekitkarn[2]

ABSTRACT

The Dwarf blue shrimp, *Caridina* c.f. *babaulti* (Bouvier, 1918), is a very popular species in the ornamental aquarium business worldwide. Recently, a native species resembling the exotic blue shrimp has been introduced. This native shrimp species has been heavily exploited in the wild, leading to attempts to promote captive culturing strategies. Our preliminary experiments suggested that the early life stage of the shrimp is related to salinity. In Experiment I, we aimed to determine the optimum salinity for nursing newly hatched larvae. Three salinity levels (15, 20, and 25 ppt) were tested, with 150 larvae exposed until they reached the juvenile stage, which took 60.33±5.03, 49.33±3.05, and 45.00±1.73 days, respectively. It was found that 20 ppt was the most suitable for this experiment ($p<0.05$). Experiment II aimed to determine the optimum rate for decreasing salinity for nursing larvae. In this experiment, salinity was maintained at 20 ppt and then sharply decreased by 2, 5, or 10 ppt per day. It was found that decreasing the salinity by 2 ppt per day resulted in a higher survival rate of the shrimp compared to the other treatments ($p<0.05$). These experiments suggest that it is possible to enhance the nursing success of the shrimp by adjusting salinity levels. It was concluded that the dwarf blue shrimp could be successfully cultivated in captivity.

Keywords: *Caridina* c.f. *babaulti* (Bouvier, 1918), Dwarf blue shrimp, Reduction rate of salinity, Survival rate

INTRODUCTION

At present, dwarf shrimp are very popular tankmate species in aquatic plant aquariums because they are assiduous algae grazer, making them highly efficient tank cleaners. Furthermore, these colorful dwarf shrimp are highly tolerant and can adapt easily to tank life. The most popular dwarf shrimp species in the Thai aquarium trade are the Amano shrimp (*Caridina multidentata*), the Cherry shrimp (*Neocaridina davidi*), and the Crystal red shrimp, (*Caridina* cf. *cantonensis*). However, these exotic species have very high retail prices and, if escape into the wild, may be harmful to the environment. Therefore, there has been recent interest in finding native species that resemble the famous dwarf shrimp species to cope with the aforementioned challenges.

One of the local species from the Mae Khlong River, which is morphologically similar to the dwarf blue shrimp species, was recently introduced into the ornamental shrimp trade. While the correct scientific name of this shrimp has not yet appeared in the crustacean species checklist of Thailand (Naiyanetr, 2007), its Thai trade name is “Kung Blue” (*Caridina* cf. *babauti*), which means blue shrimp in Thai. This native dwarf blue shrimp is restricted to the lower Mae Khlong River system in western Thailand. Higher demand for aquarium

[1]Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand
[2]Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand
[*]Corresponding author. E-mail address: ffispcp@ku.ac.th
Received 21 March 2023 / Accepted 18 February 2025

trade species has caused a large decline in the natural population. Cultivation of this species has been attempted to maintain its natural population; however, it has been challenging because the cultivation practices for this species have not been reported.

In general, the shrimp genus *Caridina*, belonging to the Family Atyidae, is distributed worldwide, with the life cycle of most species being related to saline water (Cai *et al*., 2007; Naiyanetr, 2007; Vázquez *et al*., 2016; Tu *et al*., 2021). Kondo *et al*. (2021) classified *Caridina* shrimp species into two types according to their life cycles. The first has a landlocked life cycle, spending its entire life in freshwater, while the second has an amphidromous life cycle, spending the larval stage in brackish or sea water and the juvenile and adult stages in freshwater. Generally, the dwarf blue shrimp natural habitat is not far from the brackish area of the estuary of the Mae Khlong River, which suggests that the dwarf blue shrimp species is amphidromous.

Successful breeding and husbandry in the laboratory have been reported for amphidromous *Caridina* shrimps such as *Caridina formose* by Yu *et al*. (2001), and *Caridina leucosticta, C. multidentata* and *Caridina typus* (Kondo *et al*., 2021). However, most successful results have involved temperate or subtropical species, for which the most influential parameters on the larval survival rate are temperature and salinity. As the natural habitat of the dwarf blue shrimp is in tropical rivers, the temperature factor should not be more important than salinity regarding larval survival.

The results from our preliminary experiment showed that dwarf blue shrimp larvae could not survive where the salinity exceeded 30 ppt. Hence, information on the optimum salinity, along with a suitable time for acclimatization, is likely an important factor in achieving a high survival rate of the dwarf blue shrimp, and consequently enhances successful hatchery production. Therefore, the experiments were conducted to determine the optimum salinity during nursing, and to determine the optimum rate for decreasing salinity for nursing larvae. The obtained information benefits improving hatchery practice for this species and consequently helps to reduce its exploitation in its natural habitat, which in turn could support the sustainable use of this species.

MATERIALS AND METHODS

Broodstock

A sample of 100 female dwarf blue shrimps in the berried stage (carrying fertilized eggs on the belly) was selected from a local shop selling ornamental fish in the Chatuchak Night Market, Bangkok, Thailand. The shrimps were brought back to the laboratory at Faculty of Fisheries, Kasetsart University, Bangkok, and dipped in 100 ppt formaldehyde solution to eliminate ectoparasites. Then, 20 berried females were allocated into separate 2 L aquaria supplied with oxygenation and clean freshwater. The shrimps were fed twice daily at 9 a.m. and 4 p.m. with a commercial pelleted feed (60% protein, 10% fat, and 21% carbohydrate) combined with the whole frozen mulberry leaf. The tanks were cleaned, and 30% of the water in each tank was exchanged every 3 days. The berried females were kept for 7–14 days until the larvae hatched. After that, the newly hatched larvae were collected with a scoop net and immediately transferred to the prepared experimental tanks.

Water preparation for shrimp husbandry

Water preparation commenced 14 days prior to experimental initiation. The salinity was controlled using reverse osmosis (RO) water to combine filtered tap water with commercial synthetic sea salt (Aquaraise Reef Salt brand) at rates of 13.29 $gm{\cdot}L^{-1}$, 21.71 $gm{\cdot}L^{-1}$, and 27.14 $gm{\cdot}L^{-1}$ to obtain 15, 20, and 25 ppt, respectively. Salinity was measured using a YSI multiparameter meter to ensure the correct salinity for each treatment. Upon reaching the desired salinity, the mixed solution was aerated until clear. All experimental tanks were covered with plastic wrap to prevent contamination and allowed to stand for 2 weeks to ensure that any remaining chlorine had completely evaporated.

Lived food preparation for shrimp larvae

The planktonic green alga (*Tetraselmis* sp.) was utilized as the food supply for the zoeatic shrimp as this plankton can tolerate a wide range of salinity while maintaining its high nutritional value (Agh and Sergeloos, 2007). The *Tetraselmis* was cultivated in 2,000 mL Erlenmeyer flasks nourished with Guillard f/2 medium (Guillard, 1975; Kang *et al*., 2011). The flasks were kept on incubators to control the ambient temperature within the range of 28–32 ^{o}C, with exposure to 5,000 lux of light with a 12 h·day^{-1} photoperiod for 4 days before being used as a feed supply for the shrimp larvae.

Experiment I

Our preliminary study revealed that blue shrimp larvae did not survive outside a salinity range of 10–30 ppt. To determine the optimal salinity level, we conducted an experiment using a completely randomized design (CRD) with three salinity treatments (15, 20, and 25 ppt), each in triplicate. Newly hatched shrimp larvae (150 tails·tank^{-1}) were randomly assigned to the prepared experimental tanks. The shrimp larvae were fed daily with *Tetraselmis* at 50,000 cells·mL^{-1}. All experimental tanks were exposed to 1,400 lux for 14 h with 10 h of darkness, and the ambient temperature was maintained in the range of 28–32 ^{o}C. Each day, 50% of the water was changed until all shrimp larvae had reached the juvenile stage, confirmed by their transition from the planktonic to the benthic form, which took 43–65 days depending on the treatment.

Experiment II

Newly metamorphosed blue shrimp were assigned to experimental tanks (20 tails·tank^{-1}) with 20 ppt water. The salinity was then decreased by 2, 5, or 10 ppt·day^{-1} until it reached 0 ppt. During the experiment, the shrimp were fed once a day with commercial juvenile fish feed pellets (White Crane brand, 60% protein) mixed with spirulina powder in a 2:1 ratio. Water exchange (50%) was performed daily along with the new salinity adjustment. The experiment was terminated when the salinity in the tank reached 0 ppt, at which the final number of live shrimps was counted and the duration for each experimental treatment was recorded.

Data collection and recording

Water quality parameters (salinity, water temperature, dissolved oxygen, pH, total ammonia, nitrite, nitrate, and alkalinity) were measured every week during the experimental operation. Larval or juvenile dwarf blue shrimps were counted at the beginning and end of each experiment. Differences in numbers were used to determine survival rates.

Statistical analysis

The data on survival rates and metamorphosis durations were tested for normality. If the data did not meet the statistical assumptions, a Box-Cox transformation was applied. Subsequently, an ANOVA was conducted at a 95% confidence level using SPSS software version 27. A post-hoc test (Duncan's Multiple Range Test, DMRT) was performed when statistically significances differences were detected. Results are presented as mean±standard deviation.

RESULTS

Effects of salinity on survival rates and metamorphosis rates

Among the salinity treatments, the blue shrimp larvae in water at 20 ppt exhibited the highest survival rate (64.22±8.06%), followed by those in water at 25 ppt (43.33±6.96%). The lowest survival rate was in water at 15 ppt (13.55±3.67%). The larvae first completed metamorphosis on day 43 in the 25 ppt group, while it was observed on day 46 and 55 in the 20 and 15 ppt groups, respectively. The rate of metamorphosis appeared to occur more rapidly at higher salinities, with a significantly slower completion time at 15 ppt compared to the higher salinities (Table 1).

All measured parameters for water quality among the 3 treatments were consistent with standards for shrimp culture. However, the average value of nitrate at 25 ppt was rather low (0.75±

0.39 mg·L^{-1}) compared to the other salinity levels (1.41±0.51 mg·L^{-1} at 15 ppt and 1.41±0.51 mg·L^{-1} at 20 ppt, respectively), while the alkalinity and pH were slightly higher (Table 2).

Effect of acclimatization rates on survival rate

It was apparent that the differences in the acclimatization rate impacted the survival rate of the juvenile shrimps (Figure 1). The significantly highest survival rate occurred with the decreasing rate of 2 ppt·day^{-1} (93.33±5.77%) compared to 25.00±5.00% for 5 ppt and 18.33±5.57% for 10 ppt, respectively. When the salinity decreased by 5 ppt and 10 ppt, the shrimp gradually died and fell to the tank bottom.

Table 1. Survival rate and average metamorphosis time of dwarf blue shrimp larvae reared at various salinity levels.

Salinity	Survival to Juvenile stage (%)	Duration (days) From hatching zoea to juveniles	
		Mean±SD	Range
15	13.55±3.67^{c}	60.33±5.03^{b}	55–65
20	64.22±8.06^{a}	49.33±3.05^{a}	46–52
25	43.33±6.96^{b}	45.00±1.73^{a}	43–46

Note: Mean±SD in each column superscripted with different lowercase letters are significantly ($p<0.05$) different.

Table 2. Average water quality values (mean±SD) at various salinity levels.

Salinity (ppt)	DO (mg·L^{-1})	TAN (mg·L^{-1})	Nitrite (mg·L^{-1})	Nitrate (mg·L^{-1})	Alkalinity (mg·L^{-1} as $CaCO_3$)	pH	T (°C)
15	7.38±0.28	0.235±0.086	0.043±0.019	1.41±0.51	100.71±6.21	8.27±0.04	29.0±0.75
20	7.24±0.34	0.255±0.101	0.044±0.019	1.25±0.52	134.38±6.78	8.37±0.05	29.2±0.79
25	7.20±0.36	0.301±0.118	0.048±0.023	0.75±0.39	166.57±4.79	8.55±0.05	29.3±0.79

Note: DO = dissolved oxygen; TAN = total ammonia nitrogen

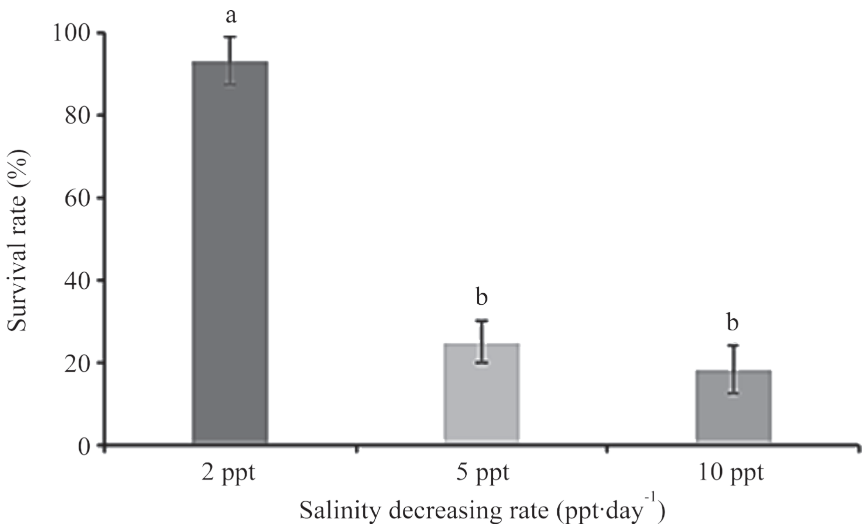

Figure 1. Bars showing survival rates of juvenile dwarf blue shrimp exposed to different acclimatization rate (ppt·day^{-1}).

Note: Error bars indicate SD; Different lowercase letters above bars indicate significantly ($p<0.05$) different among treatments.

DISCUSSION

The results indicated that the dwarf blue shrimp would have an early life cycle in the estuary, where the salinity ranged from 15 to 25 ppt. This pattern is similar to other species in the *Caridina* genus, such as *C. leucosticta, C. multidentata,* and *C. typus* (Hamasaki *et al*., 2020), *Caridina gracilirostris* (Heerbradt and Lin, 2006), and within the Atyidae family like *Atya lanipes* (Perez *et al*., 2015). This shrimp species will mate and lay eggs in freshwater environments. Here, the newly hatched zoea would drift downstream to the river estuary, where the salinity is higher and food supply is abundant. Hamasaki *et al*. (2023) noted that the shrimp zoea do not molt or absorb yolk while drifting downstream. Upon reaching the optimum salinity, shrimp zoea undergo first metamorphosis. The experiments revealed that the optimal salinity for the dwarf blue shrimp is 20 ppt, which is based on the significantly highest survival rate (64.22±8.06%). The shrimp zoea could survive in salinity levels between 15 and 25 ppt, a range similar to *Caridina japonica* (Hayashi and Hamano, 1984) but different from *C. multidentate* and *Macrobrachium rosenbergii* (Sandifer *et al*., 1975), for which the optimal salinity was 25.5 ppt, and *C. prahsahdi*, which had an optimal salinity of 17.7 ppt. Differences in optimal salinity levels limit species distribution. More salinity-tolerant species, like *C. multidentate*, are found in rivers connected to coastal areas in temperate or subtropical zones, whereas *C. prashadi* is restricted to only temperate coastal areas only. Given that normal seawater salinity exceeds 25 ppt, it can be assumed that the distribution of the dwarf blue shrimp is limited to the inner Gulf of Thailand (Honda *et al*., 2011; Hoarau, 2018; Hamasaki *et al*., 2023). This assumption is supported by the findings that the zoea at 25 ppt had the fastest metamorphosis duration (45.00±1.73 days). Finding the optimal salinity for dwarf blue shrimp is crucial for solving problems in culturing this species. Based on this study, it can be concluded that transferring zoea to 20 ppt tanks immediately after hatching is likely to enhance their survival and growth rates.

The low survival rate of shrimp zoea at 15 ppt was due to deficiencies in key elements such as Na^+, Mg^{2+}, and K^+, which are crucial for osmoregulation. This phenomenon has also been reported in other species tolerant to low salinity, such as the Whiteleg shrimp (*Litopenaeus vannamei*, Saoud *et al*., 2003) and *Caridina serratirostris* (Hoarau, 2018). Additionally, significantly low amounts of Mg^{2+} and K^+ at low salinity can negatively impact shrimp growth and survival rates (Roy *et al*., 2007; Chand *et al*., 2015). Apart from osmoregulation issue, Zhu *et al*. (2004) pointed out that a lack of K^+ led to loss of appetite, inactivity, and ultimately death. Hurtado *et al*. (2007) found that malnutrition can result in insufficient energy for daily activities, eventually leading to death.

The lower survival rate of shrimp zoea at 25 ppt was likely due to physiological stress. To maintain water and mineral balance, the zoea must use stored energy reserves in the form of protein and fat. A lack of protein and energy reserves results in insufficient energy for growth, causing the death of susceptible individuals, which significantly lowers the survival rate.

All water quality parameters (Table 2) met the standards for shrimp culture set by the NBACF (National Bureau of Agricultural Commodity and Food Standards) of Thailand. However, the levels of alkali and pH in the 25 ppt treatment were higher compared to the other treatments, likely due to greater alkaline concentrations in the soil, though still within safe ranges for aquaculture (Venkateswarlu *et al*., 2019). The nitrate level in the 25 ppt treatment was significantly lower than in the other treatments. Since ammonia is a main source of nitrate in water, lower nitrate levels may indicate lower ammonia levels, making this parameter safe for shrimp cultivation.

Salinity not only increased mortality rates, but also affected the metamorphosis duration of the shrimp. Shrimp larvae reared at the lowest salinity (15 ppt) had the longest metamorphosis duration, while those at 25 ppt had the shortest. Salinity directly impacts the metabolic rate of shrimp (Vázquez *et al*., 2016; Honda *et al*., 2021; Kondo *et al*., 2021), causing shrimp larvae at higher salinity levels to spend fewer days in metamorphosis, significantly less than those at 20 ppt. From an aquaculture point of view, the survival rate is more important, making 20 ppt the recommended salinity for dwarf blue shrimp aquaculture.

The metamorphosis rate of dwarf blue shrimp kept in water at 29 °C and 20 ppt was about 46–52 days, longer than for *C. multidentate* (21.4–24.7 days, Hamasaki *et al*., 2020) but shorter than *C. gracilirostris* (96–119 days, Heerbradt and Lin, 2006). Different durations for metamorphosis are likely due to species and salinity levels. Other research suggests that water temperature and food availability also contribute to the metamorphosis rate of caridean shrimps. For instance, Idrisi and Salman (2005) reported that the appropriate temperature for *Caridina babaulti basrensis* was 25–35 °C. However, the current study did not consider temperature as it was not expected to have a severe impact on tropical species.

The results from experiment I showed that the dwarf blue shrimp could be considered amphidromous, spending its planktonic larval stage in a river estuary. After completing metamorphosis, juvenile shrimp transition from a benthic form and begin their upstream migration to freshwater habitats. In natural habitats, juvenile shrimps gradually migrate upstream against the water flow. In contrast, tank-kept juveniles require an appropriate acclimatization rate. The recommended acclimatization rate for dwarf blue shrimp, based on the current study, is 2 ppt·day^{-1}, producing the highest survival rate (93.33±5.77%), while other treatments had survival rates below 30%. Differences in survival rates relate to osmoregulation, where lower salinity reduces the availability of key minerals required for osmoregulation, particularly alkaline earths (Na^+, K^+, and Mg^{2+}). These elements play an important role in osmoregulation and as neurotransmitters in the nervous system; inadequate levels during early life stages may cause substantial mortality (Chand *et al*., 2015; Havird *et al*., 2019).

The higher mortality rate of juveniles in the 5 ppt and 10 ppt treatments was due to a physiological response, especially in osmotic pressure following an abrupt salinity change. Somsueb *et al*. (1993) found that an immediate salinity change causes significant water leakage into the body instead of minerals, disrupting cell metabolism. Giffard-Mena *et al*. (2011) noted that water leakage resulted from a dysfunction of the protein named “aquaporin”. As this protein increases with growth, shrimps of the same size exposed to an immediate salinity change suffer more from water leakage than those treated with gradual salinity change. To mitigate water leakage, shrimps need more energy from assimilation of reserved protein and fats. Cheng and Liao (1986) and Somsueb *et al*. (1993) reported that the oxygen consumption in shrimp increased to 200% when the salinity changed from 37 ppt to 10 ppt.

This research pioneered culturing techniques and conditions for dwarf blue shrimp. However, more in-depth studies are required, including proposing a suitable scientific name, identifying appropriate feeding items for shrimp larvae, and other factors to maximize survival rates and sustain natural shrimp population.

CONCLUSIONS

The dwarf blue shrimp is an amphidromous species that can be successfully cultivated in aquariums. The zoea of the dwarf blue shrimp can survive at salinity levels ranging from 15 to 25 ppt, with the optimum salinity being 20 ppt. The appropriate acclimatization rate for juvenile dwarf blue shrimp to a salinity reduction is 2 ppt·day^{-1}. The dwarf blue shrimp requires 53–75 days to fully develop to juveniles, while the zoeal stage lasts 46–52 days. Our study pioneered the culturing of native dwarf shrimp species, and it is hoped that our findings will benefit the cultivation of other native shrimp species.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, for providing the experimental facilities.

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