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STARVATION TOLERANCE OF EARLY STAGE *LAGUNCULA PULCHELLA* (NATICIDAE)

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ABSTRACT The starvation tolerance of the moonsnail *Laguncula pulchella* was examined under nonfeeding conditions in the laboratory using individuals at the early stages of development, which comprised subadults (≤19 mm shell width) and newly hatched juveniles (≤1.7 mm). The newly hatched juveniles survived for ≤86 days at 20°C, ≤56 days at 25°C, and ≤22 days at 30°C, whereas subadults survived for ≤323 days at 20°C and ≤181 days at 30°C. These starvation tolerance ranges were relatively greater than those of other gastropods. There was a positive correlation between shell size and the duration of juvenile survival, indicating that the juveniles hatched at larger sizes have more energy stores. The high starvation tolerance of *L. pulchella* might be contributing to its invasion success in habitats with low prey availability.

KEY WORDS: *Laguncula pulchella*, naticid gastropod, nonfeeding experiment, early survival, carnivore

INTRODUCTION

Starvation can influence the feeding behavior and growth of marine molluscs. For example, the scavenging gastropod *Nassarius fraterculus* tends to eat conspecifics as starvation progresses (Zhang & Goshima 2013). Furthermore, starvation during the first few days after metamorphosis reduces the growth and survival rates of the early juveniles of the abalone *Haliotis discus hannai* (Takami et al. 2000). Starvation tolerance in marine snails has rarely been studied, but it varies largely among species. The average survival duration without feeding at 10°C is approximately 40 days for *N. fraterculus* (Zhang & Goshima 2013), ≥100 days at 21°C for the scavenging gastropods *Babylonia lutosa* and *Nassarius festivus* (Morton 1990), and approximately 15 days at 20°C for early juveniles of the herbivorous abalone *H. discus hannai* (Takami et al. 2000). In addition, the median starvation tolerance of the adults of another carnivorous snail *Nucella lamellosa* (formerly *Thais lamellosa*; Muricidae) is 91 days at 11°C (Stickle 1971). To the best of our knowledge, the starvation tolerance of Naticidae (carnivorous) has not yet been reported.

The moonsnail *Laguncula pulchella* (formerly known as *Euspira fortunei*; Naticidae) is a nonindigenous infaunal snail with the maximum shell height exceeding 50 mm that inhabits tidal flats in the intertidal zone in Japan. It is a predator of molluscs, primarily bivalves especially the asari clam *Ruditapes philippinarum* (Sato et al. 2012), and the moonsnail feeds on the soft bodies of its prey by drilling the shells of prey (Hasegawa & Sato 2009, Chiba & Sato 2012). The moonsnail was introduced to Japan after being mixed with the asari clam in imports from China and the Korean Peninsula (Okoshi 2004). The invasion of *L. pulchella* has caused intensive predation on the local asari clams in Miyagi and Fukushima prefectures of northeastern Japan (Sakai 2000, Okoshi 2004, Tomiyama et al. 2011, Sato et al. 2012, Tomiyama 2018). The density of *L. pulchella* increased from 1.0 to 14.7 individuals m^{-2} in Fukushima Prefecture from 2003 to 2010 (Tomiyama et al. 2011). Mature *L. pulchella* females lay egg collars, only once per individual per breeding season, on the surface of the tidal flats from September to November with ambient water temperature of approximately 20°C (Sakai & Suto 2005, Tomiyama 2013). Approximately 1,000–4,000 benthic juveniles hatch directly from an egg collar, and the juveniles feed on juvenile clams immediately afterward (Sakai & Suto 2005). The opportunity to encounter prey is an important factor in determining the survival of juveniles and the invasion success of this species.

This study aimed to assess the starvation tolerance of *Laguncula pulchella* in the early life stages, namely newly hatched juveniles and subadults. Starvation tolerance (number of days without food) is the basic information related to the success of invasion into new habitats.

MATERIALS AND METHODS

Animal Collection

Egg collars and subadults (<20 mm shell width, most likely 1 y old) of *Laguncula pulchella* were collected from the Misuji River estuary (34° 21' N, 132° 21' E), located in western Japan, in 2020 and 2021. The estuary forms a tidal flat with an area of approximately 60,000 m^2, which is utilized as a clam fishing ground (Yoshida et al. 2017). Annual water temperature and salinity in the estuary ranged 11°C–29°C and 17–31, respectively, in 2015 (Yoshida et al. 2019).

Moonsnails often crawl on the sand surface during ebb tides. Crawling *Laguncula pulchella* individuals were manually collected from the tidal flat in August 2020 and June 2021, when the ambient water temperature was 31.2°C and 26.4°C, respectively. Because *L. pulchella* matures at a shell width ≥21 mm (shell height of 25 mm, corresponding to 2 y of age; Okoshi & Sato-Okoshi 2011), individuals with a shell width <20 mm were considered to be subadults and were brought to the laboratory for the experiment.

To obtain newly hatched juveniles (hatchlings), egg collars were collected from the tidal flat in October 2020 and November 2021, when the ambient water temperature was 24.7°C and 21.4°C, respectively. Four egg collars were collected each year. A piece (1.3–2.9 g in wet weight) was cut from each egg collar (4.3–19.3 g in wet weight) and was placed in a 300 mL plastic bottle, which was filled with aerated seawater with a salinity of 30. The water temperature was kept at 20°C (same as the room temperature) until hatching. In 2020, 225–292 juveniles began to hatch from each egg collar piece in late November, and 84–395 juveniles hatched from each egg collar piece from

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early December 2021. These newly hatched juveniles, obtained from November 24 to 28 in 2020 and from December 8 in 2021 to January 5 in 2022, were used in the experiment.

Starvation Experiment Using Newly Hatched Juveniles

In 2020, a starvation experiment was conducted at 20°C using a total of 65 juveniles hatched from four egg collars (14–18 individuals were randomly selected from the newly hatched juveniles of each egg collar). Similar starvation experiments were conducted at 25°C and 30°C in 2021 using totals of 167 and 153 juveniles hatched from four egg collars for 25°C and 30°C, respectively (32–45 juveniles were randomly selected from the juveniles of each egg collar). The juveniles were individually transferred to a 1-cm deep cell (approximately 2 cm^2) in 24-well plates filled with artificial seawater with a salinity of 30 (Fig. 1C). The water temperature was adjusted by keeping the room temperature at 20°C or by using incubators (MIR-154-PJ, PHC Corp., Tokyo) set at 25°C and 30°C. The seawater was changed every 2 days and the survival or death of each individual was monitored. Survival or death was assessed based on the movement or heartbeat inside the shell. The shell width of dead juveniles was measured to the nearest 0.1 mm under a stereomicroscope. Changes in shell width during the experiment were considered negligible as there was no food supply. The shell width ranged from 0.6 to 1.7 mm, with an average of 1.1 mm.

Starvation Experiment Using Subadults

The collected subadult *Laguncula pulchella* (n = 20) had 10.6–19.0 mm shell widths (11.2–20.9 mm shell heights). They were kept in an aquarium measuring 315 mm × 185 mm × 240 mm (length × width × height, approximately 14 L), which was divided into two compartments using a plastic plate to reduce the risk of cannibalism. Potential water exchange between compartments was unlikely. Each compartment was filled with glass beads with a particle diameter of 4.6–5.4 mm to a depth of 7 cm, based on methods outlined in Hasegawa and Sato (2009). The seawater (salinity of 30) was added to a depth of 10 cm and was aerated. The water temperature was adjusted to 20°C or 30°C in 2020 and 2021, respectively, by keeping the room temperature at these temperatures and the water temperature was monitored every week. The actual temperatures for the 20°C and 30°C conditions were 20.5 ± 1.3°C and 29.9 ± 0.4°C, respectively. Approximately half of the seawater was replaced every 2 wk with natural seawater. Ten individuals (five individuals × two compartments) were used for each temperature (Fig. 1A). They were reared without feeding and the survival or death of each individual was observed daily. The death of *L. pulchella* was determined when it extended an unmoving part of its soft body out of the shell (Fig. 1B). Dead individuals were removed as soon as they were detected to prevent deterioration of the water quality.

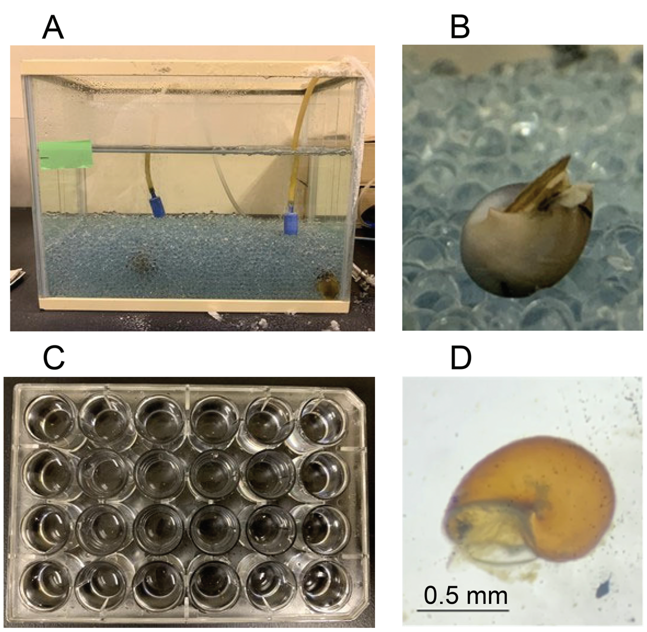

Figure 1. Starvation experiments with *Laguncula pulchella*. (A) The experiment on subadults. (B) A dead individual with a 13 mm shell width. (C) The experiment on newly hatched juveniles. (D) A dead juvenile.

Statistical Analyses

To test whether shell size and/or temperature affected the starvation tolerance of newly hatched juveniles, a linear mixed model was constructed in which survival duration in days was used as the response variable. Individual shell width, temperature, and the timing of hatching were used as initial explanatory variables, and the origin of the newly hatched juveniles (egg collar) was used as a random variable. The model was fitted using maximum likelihood and selected based on the Akaike information criterion (AIC).

Similarly, a linear model was constructed for the survival duration of subadults, using the same initial explanatory variables as those of the juveniles. The model was selected based on the AIC for small sample sizes (AICc).

To estimate 50% cumulative mortality in juveniles, a generalized linear mixed model was constructed, assuming a binomial distribution and logit link. This explains the cumulative number of dead and surviving individuals per egg collar piece relative to the temperature and days progressed. The egg collar piece was used as a random variable. A generalized linear model was constructed for the cumulative mortality of subadults and the models were selected based on the AIC.

All statistical analyses were performed using R version 4.1.0 (www.r-project.org). The experimental procedure followed the guidelines of the Hiroshima University Animal Research Committee (registration number 017A191002).

RESULTS

Starvation Tolerance of Newly Hatched Juveniles

The juveniles survived up to 86 days at 20°C, 56 days at 25°C, and 22 days at 30°C (Fig. 2A). The shell widths of juveniles, used for 20°C, 25°C, and 30°C, ranged 0.74–1.28 mm, 0.62–1.73 mm, and 0.67–1.64 mm, respectively. Six juveniles (9.2%, shell width range = 1.00–1.28 mm) survived for more than 70 days at 20°C. The survival durations (mean ± SD) were 25.8 ± 22.5, 15.4 ± 11.1, and 7.2 ± 4.9 days at 20°C, 25°C, and 30°C, respectively. Survival duration was significantly longer in individuals with larger shell widths and under lower

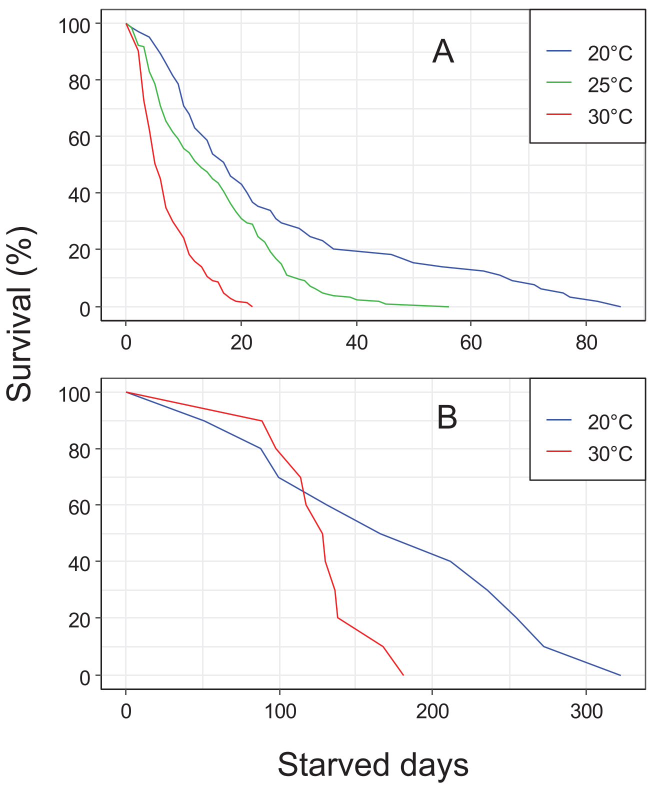

Figure 2. Temporal changes in the survival of *Laguncula pulchella*. (A) Newly hatched juveniles. (B) Subadults.

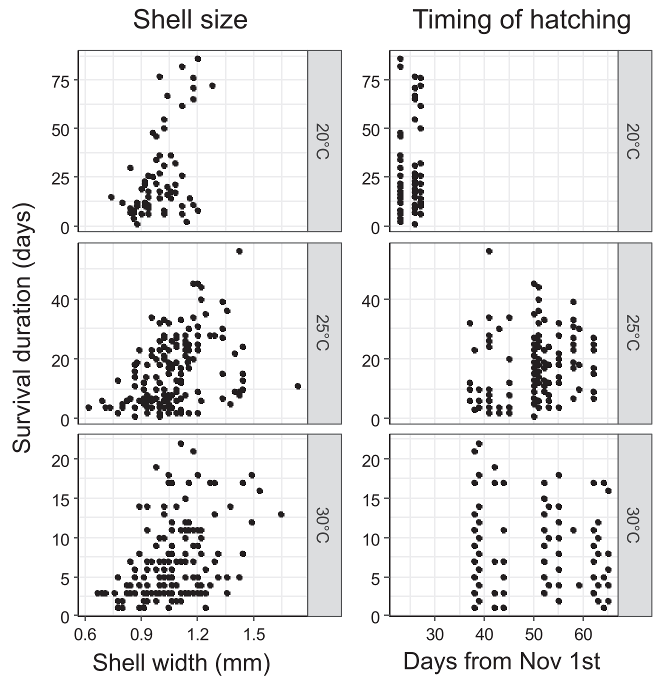

Figure 3. Relationships between survival duration of *Laguncula pulchella* and shell size or the timing of hatching in the starvation experiment on newly hatched juveniles. The timing of hatching was expressed as the days from November 1st, although the experiments were carried out in different years between 20°C and 25 or 30°C.

temperatures (Table 1, Figs. 3 and 4A). No relationship was observed between the timing of hatching and survival duration.

The cumulative mortality of juveniles increased as the days progressed. Both temperature and the number of days were included in the selected generalized linear mixed model, and a positive effect of day progression was detected (Table 2). Cumulative mortality at a given time was highest at 30°C, followed by 25°C and 20°C. The duration for 50% mortality was estimated to be 24, 13, and 2 days at 20°C, 25°C, and 30°C, respectively.

TABLE 1.

Summary (coefficient) of the selected linear mixed model or linear model for survival duration (days) of *Laguncula pulchella* under starvation.

Group	Parameter	Estimate	SE	*P*
Juvenile	Intercept	1.61	3.86	0.68
	Shell width	24.18	3.56	<0.001
	Temperature (25°C)	−11.85	1.81	<0.001
	Temperature (30°C)	−20.17	1.83	<0.001
Subadult	Intercept	183.40	21.12	<0.001
	Temperature (30°C)	−53.50	29.87	0.090

A linear mixed model was used for juveniles, whereas a linear model was used for subadults. The initial explanatory variables were shell width, temperature, and the date of hatching (only for juveniles), and the egg collars were incorporated as a random variable for juveniles. The models were selected based on the Akaike information criterion. The effect of temperature was assessed based on the result at 20°C.

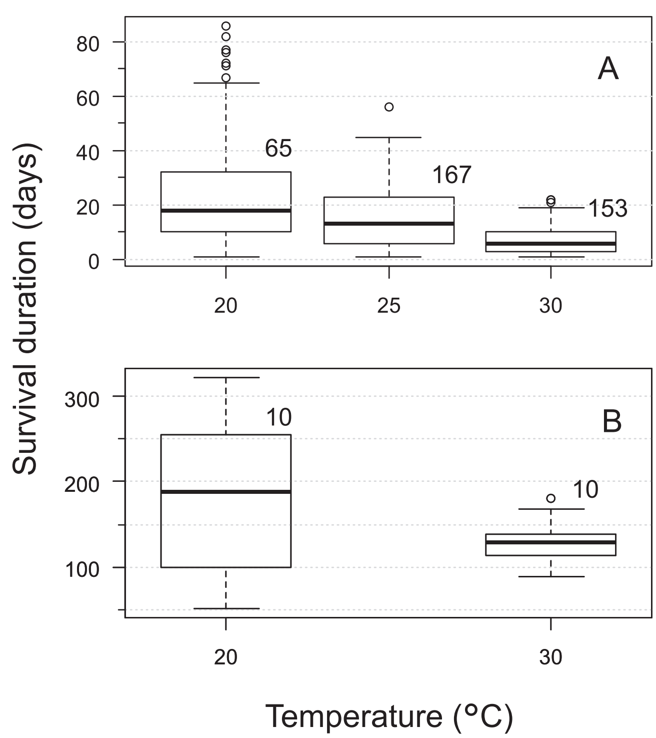

Figure 4. Boxplot showing the survival duration of *Laguncula pulchella* at 20°C–30°C. (A) Newly hatched juveniles. (B) Subadults. Boxes show the 25% and 75% quartiles and median, dashed vertical bars show the maximum and minimum, and open circles show outliers.

TABLE 2.

Summary (coefficient) of the selected generalized linear mixed model or generalized linear model for the cumulative mortality of *Laguncula pulchella* under starvation.

Group	Parameter	Estimate	SE	*P*
Juvenile	Intercept	−2.35	0.18	<0.001
	Days	0.096	0.0019	<0.001
	Temperature (25°C)	1.13	0.24	<0.001
	Temperature (30°C)	2.14	0.24	<0.001
Subadult	Intercept	−4.17	0.50	<0.001
	Days	0.023	0.0025	<0.001
	Temperature (30°C)	1.23	0.28	<0.001

A generalized linear mixed model (binomial family and logit link) was used for juveniles whereas a generalized linear model (binomial family and logit link) was used for subadults. The initial explanatory variables were the number of days and temperature, and the egg collars were incorporated as a random variable for juveniles. The models were selected based on the Akaike information criterion. The effect of temperature was assessed based on the result at 20°C.

Starvation Tolerance of Subadults

Subadults survived for 51–323 days (mean ± SD = 183 ± 90 days) at 20°C and 89–181 days (130 ± 28 days) at 30°C (Figs. 2B and 4B). Shell width was eliminated from the selected model (Table 1). The longest duration of 323 days was observed in a relatively large individual (17.6 mm shell width; collected in August 2020).

In the selected generalized linear model for the cumulative mortality of subadults, both the number of days and temperature were included (Table 2). The duration for 50% mortality was estimated to be 184 days and 130 days at 20°C and 30°C, respectively.

DISCUSSION

This study is the first to elucidate the starvation tolerance of a naticid gastropod ex-situ, with maximum durations of 323 and 86 days being observed in subadults and juveniles, respectively. The survival duration of ≤86 days in juveniles at 20°C is much longer than the approximately 15 days for early juveniles of the herbivorous abalone *Haliotis discus hannai* (Takami et al. 2000). In contrast, hatchlings of the carnivorous *Nucella emarginata* can survive for 50–120 days (Gosselin & Chia 1994). Species with a larger size at hatching showed higher starvation tolerances in some carnivorous snails (*Chorus giganteus*, *Trophon geversianus*, and *Acanthina monodon*), and *T. geversianus* juveniles that were approximately 1 mm at hatching had a 50% cumulative mortality of 45 days at 10.5°C–16°C (Gallardo et al. 2004). Therefore, a high starvation tolerance may be common among carnivorous snails. Such a high starvation tolerance is likely related to the limited opportunities to encounter prey. The density of prey clams with a 1–5 mm shell length, observed in November and December 2020 at the tidal flat, was 1,067 ± 871 individuals m^{-2} (n = 4, mean ± SD; Kinoshita, unpublished data). This indicates that starvation-induced mortality is unlikely for *Laguncula pulchella* in the Misuji River estuary. Because, in the field, juvenile *L. pulchella* experience water temperatures that are <22°C from November, juveniles can tolerate at least 13 days without feeding, which is the 50% cumulative mortality that occurred at 25°C in this study. Thus, a few days of starvation after hatching would have negligible effects on their survival, as suggested for some snails (El-Emam & Madsen 1982).

There was considerable individual variation in the survival duration of juveniles. Moreover, the significant positive effect of shell width on survival duration (Table 1) suggests that larger individuals have larger energy stores. The size variation in newly hatched juveniles may be related to the amount of food consumed before hatching. Juveniles feed on eggs that have stopped developing inside the egg collar (Okoshi & Sato-Okoshi 2011), thus, the amount of eggs consumed by embryos or pre-juveniles within the egg collar during incubation may have caused variation in the energetic status of the juveniles at and after hatching. The timing of hatching did not affect survival duration (Fig. 3), indicating that variation in the size or energy storage of juveniles is not related to the timing of hatching in egg collars. While parental condition may affect the quality of embryos and eventually the starvation tolerance of juveniles, no effect of food availability was observed on hatchling survival for the intertidal whelk *Cominella virgata* (Sman et al. 2009) or the freshwater snail *Pomacea canaliculata* (Tamburi & Martín 2011).

Subadult *Laguncula pulchella* showed greater starvation tolerance than the juveniles. Individuals used for the experiment at 20°C were collected in August, when the somatic condition of *L. pulchella* is low (Yoshida et al. 2022). Therefore, survival duration may be longer in fasting individuals with high-energy storage. To our knowledge, >300 days of survival duration under fasting conditions at 20°C is the longest period recorded for marine snails, although three individuals of a carnivorous whelk *Hemifusus tuba* (Melongenidae), which were approximately 38 mm in shell length, survived for 273 days without food at 21°C (Morton 1986). Thus, many carnivorous snails may have a high starvation tolerance, as suggested for juveniles.

Soft body weight decreases as starvation progresses. Although this study did not assess the reduction of soft body weight in juveniles or subadults under starvation conditions, the soft body weight of *Laguncula pulchella* adults (shell height = 29–44 mm, n = 4) decreased by 32% after 77 days of starvation from early July at 23.6 ± 1.1°C (mean controlled temperature ± SD) under laboratory conditions (Kinoshita, unpublished data). Simultaneously, the operculum moved deeper into the shell from the opercular lip when the soft body was retracted into the shell. Similar changes in opercular position were observed for both juveniles and subadults in this study. Thus, somatic conditions and the position of the operculum may be important considerations when assessing the starvation status of snails.

The high starvation tolerance of *Laguncula pulchella* might have been one of the factors that enabled its invasion into food-limited areas. Nevertheless, the drastic increase in the nonindigenous population of *L. pulchella* during a few years has only been observed in northern Japan (Sakai 2000, Okoshi 2004, Tomiyama et al. 2011). The limited prey density available for adult *L. pulchella* in western Japan (Yoshida et al. 2017) may affect the survival of newly hatched juveniles. Further studies on the mechanisms of invasion success and the factors regulating the population size of *L. pulchella* are needed.

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