Some Biological Aspects and Rearing of Ragged Sea Hare (*Bursatella leachii* de Blainville, 1817) in the Hatchery

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ABSTRACT

Samples of the ragged sea hare, *Bursatella leachii*, were collected in the marine shrimp ponds during December 2014–January 2015 to study some biological aspects (spawning and egg development) and condition factors (feedstock, substrates and stocking density) on reared *B. leachii*. The results showed that under hatchery conditions (salinity 32 ppt, water temperature 28.5–29.1°C, DO 4.19–5.11 mg/L and pH 8.14–8.32), *B. leachii* adults were clustered together in groups of 3–5 individuals for spawning. Egg development from brownish to dark brown took 6–7 days until newly hatched veligers (larval stage 1) were produced. The results of the condition factors experiment showed that the mean body weight of *B. leachii* fed with seaweeds (*Caulerpa* sp.) and mixed feeds (50% seaweeds, fresh weight and 50% shrimp feed No. 2, pellet size about 0.8 – 1 mm) increased, whereas it decreased when *B. leachii* was fed with shrimp feed No. 2. Rearing without or with sand substrate increased the growth of ragged sea hare, but having a clay substrate adversely affected growth. The growth and survival rates of *B. leachii* reared at stocking densities of 5, 10 and 15 sea hares/100 L (or about 2.5, 5.0 and 7.5 kg sea hares/m^3, respectively) were not significant different. This study recommends that *B. leachii* can be reared and spawned in a hatchery, with feedstock and substrates affecting growth, whereas stocking density not affecting growth and survival.

Keywords: ragged sea hare, *Bursatella leachii*, aquaculture

INTRODUCTION

Nudibranchs or sea hares are mollusks best known for displaying a fascinating range of colors and body forms. The ragged sea hare, *Bursatella leachii*, is a marine opisthobranch gastropod mollusk belonging to the family Aplysiidae and the order Anaspide (Wägele and Klussmann-Kolb, 2005). *Bursatella leachii* is a circum-tropical species, widespread in the temperate water of the Indo-Pacific Ocean, the Caribbean Sea and the Mediterranean Sea (Gofas and Zenetos, 2003; Zakhama-Sraieb *et al*., 2009). In Thailand, the distribution of this species has generally been reported from the inter-

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tidal zone in coastal areas and sometimes in marine animal pond such as shrimp ponds. Ragged sea hares are not collected for human consumption in Thailand; therefore, being less popular, they are believed to adversely affect commercial shrimp culture. However, Otero *et al*. (2013) reported that positive economic impacts include the existence of a small aquarium trade for this sea hare and the potential pharmacological use of its ink gland. The marine natural compounds with anti-cancer qualities and anti-HIV protein derived from this sea hare have been under various stages of clinical trials (Rajaganapathi *et al*., 2002; Haefner, 2003; Avila, 2006). It remains to be seen whether there will be tangible biomedical and economic benefits derived from this discovery. However, this does show its substantial potential as a candidate species for aquaculture.

The habitat, reproductive and dietary preferences of *B. leachii* are now well-known (Ramos *et al*., 1995; Gofas and Zenetos, 2003; Wägele and Klussmann-Kolb, 2005; Capper *et al*., 2006; Zakhama-Sraieb *et al*., 2009; Otero *et al*., 2013), facilitating animal husbandry. However, studies on its aquaculture are limited. Therefore, the objectives of this study were to investigate some biological aspects (spawning and egg development) and condition factors (feedstock, substrates and stocking density) affecting the growth and survival rate of *B. leachii* reared in a hatchery. This information will be useful for sea hare production in the future.

MATERIALS AND METHODS

Study site and source of experimental *B. leachii*

This study was conducted at the hatchery of the Klongwan Fisheries Research Station (KFRS), Prachuap Khiri Khan province, Thailand. *B. leachii* adults were collected along the marine shrimp ponds of KFRS during December 2014–January 2015 (Figures 1–2). The water quality during the collecting periods had the following levels: salinity 32–33 ppt, water temperature 27.2–29.4°C, DO 4.26–5.83 mg/L and pH 8.01–8.57. The specimens were transferred indoors to 2,000 L concrete tanks and fed with seaweed (*Caulerpa* sp.) until the experiment commenced.

Experimental design and set-up

Experiment 1 – Different feeds

B. leachii with body weights ranging from 20 to 30 g were transferred from concrete tanks to 10 L plastic tanks without substrates. The ragged sea hares were individually reared and fed with different feeds, namely: 1) seaweed (*Caulerpa* sp.); 2) shrimp feed No. 2 (STARTEQC™, pellet size about 0.8–1 mm, 38% protein); and 3) mixed feeds (50% seaweed, fresh weight and 50% shrimp feed No. 2). Feeding rate was 5% of body weight per day (once a day at 9.00 AM.) for 30 days, with 15 replicates per treatment.

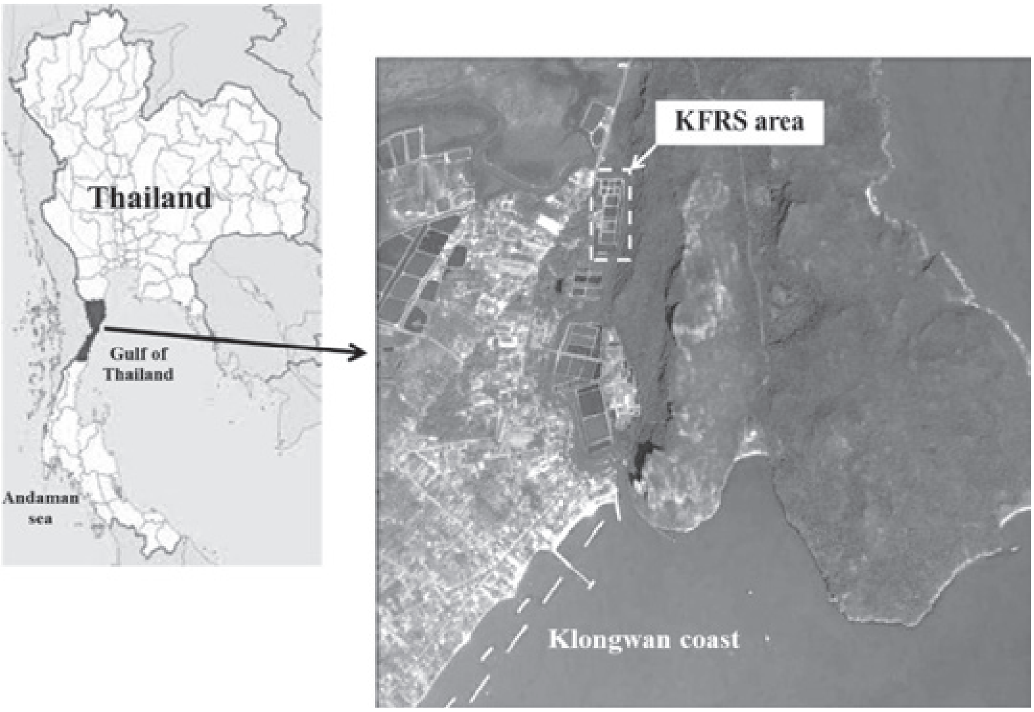
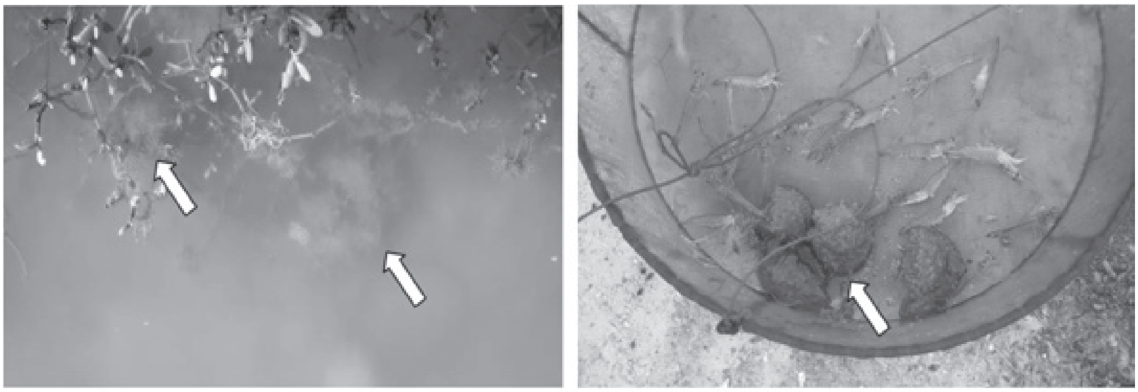


Figure 1. Sampling sites for ragged sea hare, *Bursatella leachii*, at KFRS, Prachuap Khiri Khan province, Thailand (11°44′ N, 99°47′ E).



(a) (b)

Figure 2. Ragged sea hare, *Bursatella leachii*, were found clustered together in groups of 4–8 individuals in marine shrimp ponds at KFRS: (a) around pond edges and (b) in feeding trays.

Experiment 2 – Different substrates

B. leachii with body weights ranging from 30 to 40 g were transferred from concrete tanks to 10 L plastic tanks. Plastic tank conditions varied with different substrates: 1) without substrate; 2) sand substrate; and 3) clay substrate. The ragged sea hares were individually reared and fed with seaweed (*Caulerpa* sp.) at 5% of body weight per day (once a day at 9.00 AM) for 30 days, with 15 replicates per treatment.

Experiment 3 – Different stocking densities

B. leachii with body weight of about 50 g were transferred from the concrete tanks to 100 L plastic tanks without substrates at different stocking densities: 5, 10 and 15 sea hares per 100 L tank (2.5, 5.0 and 7.5 kg sea hares/m^3, respectively). They were fed with seaweed (*Caulerpa* sp.) at about 5% of body weight per day (once a day at 9.00 AM) for 30 days, with 3 replicates per treatment.

Data collection

Data on biological spawning and egg development of *B. leachii* in the hatchery used the descriptive method. The condition factors experiment was based on the main data collected during the study trial consisting of growth and survival rates of *B. leachii* every 10 days. During the rearing period, water exchange was undertaken every 3 days at about 50% of the total volume. To remove left over feed and detritus at each water exchange, aeration was stopped temporarily and settled particles were siphoned out from the tank bottom. Water quality was analyzed every 3 days. Salinity was measured using a refractometer (Prima tech), pH using a pH meter (Cyber Scan pH 11), temperature and dissolved oxygen concentration (DO) using an oxygen meter (YSI 550A), total ammonia using Koroleff's Indophenol blue method, nitrite using the colorimetric method, and alkalinity using the titration method according to American Public Health Association *et al*. (2005).

Statistical analysis

Simple descriptive biological aspect analysis was used to draw basic conclusions about the characteristics of spawning and egg development. At the end of the experiments, for the different feeds and substrates, the difference data on the growth of *B. leachii* between before and after rearing were analyzed using paired-samples *t*- tests. For different stocking densities, data on the growth and survival rate of *B. leachii* were analyzed using one-way ANOVA and the difference between means was tested using Duncan's multiple range test at the 95% level of confidence. Data were analyzed using the IBM SPSS Statistics program.

RESULTS AND DISCUSSION

The characteristics of ragged sea hare, *Bursatella leachii*, from marine shrimp ponds are shown in Figure 3. During the collection period, the length and weight of *B. leachii* recorded were in the ranges of 45 to 140 mm and 8 to 80 g, respectively (n = 255). The average (±standard deviation) length and weight were 82.0±37.4 mm and 38.0±16.0 g, respectively. For comparison, the size of *B. leachii* recorded in northeast Queensland, Australia was 30–50 mm (Clarke, 2006), in the Mediterranean coast of Turkey, the size was 50–100 mm and up to 150 mm (Kazak and Cavas, 2007; Özvarol, 2014), while on the east coast of India, the maximum length and weight were 75 mm and 23 g, respectively (Sethi *et al*., 2015).

Spawning and egg development

Under hatchery conditions (salinity 31-32 ppt, water temperature 28.5–29.1°C, DO 4.19–5.11 mg/L and pH 8.14–8.32), *B. leachii* adults were clustered together in groups of 3–5 individuals for spawning. The eggs of *B. Leachii* were found in long brownish strings resembling spaghetti noodles, and egg development from brownish to dark brown colors took 6–7 days and then produced a newly hatched veliger, larval stage 1 (Figures 4–5). Otero *et al*. (2013) reported that *B. leachii* was a hermaphroditic species with a very fast life cycle and continuous reproduction. When mating, one individual acts as a male and crawls onto another one to fertilize it. Vue (2009) reported that *B. leachii* larval stage 1 had a maximum shell diameter of 141.1±6.9 µm and the veliger's shell grows rapidly by an average of 2 µm per day at water temperature 25°C.

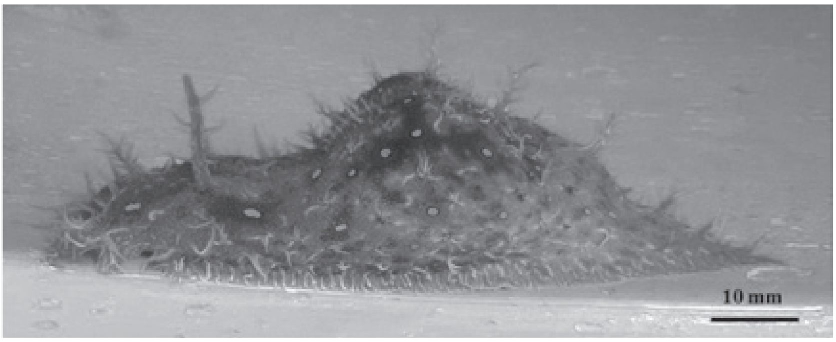
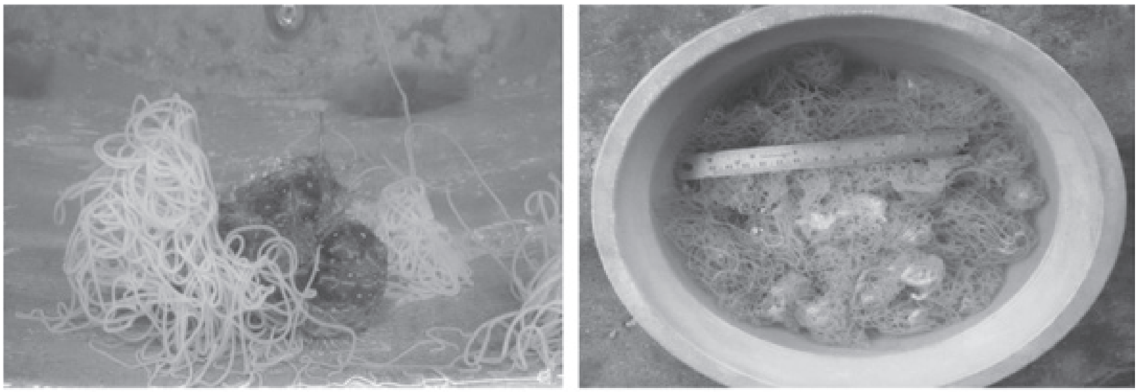


Figure 3. Characteristics of ragged sea hare *Bursatella leachii* de Blainville, 1817.



(a) (b)

Figure 4. Aggregation characteristics of ragged sea hare, *Bursatella leachii* for spawning in hatchery (a), and harvesting egg mass from *B. leachii* in hatchery (b).

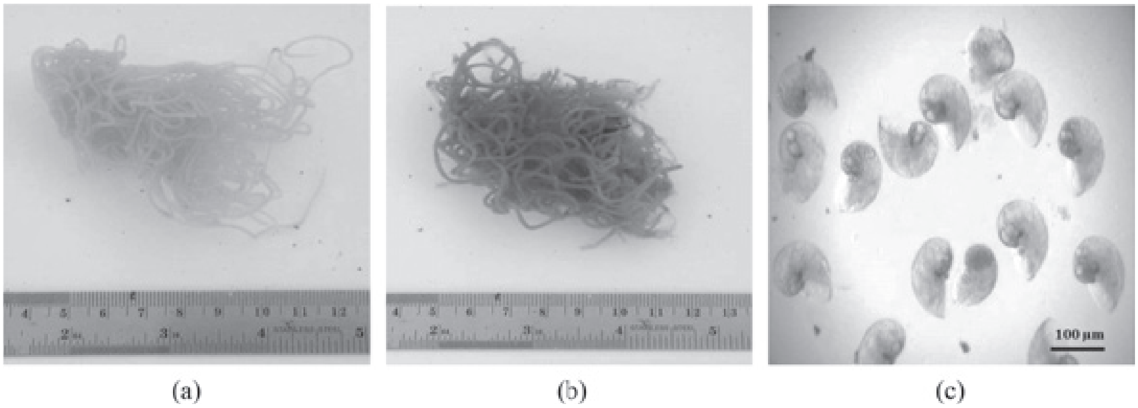


Figure 5. Egg development of ragged sea hare, *Bursatella leachii*, in hatchery: (a) long brownish strings, (b) long dark brown strings, and, (c) newly hatched veliger.

Effect of feedstock

Results showed that the mean body weight of *B. leachii* fed with seaweed (*Caulerpa* sp.) and mixed feed (50% seaweed and 50% shrimp feed No. 2) increased from 26.15±2.15 to 46.08±7.19 g (*t*- test, P = 0.000; Figure 6A) and from 26.25±2.22 to 33.33±6.85 g (*t*- test, P = 0.006; Figure 6C), respectively. In contrast, the body weight of *B. leachii* fed with shrimp feed No. 2 decreased from 25.00±1.87 g to 18.00±2.43 g (*t*- test, P = 0.003; Figure 6B), at 30 days of the experiment. This study showed that the feedstock affected the growth of *B. leachii*. Clarke (2006) reported that the feeding preferences of *B. leachii*, such as green algae, cyanobacterium, brown macroalga and red macroalga, were abundant in its natural habitat. Capper *et al*. (2006) reported that the toxic marine cyanobacterium, *Lyngbya majuscule*, was a preferred food choice for two species of sea hare, *Stylocheilus striatus* and *Bursatella leachii*, and both species preferentially consumed and grew well on an exclusive diet of *L. majuscula*.

Effect of substrate

Results showed that the mean body weights of *B. leachii* reared without substrate and with sand substrate increased from 34.82±2.27 to 47.55±4.03 g (*t*- test, P = 0.000; Figure 6D) and 35.31±2.43 to 47.62±5.88 g (*t*- test, P = 0.000; Figure 6E), respectively. However, the body weight of *B. leachii* reared using a clay substrate decreased from 34.57±3.60 to 27.86±6.36 g (*t*- test, P = 0.029; Figure 6F), at 30 days of the experiment. This study showed that rearing *B. leachii* with or without sand substrate can increase their growth while clay substrates decreased their growth. Based on field observations of *B. leachii*, many researchers reported that this species occurs most commonly in shallow waters often on sandy or muddy bottoms with *Caulerpa prolifera*, being well camouflaged in seagrass and seaweed beds (Clarke, 2006; Kazak and Cavas, 2007; Otero *et al*., 2013; Sethi *et al*., 2015).

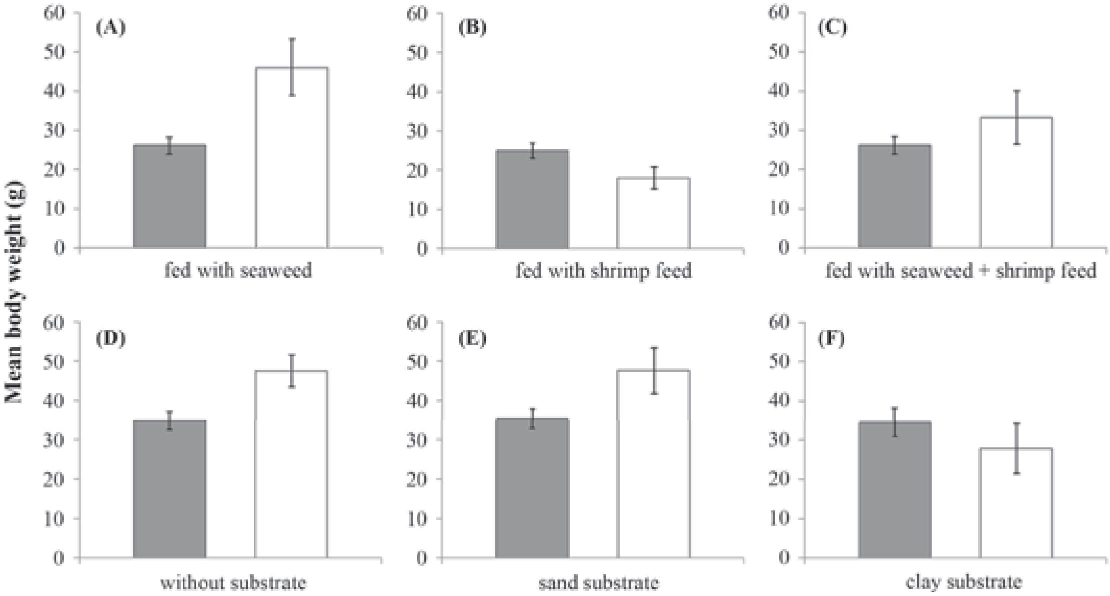


Figure 6. Growth rates of ragged sea hare, *Bursatella leachii*, reared in hatchery from initial (■) to 30 days (□) using different feedstocks (A, B and C, *n* = 15), and different substrates (D, E and F, *n* = 15). Paired sample *t*- tests were used to analyze data.

Effect of stocking density

The mean body weights of *B. leachii* reared at densities of 5, 10 and 15 sea hares/100 L increased from 51.45±3.15 to 63.43±5.08 g, 52.04±2.80 to 60.03±3.91 g, and 50.79±1.92 to 62.50±4.02 g, respectively, at 30 days of experiment, but these results were not significantly different (ANOVA, *P* = 0.625; Figure 7A). In addition, the survival rates of *B. leachii* reared at densities of 5 (86.66±11.54%), 10 (90.00±10.00%) and 15 (90.66±8.08%) sea hares/100 L were not significantly different (ANOVA, *P* = 0.873; Figure 7B). This study showed that stocking density did not affect growth and survival of *B. leachii*.

Water quality

Water quality analysis during the rearing period showed the following levels: salinity 31–32 ppt, water temperature 28.9–30.2°C, DO 4.49–5.58 mg/L, pH 8.17–8.52, total ammonia 0.000–0.213 mg/L, nitrite 0.000–0.159 mg/L and alkalinity 111–134 mg/L as $CaCO_3$. Vue *et al*. (2014) reported that the optimum seawater temperature for *B. leachii* aquaculture was 25ºC. However, desirable levels for the other water parameters were not clearly reported.

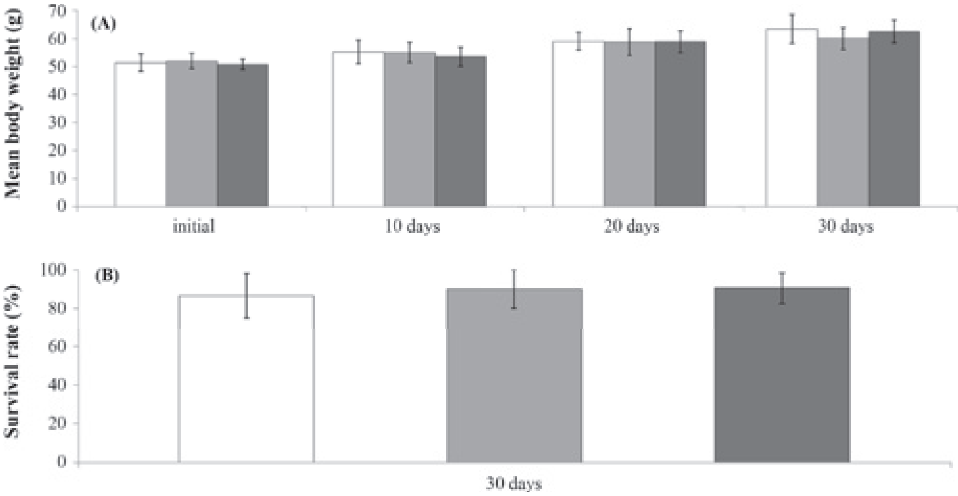


Figure 7. Growth (A) and survival (B) rates of ragged sea hare, *Bursatella leachii*, reared in hatchery at different stocking densities: 5 (□), 10 (■) and 15 (■) sea hares/100 L, *n* = 3. One-way ANOVA was used to analyze data.

CONCLUSION

The ragged sea hare, *Bursatella leachii*, can be reared and spawned under hatchery conditions. In addition, at 30 days rearing period, the mean body weights of *B. leachii* increased when fed with seaweeds and mixed feeds (from 26.15±2.15 to 46.08 ±7.19 g and from 26.25±2.22 to 33.33±6.85 g, respectively), but decreased when fed with shrimp feed No. 2 (from 25.00±1.87 to 18.00±2.43 g). The mean body weights of *B. leachii* reared without substrate and with sand substrate increased (from 34.82±2.27 to 47.55±4.03 g and 35.31±2.43 to 47.62± 5.88 g, respectively) but decreased when a clay substrate was used (from 34.57±3.60 to 27.86±6.36 g). The growth and survival rate of *B. leachii* reared at stocking densities of 5 (63.43±5.08 g and 86.66±11.54%), 10 (60.03±3.91 g and 90.00±10.00%) and 15 (62.50±4.02 g and 90.66±8.08%) sea hares/ 100 L were not significantly different. This study demonstrated that the feedstock and substrates affected growth but stocking density did not affect growth and survival of *B. leachii*. However, more extensive research has to be done to determine the effects of other factors (such as nutritional quality of feed and water quality) that affect seed production of *B. leachii*, and to improve rearing management.

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