Introduction

The ball-like white sea urchin, *Salmacis sphaeroides* (Echinodermata: Echinoidea: Temnopleuridae), is one of the regular echinoids, occurs in tropical Indo-West Pacific ocean where it can be found from China to Solomon Islands and Australia (Schoppe, 2000; Miskelly, 2002) and Singapore (Tan and Ng, 1988). It can also be found in the warm temperate regions including Johor States, between Malaysia and Singapore (Rahman et al., 2012). This species can occur at depth ranging between 0 to 90 m, but it is mostly found in shallow waters, especially amongst seagrass meadows, in muddy sublittoral zone or washed ashore and in coral reef areas (Tan and Ng, 1988; Schoppe, 2000). *Salmacis sphaeroides* finds their food from algae, bryozoans, seaweeds and detritus (Miskelly, 2002). It has important biological, ecological, aquacultural, nutritional and pharmaceutical significance (Rahman et al., 2012, 2013a).

The gonads of sea urchin commonly referred to as “sea urchin roe” or ‘uni’ for long has been considered as a luxury food in Japan (Shimabukuro, 1991). Although, S.

Growth and survival of the tropical sea urchin, *Salmacis sphaeroides* fed with different macroalgae in captive rearing condition

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**Abstract**

An experiment was undertaken to develop appropriate culture techniques for high-valued tropical sea urchin, *Salmacis sphaeroides* under captive aqua-rearing conditions. Three-month-old juveniles produced through induced breeding, larval rearing and metamorphic induction were stocked with 20 juveniles in each of nine well-aerated glass aquaria (46 x 30 x 30 cm). Juveniles fed with red alga (*Amphiroa fragilissima*) were designated as Treatment-1 (T1), brown alga (*Sargassum polysystum*) as Treatment-2 (T2) and sea grass (*Enhalus acoroides*) as Treatment-3 (T3). At the time of stocking, juveniles were under the same age group and batch-reared with a mean length and weight of 9.98 ± 0.56 mm and 0.49 ± 0.11 g, respectively. The juveniles were fed *ad libitum*, and the seawater in each rearing aquarium was changed at bi-monthly intervals. The culture was carried out for one year during which time the juveniles attained sexual maturity. Growth performances (viz., final weight, weight gain, final length, length gain, specific growth rate and daily growth rate) and survival of adults were significantly higher (P < 0.05) in T1 than those in T2 and T3, respectively. Gonad production, in terms of wet gonad weight and gonad index, also followed the same trend as that for growth. Hence, of the three algal feed evaluated, red alga appeared to be the most suitable food for rearing of *S. sphaeroides* under captive conditions. The present study is the first demonstration of successful culturing of *S. sphaeroides* in a static aquarium system, the findings of which could be helpful towards the commercial sea urchin aquaculture.

**Key words**

Growth, Macroalgae, Production, Sea urchin aquaculture, *Salmacis sphaeroides*, Survival
sphaeroides has not yet been used as edible species in Malaysia, it has been found to serve as a delicacy food item in local seafood restaurants in Hong Kong (Chen et al., 2010; Rahman et al., 2012). Gonads of sea urchins are also rich in important biologically active compounds such as polyunsaturated fatty acids (PUFA) and \( \beta \)-carotene (Dincer and Cakli, 2007). The PUFA, particularly eicosapentaenoic acid (EPA, C20:5 (n-3)) and docosahexaenoic acid (DHA, C22:6 (n-6)), have substantial protective effects on cardiovascular disease, arrhythmia and cancer (Pulz and Gross, 2004). Some xanthophylls and \( \beta \)-Carotene have potential pro-vitamin A activity and can be utilized to prevent light sensitivity and tumor development (Britton et al., 2004).

High amount of arachidonic acid and EPA eicosapentaenoic acid recently identified in S. sphaeroides (Chen et al., 2010), support the development of aquaculture of this important sea urchin, since PUPAs are essential for human nutrition (Lawrence, 2007). In recent years, the fisheries of sea urchin have extended greatly that their population around the world have been depleted due to overfishing (Andrewed et al., 2002, 2004; Rahman et al., 2012, 2013a). These declining patterns clearly indicate overexploitation of major fishery grounds and focus the necessity for proper conservation strategies, stock enhancement, fishery management and aquaculture development to fill-up the potential gap between the demand and supply.

Considering the potential importance of S. sphaeroides, few studies on its population characteristics, distribution, feeding, breeding and development have recently been carried out (Klumpp et al., 1993; Yulin, 1998; Lane et al., 2000; Tsuchiya et al., 2009; Rahman et al., 2012, 2013a), however no systematic studies have yet been conducted to optimize the juvenile and adult growth and production in culture system. Therefore, an attempt was made to develop suitable techniques for aquaculture and stock improvement of S. sphaeroides under captive rearing condition.

### Materials and Methods

#### Sample collection and maintenance: 
Sexually matured specimens of sea urchin, S. sphaeroides, weighing 90 to 200 g, were collected from Merambong shoal off Tanjung Kupang (01°34' N; 103°60' E), Johor, Malaysia at low tide during their natural spawning season from July to October, 2011. Soon after collection, live sea urchins were transported to the Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, where they were maintained in aerated aquaria at least 7-10 days before used for breeding trails.

#### Spawning and fertilization: 
Gametes were obtained from matured urchins following injection of KCl (0.5 M) into the body cavity. Matured eggs were collected by placing the inverted female individuals on a glass beaker filled with filtered (2.5 µ) sea water, while the concentrated sperms were pipetted off the genital pore from the male individuals. Fertilization was carried out by mixing the eggs with a 10\(^{-5}\) dilution of “dry” sperm (Rahman et al., 2000, 2001, 2005). Sperms were left for at least 10 min to ensure that all the eggs were encountered by sperms during the fertilization process. Excess sperms and debris were then removed from the inseminated eggs by 3 to 4 consecutive washes with filtered sea water (Rahman and Uehara, 2004; Rahman et al., 2004, 2012).

#### Rearing of embryos and larvae: 
Fertilized eggs were incubated in 500 ml glass beakers containing filtered sea water at ambient temperature (25-26°C) until they attained free swimming blastula. They were then reared for 2 days at the same temperature in 500-ml glass bottles containing filtered sea water on 10 rpm rotating motors. Rearing densities of larvae up to four-armed pluteus stage were kept at 3-4 individuals/ml for 2 days, following the protocols described by Rahman et al. (2000, 2005). At the time when larvae attained four-armed pluteus stage, they were reared at a larval density of 1 individual/ml in 1000 ml containing glass.

### Table 1: Mean (± SD) and range values of water quality parameter over the 1-year rearing period of S. sphaeroides in captive aqua-rearing condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1 (Red alga)</th>
<th>T2 (Brown alga)</th>
<th>T3 (Sea grass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>28.86 ± 0.50 (27.89-29.52)</td>
<td>28.84 ± 0.49 (27.85-29.50)</td>
<td>28.82 ± 0.52 (27.80-29.61)</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>30.73 ± 0.73 (29.95-31.77)</td>
<td>30.77 ± 0.83 (29.40-31.89)</td>
<td>30.90 ± 0.80 (29.55-31.98)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg l(^{-1}))</td>
<td>6.98 ± 0.29 (6.40-7.55)</td>
<td>6.92 ± 0.27 (6.35-7.38)</td>
<td>6.86 ± 0.30 (6.25-7.35)</td>
</tr>
<tr>
<td>pH</td>
<td>8.16 ± 0.22 (7.56-8.35)</td>
<td>8.13 ± 0.22 (7.53-8.31)</td>
<td>8.07 ± 0.23 (7.50-8.27)</td>
</tr>
<tr>
<td>Total alkalinity (mg l(^{-1}))</td>
<td>143.96 ± 7.70 (132.80-155.50)</td>
<td>140.44 ± 7.40 (130.40-151.60)</td>
<td>137.92 ± 7.10 (130.20-150.70)</td>
</tr>
<tr>
<td>Ammonia nitrogen (mg l(^{-1}))</td>
<td>0.10 ± 0.06 (0.00-0.15)</td>
<td>0.12 ± 0.07 (0.00-0.20)</td>
<td>0.14 ± 0.08 (0.00-0.25)</td>
</tr>
<tr>
<td>Nitrate nitrogen (mg l(^{-1}))</td>
<td>1.18 ± 0.21 (0.90-1.40)</td>
<td>1.26 ± 0.20 (0.95-1.50)</td>
<td>1.32 ± 0.20 (1.00-1.60)</td>
</tr>
<tr>
<td>Nitrite nitrogen (mg l(^{-1}))</td>
<td>0.14 ± 0.04 (0.10-0.20)</td>
<td>0.17 ± 0.06 (0.10-0.25)</td>
<td>0.19 ± 0.07 (0.10-0.30)</td>
</tr>
<tr>
<td>Phosphate phosphorous (mg l(^{-1}))</td>
<td>1.32 ± 0.22 (1.00-1.60)</td>
<td>1.37 ± 0.21 (1.10-1.65)</td>
<td>1.42 ± 0.21 (1.15-1.70)</td>
</tr>
</tbody>
</table>

Mean values in same row with same superscripts are not significantly different (Tukey's test, \( P > 0.05 \))
bottles. Larvae were supplemented with a cultured phytoplankton, Chaetoceros calcitrans at the rates of 5000, 10000 and 15000 cells/ml per day at 4-, 6- and 8-armed pedicellaria stage, respectively by adjusting the food levels until attaining the metamorphic competence (Rahman et al., 2000, 2012).

**Induction of metamorphosis**: After 30–34 days of larval rearing, once the matured larvae attained competent stage, they were then used for the induction of settlement tests. Competency was judged by confirming large juvenile rudiment and higher rate of metamorphosis. (Rahman and Uehara, 2001). Settlement induction and metamorphosis of competent larvae were done on coraline red algal extracts in plastic petri dishes (9.0 x 3.0 cm) containing filtered sea water. Density of larvae at this trail was maintained at 1 individual/2ml filtered sea water, following the method of Rahman and Uehara (2001), and Rahman et al., (2012). Metamorphosis usually took around 1 hr 30 min from attachment on the substratum to complete regression of larval tissues and development of complete juvenile structure with growing adult spines, extended tube feet and well-developed pedicellaria, and the entire event usually took place within 1 day post-settlement (Rahman et al., 2012).

**Culture of juveniles and adults**: The newly metamorphosed juveniles were cultured for 3 months in small plastic aquaria (25 x 20 x 12 cm) with aerated FSW, and the coral skeletons with calcareous red algae were provided as food ad libitum (Rahman et al., 2000; 2005). Sea water from each aquarium was partially changed twice a month with new filtered sea water. This procedure was followed up to three months, by which time the juveniles reached to 9.0–10.0 mm test diameter. The 3-month-old juvenile urchins having a mean length and weight of 9.63±0.31 mm and 0.39±0.05 g, were then cultured for 1 year in 9 replicate glass aquaria (46 x 30 x 30 cm), each of which was provided with aerated seawater at the grow-out culture unit of the Institute of Bioscience, Universiti Putra Malaysia. The stocking density was maintained at 20 juveniles per aquarium. Juveniles fed with red alga (Amphiroa fragilissima) were designated as Treatment-1 (T₁), brown alga (Sargassum polysystum) as Treatment-2 (T₂) and sea grass (Enhalus acoroides) as Treatment-3 (T₃), respectively. All the juveniles were fed ad libitum, and the uneaten feed and feces were removed on weekly basis. Seawater in each culturing aquaria was changed completely at every 2-3 months until the end of the grow-out trials.

**In situ** water quality parameters were measured fortnightly between 09:00 and 10:00 hr. Water temperature, dissolved oxygen (mg l⁻¹), salinity (ppt) and pH were determined directly by using a digital water quality analyzer (YSI, MODEL 58; YELLOW Spring, OH, USA), and ammonia nitrogen, nitrate-nitrogen, nitrite-nitrogen and phosphate-phosphorous by a HACH water analysis kit (DR 2000; Hach Company, Loveland, CO, USA). Total alkalinity was estimated following the standard procedure (Stirling, 2000; Hach Company, Loveland, CO, USA). Total alkalinity was estimated following the standard procedure (Stirling, 2000; Hach Company, Loveland, CO, USA).

**Table 2**: Comparison of growth performance, survival and production of S. sphaeroides fed with different algal feeds at the end of one year culture period.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T₁(Red alga)</th>
<th>T₂(Brown alga)</th>
<th>T₃(Sea grass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial length (cm)</td>
<td>9.98 ±0.56  (9.15–12.00)</td>
<td>9.98 ±0.56  (9.15–12.00)</td>
<td>9.98 ±0.56  (9.15–12.00)</td>
</tr>
<tr>
<td>Final length (cm)</td>
<td>38.74 ±0.35  (38.41–38.73)</td>
<td>34.19 ±0.11  (33.69–34.78)</td>
<td>30.04 ±1.09  (29.16–31.26)</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>20.82 ±0.42  (20.43–21.27)</td>
<td>15.16 ±0.67  (14.55–15.88)</td>
<td>10.78 ±0.65  (10.19–11.48)</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>20.33 ±0.42  (19.94–20.78)</td>
<td>14.67 ±0.67  (14.06–15.39)</td>
<td>10.29 ±1.09  (9.70–10.99)</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>4149.66 ±0.35  (4069.39–4240.82)</td>
<td>2993.20 ±1.09  (2869.39–3140.82)</td>
<td>2100.00 ±1.09  (1979.59–2242.86)</td>
</tr>
<tr>
<td>Specific growth rate (SGR) (%/day)</td>
<td>1.39 ±0.01  (1.38–1.40)</td>
<td>1.27 ±0.02  (1.26–1.29)</td>
<td>1.14 ±0.03  (1.12–1.17)</td>
</tr>
<tr>
<td>Daily growth rate (DGR) (%/day)</td>
<td>7.53 ±0.16  (7.39–7.70)</td>
<td>5.43 ±0.25  (5.21–5.70)</td>
<td>3.81 ±0.24  (3.59–4.07)</td>
</tr>
<tr>
<td>Wet gonad weight (g)</td>
<td>2.92 ±0.13  (2.80–3.05)</td>
<td>1.90 ±0.11  (1.75–2.08)</td>
<td>1.16 ±0.12  (1.05–1.29)</td>
</tr>
<tr>
<td>Gonad index (%)</td>
<td>18.49 ±0.46  (18.05–18.96)</td>
<td>16.47 ±0.47  (16.05–16.97)</td>
<td>14.64 ±0.45  (14.23–15.12)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>90.00 ±5.00  (85.00–90.00)</td>
<td>73.33 ±7.64  (65.00–75.00)</td>
<td>58.33 ±7.64  (50.00–65.00)</td>
</tr>
</tbody>
</table>

Thirty specimens were measured for each parameter with 10 randomly selected individuals per replicate for each experimental treatment. All the values represent mean ± SE with ranges in parentheses; Mean values in same row with same superscripts are not significantly different (Tukey’s test, *P* > 0.05)
Growth performance (length and weight) and health condition (based on physical observation) of the cultured urchins were monitored regularly. Ten individuals from each aquarium were measured monthly for the length and weight till they attained the adult stage. The culture was continued for one year and terminated on December 2012, during that time the sea urchins achieved sexual maturity and contained ripe gametes. Growth in respects of final weight and length, weight gain, length gain and survival (%) was estimated following standard methods (Rehman et al., 2000, 2005) while specific growth rate (SGR) and daily growth rate (DGR) were calculated according to Brown (1975), and De Silva and Anderson (1995), respectively. Gonad production in terms of wet gonad weight was estimated following Rahman et al. (2005), while gonad index (GI) was computed according to the formula (Lamare and Stewart, 1998; Meidel and Scheibling, 1998; Walker and Lesser, 1998) given below:

\[
GI = \frac{\text{Wet weight of the gonad (g)}}{\text{Drained weight of the urchin (g)}} \times 100
\]

Data analysis: Percentage data was arcsine transformed before statistical analysis. This transformation helped to normalize the data and also reduce the heterogeneity in variances. The Bartlett’s-test was used to analyze homogeneity of variances (Barlett, 1937). When variances were not significantly heterogeneous and no major departures from normality, one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test Statistical significant level was set at 0.05.

Results and Discussion

Physico-chemical parameters: The average and range values of water quality parameters throughout one-year culture period of S. sphaeroides are summarized in Table 1. The mean temperature (°C), salinity (ppt), dissolved oxygen (mg l⁻¹), total alkalinity (mg l⁻¹), ammonia nitrogen (mg l⁻¹), nitrate nitrogen, nitrite nitrogen (mg l⁻¹), and phosphate phosphorous (mg l⁻¹) among the treatments did not differ significantly (Tukey’s test, P > 0.05).

Growth and production performances: Monthly growth (length and weight) trends of S. sphaeroides are depicted in Figs 1 and 2. The length and weight increment was highest in T₁, followed by T₂, and T₃ (Tukey’s test, P < 0.05). The detailed growth and production performances (initial weight, final weight, final length, weight gain, length gain, specific growth rate, daily growth rate, gonad weight, gonad index) and survival of the urchins under different treatments at the end of the one-year culture period are summarized in Table 2. The mean final length and weight of urchins in T₁ were significantly higher (Tukey’s test, P<0.05) than those in T₂ and T₃. Similar results were also found in weight and length gains. Percent weight gain and length gain were significantly highest in T₁ and lowest in T₃ (Table 2). Specific growth (SGR) and daily growth rate (DGR) were also significantly higher (Tukey’s test, P<0.05) in T₁ than in T₂ and T₃.

The production of edible gonads (wet gonad weight) was significant higher (Tukey’s test, P<0.05) in T₁ than those in T₂ and T₃ (Table 2). Gonad index (percent gonad weight in respect to drained body weight) was highest in T₁, followed by T₂ and lowest in T₃ (Tukey’s test, P<0.05). The production of gonads in sea urchins fed with red alga (T₁) showed an increment of 152.58% over sea grass (T₂) and 54.18% over brown alga (T₃) fed urchins, while it showed an 64.17% increase over sea grass when they were fed with brown alga. Survival (%) was significantly highest (Tukey’s test, P < 0.05) in T₁ followed by T₂ and T₃ (Table 2).

The water temperature (27.80-29.61 °C) and salinity (29.40-31.98 ppt) in the experimental aquaria were within...
suitable levels for sea urchin culture, which agrees well with the results obtained by Rahman et al. (2000, 2001, 2005) and Asia (2009). The dissolved oxygen (DO) levels (6.25-7.35 mg l⁻¹) were higher than that recorded (4.57 to 5.98 mg l⁻¹) Asia (2009), while culturing sea urchin (Tripneustes gratilla) in vitro using glass aquaria were within the appropriate levels for grow-out sea urchin culture. The measured pH ranged from 7.56 to 8.35, demonstrating a productive condition appropriate for sea urchins, which are more or less similar to what Asia (2009) found in the sea urchin culture within aquaria in the Philippines. Coral reefs are considered to be important habitats for most marine vertebrate and invertebrate organisms. Survival, reproduction and development of marine organisms highly depend on environmental factors such as water temperature, salinity, pH and minerals (Grosjean et al., 1996; Alsaffar and Lone, 2000, Chen et al., 2000; Kennedy et al., 2007; Sarifudin et al., 2014). Apart from these, the effects of other water quality parameters (such as total alkalinity, ammonia nitrogen, nitrate nitrogen and phosphate phosphorous) on the body growth of sea urchins have not been thoroughly examined before. However, during the present study, these parameters were investigated for the first time in sea urchins and all of them were found to be suitable for their aquaculture in captivity, as similar to those reported from various fish rearing ponds (Islam, 2002; Kohinoor et al., 2004; Rahman et al., 2008a, 2008b, 2011a, 2011b, 2012, 2013b, 2013c, 2014; Rahman and Marimuthu, 2010).

The growth performance, gonad production, and survival of one-year-old S. sphaeroides adults were significantly higher (P < 0.05) in T₁ when juvenile urchins were fed with red alga (A. fragilissima) than those fed with brown alga (S. polysystum) (T₂) or green sea grass (E. menziesii) (T₃), respectively. The reason behind this might be due to the fact that red alga was preferred and palatable food and therefore, accelerated the growth performances of S. sphaeroides compared with other algal diets. Similarly, coralline red algae was found to be the best algal food for promoting survival, as well as growth in terms of weight, length, and gonad production for the grow-out adults of conspecific parents and their reciprocal hybrids among the different Echinometra spp. in Okinawa (Rahman et al., 2000, 2004, 2005). The results of the present study are more or less in agreement with the findings of Steinberg (1988), Kenner (1992) and Sonnenholzner et al. (2011), who advocated that echinoids could change their food rations when the favored macrophytes were rare or absent, however, growth and survival could be considerably affected. The findings of the present study is in contrast with Sonnenholzner et al. (2011), who also found that the sea urchin (Strongylocentrotus purpuratus) fed with coralline algae (Bosellia orbigniana) and elggrass (Phyllospadix scouleri) had substantially lower growth in size and weight, and actually these plants did not promote gonadal growth in juvenile urchin. Moreover, it was observed that a mixed diet, comprised of the above three species, was better for the sub-adult S. purpuratus as compared to a single diet of coralline algae (B. orbigniana) or elggrass (P. scouleri).

From nutritional point of view, nitrogen has been considered as a vital constituent for reproduction and growth of herbivores (Mattson, 1980). However, nitrogen (hereafter referred to as protein) is often low in all types of marine plants and seems likely to be the nutritional component that most frequently impacts on food selections by herbivores as a whole (Neighbor and Hom, 1991; Sonnenholzner et al., 2011). For example, kelp (E. menziesii) and eelgrass (P. scouleri) contain similar amount of proteins, (8-12%) lipids (0.8-1.5%) and carbohydrates (40-45%) (dry weight basis), respectively, while coralline alga, B. orbigniana has lower protein (~3% lipd (0.5%) and carbohydrate (8-10%) contents respectively. Despite the fact that green eelgrass, P. scouleri is considered as a protein source comparable to brown kelp, E. menziesii, the highest growth rate was obtained for S. purpuratus, when they were fed with kelp (Sonnenholzner et al., 2011). Although, we have not been able to estimate the proximate composition of algal plants used in the present study, it should not be ignored that these plants can present significant differences in the contents of

![Fig. 2: Mean live weight increment of Salmacis sphaeroides fed with different macroalgae over one year of culture period](image-url)
most of their nutritional components, which deserves further investigations.

Algae release extracellular organic matter, which may increase with stress (Sieburth, 1969; Kores, 1970; Rahman and Uehara, 2001). Brown and green algae usually release higher amount of polyphenols than red algae (Sieburth, 1969), which was most probably accounted for relatively lower percent of metamorphosis and survival of juvenile urchins in treatments with these algae than red algae (Rahman and Uehara, 2001). Other studies have determined that brown algae and green sea grass can seasonally produce some chemical deterrants like condensed tannins and phenolic acids which can reduce palatability or increase toxicity to herbivores and thus, reduce their nutritional value (Zapata and McMillan, 1979; Goecker et al., 2005; Iken and Dubois, 2006). This was possibly a vital key factor in the present experiment for restraining intake of *S. polysystum* and *E. acoroides* by the juvenile and adult sea urchin, *S. sphaeroides*.

Overall, the highest growth performance, edible gonad production and survival of *S. sphaeroides* were found when, the urchins were fed with red alga than those fed with brown alga or green sea grass respectively. This is a first attempt in successful culture of adult *S. sphaeroides* in a static aquarium system, and these findings will likely be of great help in the development of sea urchin aquaculture. Further studies are also needed to determine more suitable stocking densities, feeding regimes and culture techniques of this high-valued sea urchin fishery.

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**References**


Growth and survival of tropical sea urchin


